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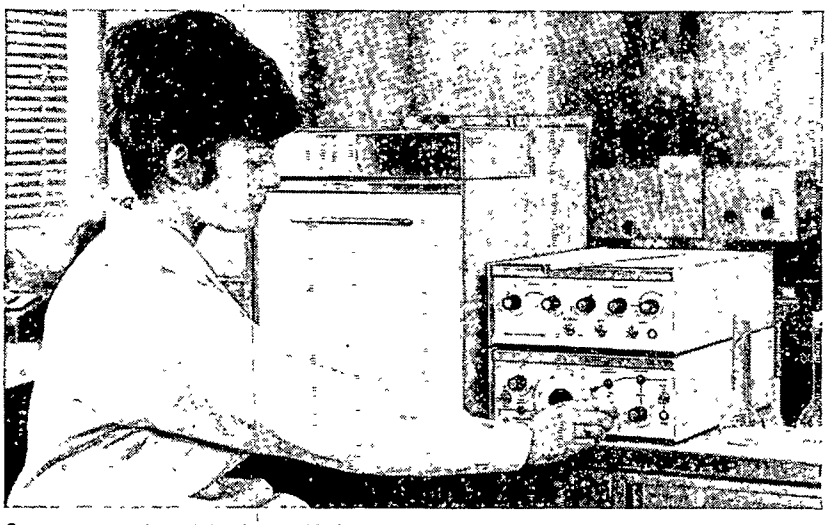
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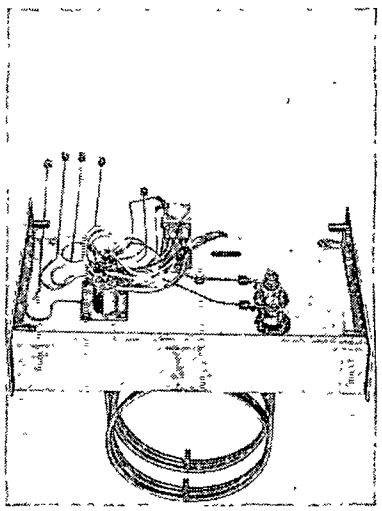
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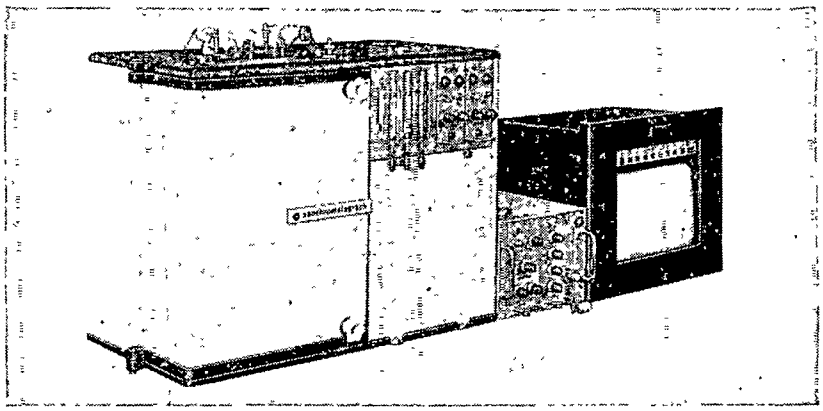
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STABILIZING THE RESEARCH EFFORT

IN its summer issue, devoted to the subject of "Creativity and Learning", *Daedalus*, the journal of the American Academy of Arts and Sciences, introduces a new section, entitled "Documents". This includes Prof. J. B. Kistiakowsky's "On Federal Support of Basic Research", which originally appeared as part of an investigation undertaken by the American Academy of Sciences at the invitation of the Committee on Science and Astronautics of the United States House of Representatives. This is a thoroughly pragmatic discussion of the relations between pure and applied research and is the more interesting in the light of a report on "Basic Research and National Goals" made by the Academy of Sciences to the same Committee, which has now been published in a corresponding section of the Summer 1965 issue of *Minerva*.

Prof. Kistiakowsky begins by referring to the serious danger created by the increasing spread of the idea that basic research can be planned in detail, and that money need be allocated only to specific topics in order to provide the necessary scientific knowledge for the advance of technology. Citing as examples the discovery of the laser and the maser, the relations between genetics and chemistry in molecular biology, and the impact of high-polymer research, he points out that, on the contrary, while clearly defined mission-oriented research has value, by itself it is insufficient and is incapable of developing really new ideas and new principles on which each particular mission will ultimately be based. If the social climate and the mechanisms of support do not rapidly and effectively encourage the free exploration of new ideas, technology will die on the vine because, in the absence of the results of new, undirected basic research, applied work tends to become confined increasingly to expensive refinements and elaborations of old ideas.

Prof. Kistiakowsky points out that scientific activities and their financial support cannot continue to grow indefinitely at the present pace. Nevertheless, it is in the nature of technology not only to solve human problems, but also to generate new ones. Here he is thinking more especially of the human and social problems which arise and, while he insists that the major problems of humanity will not be solved by technological innovations (educational and other social progress being probably more important), he concludes that we need a better understanding of relevant social and behavioural problems and consequently more research in these sciences. The progress of human society must be based on social change keeping pace with technological progress.

Prof. Kistiakowsky goes on to discuss the criteria for determining fiscal support of scientific research. Basic research, he estimates, accounts for only a few tenths of 1 per cent of the gross national product of the United States. Expenditure on research and development, however, is estimated at nearly 20,000 million dollars—some 90 per cent of this being expended on efforts to achieve specific practical objectives, many related to the military and space programmes. Fewer than 100,000 scientists are engaged in basic research out of a technical community of more than 2 million engineers and scientists. It is at this point that Prof. Kistiakowsky considers the two distinct questions posed by the House Committee on Science and Astronautics, which are the subject of the reports of Prof. H. G. Johnson and Dr. A. M. Weinberg in *Minerva*: the requirements for overall support of basic research and the proper balance of support among the several sciences. He approaches both questions by dividing basic research into four categories and suggesting a basis for criteria of support for each.

His first category is characterized by the small size of its constituent autonomous research units, in contrast with his second category which involves large co-ordinated effort, and usually very costly research facilities as well. Both these categories are mainly identified with academic institutions. His third category is basic research in mission-oriented Government establishments, and his fourth in industrial laboratories. He regards projections for a break-down by separate sciences as well-nigh impossible and adds a caveat that the purpose of planning for science is to chart in advance the evolution of a scientific environment and climate in which innovation and creativity can flourish.

About 57 per cent of research in the first category is supported by grants and contracts from the Federal Government to universities, and although Prof. Kistiakowsky estimates that less than 100,000 people are engaged on a full-time basis, it is responsible for great contributions to scientific knowledge, as well as for training most of the new scientists. The 7,000 or so Ph.D.s in science now being produced annually—a figure growing by 6 or 7 per cent each year—are responsible for research work costing on average perhaps 15,000–20,000 dollars per annum per man, and he makes the interesting comment that more should be expected from research institutes or laboratories in scientific output if, unlike the universities, they do not contribute to the training of new manpower. On the financing of such work by large contracts or block grants, he emphasizes the danger that this method can lead to distinctly second-rate or poor research being hidden and protected by the good work of a few leaders.

The costs of scientific research are steadily increasing, partly because the problems are becoming more difficult; although, on the other hand, because of efficient instruments, problems that formerly appeared formidable can sometimes be solved in days instead of years. As to the criteria for determining actual expenditure in this category, he suggests first that, since continuity is important, effective expenditure in the past year should be a large factor in deciding expenditure in the next. The second, he suggests, is to assure future students with scientific aptitudes of the opportunity to learn productive research. His third suggestion concerns the level of support in the rest of the world—a criterion which, if valid for the United States, is open to question for most other countries. He believes that more basic research is needed across the whole range of scientific fields, including the behavioural sciences, and to meet this need he tentatively suggests an overall growth of 15 per cent over the next 5–10 years, which would double by 1968 his estimate of a total 600 million dollars in 1963.

As to the allocation of the total to individual problems, he insists that every effort should be made to minimize detailed centralized planning and control. The detailed

allocation of support should be based on the multitude of individual choices made by individual investigators.

The proper rate of growth in his second category should be determined by other considerations, and here he is largely guided by Dr. Weinberg's observations in an earlier article of 1963. In this field the commitment of scientific personnel per project is rising comparatively slowly and costs are concentrated in the engineering effort of constructing the special facilities required, and in their maintenance, operation and improvement. Nevertheless, the cost for each active scientist or scientific paper is very much higher than in his first category, and Prof. Kistiakowsky suggests that, before weighing seriously the comparative value of a new project in this field, it is essential to verify that it is technically feasible in the judgment of engineering experts and that a qualified group of scientists regard it as important enough to warrant support with firm personal commitments.

Beyond this, Prof. Kistiakowsky would, like Dr. Weinberg, give some weight to the impact of a given type of research on related and significant branches of science and to its impact on practical application. He does not entirely reject the factor of national prestige and international influence, and feels that the broad cultural impact of scientific research is less susceptible of quantitative evaluation, although he attaches some importance to it in the particular fields of galactic astronomy, molecular genetics and high-energy physics. Judgments, however, are fallible and should, he points out, be in terms of various degrees of value. He believes that this category of research should not grow at the expense of his first category but is undecided whether it should grow more or less rapidly than the latter.

While firmly endorsing the need for, and value of, mission-oriented research, he has important reservations about the introduction of general basic research into Government establishments and regards the substitution therein of such research for mission-oriented research as unsound both from the scientific and the economic points of view. Much the same considerations, he believes, should apply to his fourth category—that of research financed by the Federal Government in private industrial research laboratories. He would accordingly relate such research to practical objectives and it would seem that he would have some reservations about the grant-aided industrial research association as practised in Britain. For all his conviction that basic research requires support over the whole spectrum of sciences, he is emphatic that the main justification for Federal support on the present scale lies in its practical application of benefit to the nation.

The first of the two reports in *Minerva* is concerned with some economic issues involved in the Federal support of basic research. Prof. Johnson accepts that some meaning can be attached to the distinction drawn between basic and applied research, though it is not so much the imprecision of that distinction as the extreme uncertainty of both the contribution to knowledge resulting from a particular line of research and the ultimate practical utility of that contribution which is the main source of difficulty in formulating public policy with respect to basic research. He sees, however, no necessary connexion between leadership in basic science and leadership in the applications of science: there may indeed be conflict between basic research and applied science. The two main arguments for Government support of science are cultural–social and economic.

Prof. Johnson examines both these arguments: the latter at some length. The former emphasizes scientific activity as a form of social consumption of wealth and raises the political question of whether other uses of the resources involved would contribute more to the greatness of the society. Accordingly, the decision cannot be left to the scientist alone. The economic argument regards scientific research as a form of investment for the future and raises the questions of what the rate of return on such investment is, and whether and to what extent Government support is needed.

Economic research has so far provided few relevant results. Prof. Johnson points out that there are good theoretical reasons for expecting that, left to itself, the market would tend both to allocate too few resources to research in general and to prejudice the allocation against basic research as contrasted with applied research, and towards research in scientific areas related to the technology of industries dominated by large multi-product firms. This expectation seems to be substantially confirmed by the industrial distribution of expenditure on research and development. (The analysis, incidentally, offers an explanation of the lack of research effort in the smaller firm.) This discussion, moreover, brings out several important questions to which answers should be sought before firm conclusions can be reached. These relate to the extent or the distribution of support of basic research. For the latter, such questions arise as the likelihood of proposed new lines of research producing new contributions to knowledge and the possible social value of these contributions in relation to their cost; and the probability of any particular project being undertaken elsewhere, and the consequences. The former raises questions as to the rapidity with which returns on research diminish; how far increased expenditure on research increases the quantity of research results and how far it merely raises the operating costs.

There is also the final question of the extent to which Government support and private support of basic research are substitutes for one another. Answers to all these questions are necessary to judge the adequacy of Federal support of basic research from the economic point of view. Prof. Johnson does not attempt to provide a firm answer to the question of the amount and distribution of such Federal support. He is content to emphasize the dangers attending any answer in the absence of further and firm knowledge which might provide answers to the subsidiary question. He directs attention to the importance which the United States attaches to the geographical distribution of support and urges that so long as public funds are allocated to basic research, the geographical allocation of those funds should take account of the social effects of their expenditure—an argument which, to a limited extent, appears to be reflected in some of the recent regional plans for Britain's economy.

Dr. Weinberg, in contrast, addresses himself primarily to the question of scientific choice in both basic science and applied missions, and while he admits his indebtedness to Dr. Toulmin's paper on this subject (see *Nature*, 205, 426; 1965) he largely follows his own earlier exposition (*Nature*, 200, 503, 1030; 1963). He reiterates, for example, his distinction between political choices and administrative choices and emphasizes the importance of making choices between political alternatives and administrative alternatives; this factor, rather than the question of who makes the choice, is the key problem. He insists once again on the prior need to separate

research pursued for non-scientific ends from that pursued to further science. He also regards it as essential that each mission-oriented agency should assume the responsibility for supporting basic science in fields generally relevant to the agency's research and at a level bearing some relation to the size of its budget for applied science. It is beyond this level that the National Science Foundation should assume responsibility for supporting basic science where necessary.

Dr. Weinberg does not really answer the question of how to allocate support for basic science, other than by proposing a pattern of organization to handle the allocation, and he has little fear that to place the motivation for supporting basic research mainly on the hope of some eventual benefit from its application will result in basic research being unduly curtailed in time of falling budgets. Furthermore, for all the reliance he places on the National Science Foundation for securing a sound distribution of the national expenditure on basic research, he is convinced that such support should be drawn from numerous sources, and not just one or two. Nevertheless, he is opposed to any expansion of basic physical research merely to further the objectives of, for example, the Atomic Energy Commission, the Department of Defence, or the National Aeronautics and Space Administration.

The relevance of these views to discussion on expenditure on civil research in Britain, for which the new Council for Scientific Policy has recommended some £54 million a year, with a continuing growth for five years at the same rate as in the past decade, or 15 per cent per annum, is obvious. However, before considering specific implications to complete the picture we may appropriately note Dr. Weinberg's further observations in a paper, "Government, Education and Civilian Technology", which he contributed to a Seminar on Technology and Social Change at Columbia University. This paper has now been published, together with the other six papers, an introduction by C. Wright, notes on the discussions on each paper, and a summary by A. W. Warner, under the title *The Impact of Science on Technology* *. Some of the points which Dr. Weinberg makes here concern the dangers inherent in the reform of curricula in universities unless the reforms are in the hands of men of wide outlook sympathetic to applied science and resistant to the fragmentation of science. Otherwise we may continue to produce graduates with little sympathy outside their own special field, who are thus less competent for technological research. He repeats his warning that the value of the by-products of military research and development tends to be exaggerated and that civil industry should not rely to any extent on such 'fall-out'. He reiterates his concern at the consequences of neglecting areas of the scientific field because they are less fashionable and emphasizes the importance of scientists paying due attention to the social implications of their work.

In the first two papers presented at the symposium, Prof. I. I. Rabi and Dean Harvey Brooks contribute differing views of the interaction of science and technology. Prof. Rabi questions the effect of science on older industry until the latter has developed to a very sophisticated degree: the more important effect has been to create new industries, and supply, along with new knowledge, new components and new techniques. An important factor in the growth of industry and technology has been the adoption of instruments originally developed for scientific advance itself. He questions, however, whether the cultural climate in the United States really encourages the originality and eccentricity which often mark the innovator. On the other hand, it does encourage team work, the value of which he firmly acknowledges. Like Dr. Weinberg, he is equally emphatic, while emphasizing the dependence of the future of society on science, that the scientist should be concerned with society and the arts, if only for their contribution to imagination and inspiration. In the discussion he adds, too, a warning against the assumption that increasing the number of scientists is likely to increase proportionately the number of outstanding people.

Dean Brooks disagrees to some extent with Prof. Rabi as to the factors promoting innovation. He regards the role of science in the development of technology as being to provide the environment in which technological ideas can be exploited, rather than being itself the origin of technological ideas. The same qualities of mind go into science as go into invention, and whether a man will be an inventor or a scientist is determined by his emotional attitude to the subject. While the same qualities that go into invention may also go into the creation of new techniques, emotionally the two activities are poles apart. Accordingly, while the creation of new science is necessary, it is not a sufficient condition for creating new technology.

Without convincingly establishing this view, Dean Brooks lists several factors conducive to technological innovation which originate in science and are highly pertinent to the consideration of the expansion of higher education in Britain, as well as to that of the scale and distribution of British scientific effort. First and most important is widespread scientific literacy. Next he places the creation of a scientifically literate management with an instinct for possibilities in the technical area. It might be interpreted that this is a first condition for the effective distribution of our scientific effort in general and for informed discussion of structure and distribution. This is especially so in view of the fact that the House of Commons, in debating on October 27 the report of the Select Committee on Procedure, slammed the door on any such idea, in spite of the recommendation from the Parliamentary and Scientific Committee.

Thirdly, he puts mobility among the intellectual disciplines, and firmly condemns specialization as the enemy of technological innovation. He adds that application of science often arises from the movement of people from basic science into technology—a point of view which may well lie behind Prof. Rabi's belief in the value of team work. Like Prof. Rabi, when emphasizing the great importance for innovation of the diffusion of graduates from science into technology, Dean Brooks suggests that some over-production of scientists may be an essential condition of continuing technological innovation. Put in another way, this amounts to condemnation of the practice of regarding university education or technological education as primarily professional training for a particular occupation instead of being aimed at producing educated men and women competent to apply themselves to a range of professional occupations.

Here again we note the emphasis on the social aspects and on the vital importance of a broad-based education. Moreover, there is a limit, which should be noted, of the effect of science and technology on institutional innovation. This recurs to some extent in a fourth paper from F. de Hoffmann on "Advanced Technology and American

* *The Impact of Science on Technology*. Edited by Prof. Aaron W. Warner, Dean Morse and Alfred S. Eichner. Pp. vi+221. (New York and London. Columbia University Press, 1965.) 50*s*.

Business: Friends or Foes?" He is mainly concerned with questions of management and organization, for while he holds that creativity and organization can be harmonized in group or team efforts, creativity and regimentation cannot. Again, therefore, we find emphasis placed on the importance of wise management in organizing interdisciplinary effort and, by implication, on the broad general education which promotes scientific understanding in the management field. One constructive suggestion that he makes is that decisions regarding large-scale research and development should be the responsibility of several centres rather than one alone, and that industry should co-operate with Government to that end. The importance of entrusting responsibility to technical people could not be more strongly emphasized, nor indeed could the value of ensuring that the scientist works in an atmosphere conducive to creative work, with the assurance that problems, so far as possible, are approached in a scientific manner.

Mr. de Hoffmann's paper is full of warnings that the conditions for effective scientific work must be respected, and much of the paper is in line with the views of Dr. Stoll. Nor does he offer much support to the idea of the co-operative research association in industry. A further paper, from Mr. J. H. Hollomon, on "Science and the Civilian Technology" directs attention to some relevant developments in the social and economic structure of the United States of which account must be taken in any planning in this or in any other field. He, too, emphasizes the limitations which are placed on technological advance by any lack of those who understand both science and technology on one hand, or, on the other, the business aspect of a technological enterprise. His most serious warning, however, concerns the high social cost of technological change, and the importance of the adequate use of technology in decision-making at all levels.

The remaining two papers are much more clearly concerned with the American scene. Mr. J. Brademas sets forth a Congressman's view of technology and social change, but Dean R. S. Halford's paper on modern science and its implications for the university, while largely concerned with the effect of the increasing scale of Federal support for research in the American universities and the way this is being handled through the formation by a group of universities of non-profit corporations, has some interest for the organization of research in Britain. As a further safeguard against possible distortion of the work of a university through Federal grants for research, Dean Halford stresses the firm insistence on the recovery of all indirect costs related to the conduct of research sponsored by Government agencies, and the need to relate requirements for a faculty to the instructional requirements of the teaching programme.

Some of the points raised in the seminar which are most pertinent to present-day debates in Britain are well brought out by Prof. A. W. Warner in his summing up. He does not, however, mention the fact that within the previous 6 months Columbia University had found itself under-spending funds committed to it for research, just when, in the United States generally, the level of expenditure on research was being questioned. This may well be relevant if, in fact, the growth of expenditure on research in Britain is to be checked under the Government's plans. Conditions in the two countries, however, are not parallel and much clear thinking will be required if short-sighted curtailment of expenditure on basic research is not to do serious harm. That there are definite limits to the amount which any nation can wisely expend on scientific research is not disputed. How that limit is to be determined, and still more, how the amount available should be distributed among the different fields are other questions altogether; it is obvious from the papers noted here that the United States is still seeking for answers to these.

What should be evident, so far as Britain is concerned, is that she is as yet a long way from the limit. In the social sciences and in education in particular expenditure on research is trifling, and far short of what the national interest demands. That is also largely true of research in the physical sciences which bears on the utilization of natural resources. Here, for example, the Government's reported decision to hold up the pilot studies on the Solway and Morecambe Bay barrage schemes on the excuse of economy is disturbing and indicates the persistence of the 'penny wise, pound foolish' attitude for which Britain has paid dearly in the past. For all the protestations of the present Government and its predecessor, it is hard to point to any imaginative and far-sighted decisions which indicate an awareness of the potential contributions which scientific research could make in Britain's economy, or readiness to take the action demanded by the fact and not that demanded by party prejudices. However low the level of expenditure on fundamental and applied research may be set, it will still call for sacrifices elsewhere. Scientists are unlikely to be convinced that any Government is really prepared to support such expenditure on an adequate scale while the present attitude of the Ministry of Technology persists.

THE ACHIEVEMENTS OF LORD RAYLEIGH

Scientific Papers

By Lord Rayleigh (John William Strutt). Vol. 1: 1869–1881. Pp. xiii+562. Vol. 2. 1881–1887. Pp. x+598+16 plates. Vol. 3: 1887–1892. Pp. xiv+596. Vol. 4: 1892–1901. Pp. xiv+568. Vol. 5: 1902–1910. Pp. xvi+624. Vol. 6: 1911–1919. Pp. xiii+718. (New York: Dover Publications, Inc.; London: Constable and Co., Ltd., 1964.) 30 dollars; 240*s*. The six volumes bound as three.

LORD RAYLEIGH was a man whose range of research in the world of physical science has, it may be ventured, no equal among those active at the present time. In the first place, he was equally distinguished for his work in the mathematical field and for his experimental achievements. In the second place he made notable advances in all branches of what is known as classical physics: heat, light, sound, properties of matter, magnetism and electricity—and in chemistry too, if we consider the leading part which he played in the discovery of argon.

To mention but a few contrasting accomplishments, he made a fundamental experimental re-determination of electrical standards and wrote what is still a standard book, containing much of which he was the originator, on sound; certain advances which he made in wave theory explained the blue of the sky and others contributed to the study of earthquakes; he was equally authoritative in hydrodynamics and thermodynamics; he gave a celebrated presidential address to the Society for Psychical Research and took a leading part in the foundation and early administration of the National Physical Laboratory. These odd citations suffice, perhaps, to revive memories of his varied mastership.

His collected *Scientific Papers* were originally published in six large quarto volumes by the Cambridge University

Press, the first volume having appeared in 1899 and the final one, posthumously, in 1920. Rayleigh made an amusing comment in connexion with the publication of the first volume: "When I was bringing out my *Scientific Papers* I proposed a motto from the Psalms, 'The works of the Lord are great, sought out of all them that have pleasure therein'. The Secretary to the Press suggested with many apologies that the reader might suppose that *I* was the Lord." This, no doubt, is the reason that, while the scriptural quotation is used, it appears not on the title page but in isolation on a separate page just before the first paper.

The atmosphere of the great age of classical physics to which these papers pertain may be recalled by reference to the name index which appears at the end of the final volume. Citations, merely page numbers, of Stokes occupy 13 lines, of Maxwell 12, of Kelvin 11, of Helmholtz 9, of Lamb 7 and of Airy 5 lines—a formidable company.

These collected papers are still constantly used for reference, since, among other things, when Rayleigh had written at length on a subject he had, in general, tidied it up and left it in a state clearly recording the position at the time. One can scarcely do better than quote J. J. Thomson's pronouncement: "While most collections of scientific papers rest undisturbed on their shelves, and are monuments, rather than parts of one's working library, there are no books I refer to so frequently as Rayleigh's collected papers. His papers deserve the description, which Maxwell applied to those of Ampère, as being 'perfect in form and unassailable in accuracy'. The style is very clear, so clear in fact that the reader may not realize how difficult the problem was unless he had attacked it himself before reading the paper".

The original issue of the *Scientific Papers* has, with the exception of Volume 6, been out of print for some time. Fortunately a re-issue of the six volumes, bound in pairs so as to make three books, has recently been published at a very reasonable price. The text would appear to have been reproduced by a photographic process, since whenever a page has been compared with the original the two are exactly the same. However, the introduction states that the edition is an unabridged and corrected republication of the work first published by the Cambridge University Press, which would seem to imply that there have been changes, if minor ones.

This new edition contains an interesting supplement of photographs pertaining to Rayleigh's life—portraits, pictures of his laboratory, of pages from his notebook, and so on. A great improvement could have been effected by the provision of a serviceable index. There is a name index, which suffers, as is so often the case, from a lack of the slightest indication as to what is in question under each of the large number of pages listed under a given name; more than a hundred, for example, in the case of Maxwell. There is no subject index at all, unless the list of titles of papers, grouped under twelve different headings, such as "Mathematics", "General Mechanics", "Elastic Solids", and so on, can be so called. This list, however, is not very useful if one desires to find quickly what Rayleigh has written on a particular subject: thus the only references to argon are in the titles of certain papers listed under the heading "Properties of Gases", but there is no indication that the viscosity of argon is considered in papers listed under "Dynamical Theory of Gases". Possibly the publishers will contemplate adding to the value of this welcome reprint by issuing in a small supplementary volume a detailed index giving not only a properly classified subject schedule but also a name index with some indication as to what, to take an example, is concerned in each entry under the name Maxwell. The preparation of such an index, which would require considerable knowledge and study, might possibly qualify a student for a degree in the history of science.

E. N. DA C. ANDRADE

A ROUTE MAP THROUGH THE SPECTRAL LABYRINTH

Atlas of Steroid Spectra

By W. Neudert and H. Röpke. Translated by J. B. Leane. Pp. viii+471. (Berlin, Heidelberg and New York: Springer-Verlag, 1965.) D.M. 144.

THE advent during recent years of various sophisticated, physical techniques, as an adjunct to structural investigations in organic chemistry, has led to its own special problems. In particular, the data thus accruing are frequently very difficult to locate in the literature and, for a variety of reasons, are often of doubtful comparative value for the identification of closely associated compounds.

Nowhere is this problem more acute than in the vast literature appertaining to the steroids. The *Atlas of Steroid Spectra* is a gallant and monumental attempt to bring some rationale into an otherwise confused situation. The production of this weighty (in both senses of the word) volume was undertaken because the majority of existing steroid spectra originated from various sources, and therefore could not be compared with each other directly. The great merit of this volume, which has been produced with typically Teutonic attention to detail and accuracy, is that it records the spectra of 900 specially selected steroids. The samples were prepared under directly comparable conditions and the spectra measured on the same instrument in order to ensure that the results are directly comparable. The information recorded in this *magnum opus* has been accumulated during the past twelve years, which the authors have spent in the structural investigation of steroids.

The first section includes the infra-red spectra and other relevant physical data, such as molecular weight, melting-point and characteristic ultra-violet absorption maxima for 900 steroids. Then follow various typical ultra-violet absorption curves for 41 representative steroids. The third section records the nuclear magnetic resonance spectra of 95 steroids. The remainder of the book consists of chapters concerning the relevant theory and the methods by which these various spectral determinations may be performed, together with a veritable wealth of associated information such as, for example, the effect of temperature on spectra. The final section of the book contains the basis of a versatile catalogue system for recording the detailed spectral information.

A most useful feature of the *Atlas* is that the German text has an English translation alongside.

This book is essentially a handbook or dictionary, which will serve as a most valuable reference work for all research laboratories. By nature of its size, price and content, it is scarcely likely to appeal to the individual purchaser. A particularly noteworthy feature of this *Atlas* is that it is based entirely on work carried out in the laboratories of an industrial company, namely, Schering *A.G.* (Berlin). Its authors, to whom all steroid chemists owe an immense debt of gratitude for their painstaking effort, are also employed by the same company.

This book maintains the extremely high standard which has been established by its publishers. W. B. WHALLEY

RADIOACTIVE TRACER TECHNIQUE IN BIOLOGY

Radiotracer Methodology in Biological Science

By C. H. Wang and D. L. Willis. (Biological Science Series.) Pp. xvii+382. (Englewood Cliffs, New Jersey and London: Prentice-Hall International, 1965.) 96*s*.

RADIOTRACER Methodology in Biological Science is an excellent book. Radioactive tracers are commonplace and indispensable in research laboratories, but their

experimental methods find little place as yet in teaching courses. There must be many readers who need reliable, sensible and up-to-date information and guidance, and they will be grateful to Profs. Wang and Willis for providing it.

The authors write that "the organization of the text stems both from the authors' research interests and, more importantly, from teaching experience involving students from the various fields of biology". This is apparent from the choice and treatment of the material.

The first and largest part of the book (266 pp.) deals with the origin and basic properties of radioactive materials, methods of detection and other practical considerations. The treatment is clear, systematic and concise. The typical reader, who may have little or no acquaintance with radioactive tracers, will find it well suited to his needs, both for instruction and as a first book of reference. Proper emphasis is laid on the best techniques at present available in a well-equipped laboratory, but older or simpler methods, which are still in use or which teach important principles, are also adequately treated.

The second part (44 pp.) consists of basic experiments in the measurement of radioactivity. It covers Geiger, proportional, solid-scintillation and liquid-scintillation counters, the nature of radioactivity decay, and interaction of radiation with matter.

The final section (56 pp.) is, in some respects, the most interesting. It comprises six illustrative radiotracer experiments, selected for the likely interests of the reader; these are: "Incorporation of $^{14}CO_2$ into Amino Acids in Yeast"; "A Time Course Study of $^{14}CO_2$ Production from Rats Metabolizing Glucose-^{14}C Substrate"; "The Effect of X Irradiation on Bone Marrow Activity in Rats as Measured by Iron-59 Incorporation into Erythrocytes"; "An Investigation of Sodium Ion Regulation in Crayfish using Sodium-22"; "Determination of Coefficients of Zinc-65 Accumulation in Freshwater Plants"; "Determination of the Content of Aspartic Acid in Cheese by Means of the Isotope Dilution Method". Each is worked out quite thoroughly, using the information and methods given earlier in the text: for example, survey of the problem; experimental design (scale and requirements for radioactivity); materials and equipment required; experimental procedure; experimental notes. Questions interpolated here and there will help to keep the student aware of what he is doing, and why.

The bibliographies for the various chapters are good, and there is an adequate index. The book is well but not extravagantly produced.

There are a number of specific points about this book which I liked. The English style is clear and readable. The text abounds in brief, commonsense observations which attract attention and set the reader on the right lines: "autoradiography is as much an art as a science"; "proper preparation of counting samples is as important—as the counting operation"; "radiotracers must always be regarded as only a tool, not a panacea"; "the investigator must take an open-minded, unprejudiced approach—"; "the opportunities for misinterpretation are probably much greater (in tracer experiments) than in the traditional approaches". I can endorse the validity of these and of many similar observations, and the need to present them and to insist on them, even at the present date.

The selection of material shows also that the authors are looking forward and not backward. The technique of 'radiorespirometry' (kinetic studies of respiratory metabolism, detecting $^{14}CO_2$ from suitably labelled substrates), patiently promoted for some years by a handful of enthusiasts, is still in very limited use, and good equipment is still rare and expensive. Profs. Wang and Willis give it nevertheless a fairly thorough treatment, as a method of which every serious student should have some knowledge.

Inevitably, I found details with which I do not agree, but they are not important enough to be discussed in a review of this size

The price may appear rather high to readers in Britain, but for a book of this kind and standard it is reasonable.

John R. Catch

CRYSTALLOGRAPHY: TRANSLATION OF A RUSSIAN DATA COMPILATION

Handbook of X-ray Analysis of Polycrystalline Materials

By Lev Iosifovich Mirkin. Authorized translation from the Russian by Dr. J. E. S. Bradley. Pp. xx+731. (New York: Consultants Bureau, 1964.) 35 dollars.

HOW different the appraisal of the English edition of Dr. Mirkin's *Handbook of X-ray Analysis of Polycrystalline Materials* must be from that of the original Russian text of 1961 is shown by Prof. Ya. S. Umanskii's remark, in the foreword, that apart from Kitaigorodski's classical compilation of twenty years earlier this was the only general reference work on X-ray analysis published in the U.S.S.R. In the English language it is with a bookshelf already containing the *International Tables for X-ray Crystallography* and other well-known specialized compilations that we must judge the usefulness of any new compilation.

Dr. Mirkin's book is divided into two roughly equal parts entitled "General Methods of X-ray Structure Analysis" and "Some Special Problems and Methods in X-ray Structure Analysis". These titles would be thoroughly misleading were it not for the overall title, Part 1 in fact being directed towards the powder method, and Part 2 mainly concerning identification, accurate measurement of structure cell dimensions, and certain extra-structural features.

In choosing the order of presentation in Part 1, the author has borne in mind the sequence of events in an analysis. Thus we find that X-ray wave-lengths, absorption coefficients and similar data come first. A short survey of the main types of Russian equipment (*c.* 1961) is also included and nomograms and charts are provided to facilitate the setting up of cameras and monochromators.

Having, so to speak, obtained diffraction data, one arrives at tables relating Bragg angles with ring diameters for a range of specimen-to-(flat)film distances. If a cylindrical camera is used then corrections for specimen eccentricity and thickness are here to hand. The usual extrapolation functions, however, are kept for a chapter in Part 2 and it is only by browsing that one finds them—the index being of little help. Commendably, charts for $\theta - d$ conversion have been omitted, these being available in a more suitable larger format elsewhere. Intensity measurement receives little attention apart from the usual guidance in evaluating errors in counter methods.

More than half of Part 1 concerns various ways of indexing powder patterns. In common with the remainder of the book there are short explanatory sections followed by the relevant data; in the present instance, tables of the necessary trigonometric functions, *d.* v. $1/d^2$, quadratic forms, space group data, Cernohorsky's nomograms, and Hull–Davey and other charts. There are also theoretical line-patterns for some structure types, but these are inferior to those in Part 2 to which, unfortunately, there appears to be no cross-reference. Indexing in the monoclinic and triclinic systems is limited almost exclusively to the use of homology, with only brief mention of, for example, reciprocal-lattice methods: as in other sections, one is reminded that the contents are essentially pre-1961.

Part 1 concludes with standard data and aids required for the calculation of intensities and includes atomic co-ordinates for a number of structure types, and structure factors for a selection of space groups.

Inorganic 'phase analysis' forms a major topic of the second part, and includes Sagel's list (1958) giving structure type and cell dimensions for 1,200 inorganic sub-

stances. The original Russian text also included 126 pages of data from the *A.S.T.M. Powder Data File* (c. 1957) but very sensibly these have been omitted from the present English edition because more accurate and extensive data have become available from the American Society for Testing Materials and also because continuance of the *File* depends on its separate publication. For the cubic, tetragonal and hexagonal systems, however, there are Frevel's theoretical line-patterns for recognizing and indexing many structure types, with tables for identifying some hundreds of compounds in terms of structure type and cell dimensions. Tables of 'homologous pairs' for some two-phase mixtures, and others for the general conversion from weight per cent to atomic per cent, would, like so many of the entries, become useful if one knew they were there.

Precision measurement of lattice constants is another topic receiving special attention with tables of $\frac{1}{2}\lambda\sqrt{N}$, refraction corrections, charts for selection of most suitable wave-length, and description of the classical aspects such as extrapolation procedures and use of least squares. The relatively recent refinements in this field were, of course, only just appearing in the literature at the time of the original publication but, even so, brief mention is made and some references are given.

Three chapters are concerned with extra-structural features such as stress, crystal size, lattice distortion, preferred orientation. To aid stress measurement in iron, copper, aluminium and their alloys, for example, there are Zhmudski's tables (1953) for determining cell dimensions directly from the Bragg angles of certain high-angle reflexions. Investigation of crystallite size, lattice imperfections and lattice distortion are dealt with collectively and there are useful data for profile analysis. Chapters on diffuse and small-angle scattering and on the determination of preferred orientation complete the coverage of X-ray techniques, and the book concludes with short summaries on electron and neutron diffraction.

Dr. Mirkin's compilation, of necessity, overlaps the coverage for the *International Tables for Crystallography* and other established sources, but then extends to give a unique collection of data for the analysis of polycrystalline materials. Where it has drawn, for example, on the *International Tables* one will continue to use the original (which, in some instances, probably contain more complete or up-to-date data). It is for the compilation in English of material from Russian and other sources (notably Sagel's *Tabellen zur Roentgenstrukturanalyse*) not otherwise available in English that the book is so obviously of particular value. Furthermore, some of the tables are here published for the first time or have been expanded for this edition. Unfortunately there are a number of inconsistencies in the book and also a lack of critical assessment which could mislead the inexperienced user.

While easing the retrieval of data from scattered sources this book does, between its own covers, pose a problem in retrieval—especially of the less conspicuous items. The subject index is totally inadequate and there is no author index—the 464 references being listed only by number.

E. G. Steward

RESEARCH IN LEUKAEMIA

Current Research in Leukaemia

Edited by F. G. J. Hayhoe. Pp. xii + 306. (Cambridge : At the University Press, 1965.) 120*s*.

THIS book contains all but four of the papers delivered at a postgraduate medical course in Cambridge in August 1964 by workers mainly drawn from England and Italy, with a few from France, the United States, the U.S.S.R. and Germany.

In the first section of four papers, Merker considers the alkaline phosphatase score, the chromosomes, and neutrophil migration, particularly in chronic granulocytic leukaemia. The phagocytic studies in myeloid forms of leukaemia reported by Marmont and Damasio are interesting but restricted by inadequate quantitative methods. The excellent report by Dmochowski on electron microscopy applied to the viral and mycoplasma aetiology of human and animal leukaemia is unhappily marred by an incorrect order of the 75 clear, well-chosen photomicrographs (this is being corrected in later printings). Harris gives a sound outline of virological studies in avian and murine leukaemia and advises caution in attempting extrapolation to man.

Of the three reports dealing with cytogenetic investigations in leukaemia, that by Hayhoe and Hammouda is the most valuable; it correlates chromosome aberrations with clinical and cytochemical findings in leukaemia and other haematological malignancies. Tough compares the cytogenetics of chronic myeloid leukaemia with and without a significant radiation history, but there are few cases in the former group.

The eight articles in the third and longest section on cell growth and division indicate the recent exuberance of interest in this basic field. Hale and Cooper used autoradiography to investigate the DNA replication of leukaemia cells; Storti and Torelli speculate on differing metabolic types of RNA and its relation to protein synthesis, and Gavosto *et al.* used autoradiography to study DNA synthesis in the chromosomes, extra-chromosomes, and at different times in the *S*-period of mitosis in acute leukaemia.

In the papers dealing with *in vitro* culture systems and phytohaemagglutinin stimulation of leukaemic leucocytes, the metabolic correlations obtained by Quaglino and Hayhoe using autoradiographic and cytochemical methods, combined with the assessment of proliferative capacity by Astaldi *et al.*, form two valuable and complementary contributions. Elves discusses the transformation of lymphocytes to blasts and macrophages, and speculates on haematopoiesis, a subject discussed later by Loutit.

In the longest paper in this symposium, on biochemical aspects of leukaemic cells, Seitz reviews much work on nucleic acid and glycogen metabolism carried out in his laboratory, and published only in the Russian literature. Salvidio and Baldini suggest mechanisms for the differing patterns of enzyme-levels in lyophilized spleens in a variety of conditions. The 'therapeutic experiment' of allogeneic marrow grafting for the treatment of acute leukaemia in animals and man is discussed by Mathé and Amiel.

The final section relates to epidemiological aspects of leukaemia: it includes investigations of the 'summer peak' by Lee and Gardner; on the 'clustering' of cases by Knox; and on incidence and distribution of leukaemia by Doll.

The editor, contributors and publishers are to be congratulated on the speedy production of this volume, which can be recommended to those wishing to have up-to-date reviews in the different fields of leukaemia research. References and the index are good, but throughout one feels the absence of discussion despite the explanation on the cover.

R. Penny

ADHESION AND ADHESIVES

Adhesion and Adhesives

Edited by Dr. R. Houwink and Dr. G. Salomon. Vol. 1: Adhesives. Second, completely revised edition. Pp. xvi + 548. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 135*s*.

IT is fourteen years since N. A. de Bruyne and R. Houwink produced the first edition of *Adhesion and Adhesives*, and it would, therefore, seem an appropriate moment for publication of a second enlarged edition in view of the tremendous developments which have occurred

in this period. Full appreciation of the present volume will await the publication of the complementary volume and will be tempered by the emergence over the years of a number of books on the same topics.

The general arrangement of the book is similar to that of the first edition with articles contributed by a team of American, British and Dutch authors, this time largely under the direction of G. Salomon, who writes the first chapter on 'Adhesion'. The subject-matter of this chapter ranges far and wide in order to provide a comprehensive survey of many aspects of adhesive and appropriate polymer science and, consequently, certain areas suffer a somewhat sketchy treatment. However, the subject-matter is well supported by a classified system of 296 references with a brief critique of a number of the more recent books on various aspects of adhesives and adhesion. The succeeding chapters concern themselves with specific classes of adhesives and contain a varying but minor emphasis on adhesion, leaving the subject of applications to Volume 2. Chapters on animal glue and related protein adhesives (A. M. Kragh and J. Wootton) and vegetable adhesives (K. W. Kirby) are followed by a long chapter on synthetic organic adhesives (C. A. A. Rayner), and the length of this and the first chapter, which together comprise more than half of Volume 1, reflect the enormous interest in these fields over recent years. Not only are the important adhesives in the class discussed and an account given of two-polymer adhesives, but again there is a monumental and classified system of references. Chapters on bituminous binders and coatings (R. N. J. Saal) and rubbers (W. C. Wake), the latter including a section on tack, complete the field of organic adhesives. The remaining chapters are on glass, enamels and ceramics (W. van der Colk), inorganic adhesives and cements (J. H. Wills) and, finally, metallic adhesives (W. R. Lewis), including welding.

The overall presentation of the volume is good, and textual errors appear to be few. It supersedes the first edition in that, with the exception of the final chapter, the majority of references given are post-1951. Some readers might have welcomed more chemical formulae in chapters outside their own experience and it seems a pity that, in a book which will no doubt be regarded as both reference and text-book, no author index is provided. All in all, although the present book will not stand out as much as its predecessor in view of other publications on the same subjects, nevertheless it is to be recommended to all interested in adhesion and adhesives, and I for one await publication of the second volume with interest.

R. P. Sheldon

PEASANT FARMING SYSTEMS IN AFRICA

The African Husbandman

By William Allan. Pp. xii + 505 + 7 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 63*s*. net.

At the present stage of agricultural development in Africa, William Allan could scarcely have written a more basic and pertinent book. African Governments, with the tremendous demands made on them by development, are turning to their countries' agricultural resources for a greater contribution to the economy. They are faced with an enormous proportion of their people dependent on subsistence farming, and increasing population pressures on the land which will rapidly reduce its productivity. It is a vital first step to assess the carrying capacity of land, to give not only a basis for the control of exploitation under existing systems, but also an indication of the potential of the land with regard to improved farming schemes.

Allan set out to measure the carrying capacity of the land, which he terms the "critical population density". He has calculated the critical population density for African peasant systems on the basis of his work and experience with the subsistence cultivators of Northern Rhodesia, now Zambia.

In Part 1 he discusses in detail how the formula for estimating critical population density is built up. He describes the method of classifying the land on an ecological basis, and shows how this relates to the systems of land use practised. He then calculates the critical population density, which is the maximum density that is consistent with the maintenance of the agricultural system without degeneration of the land. Allan describes the different cultivation practices of Northern Rhodesia, and shows how critical population densities were worked out and applied to these systems. This is a very important section and could well be regarded as a working paper for anyone involved in rural development in Africa.

In Part 2 the traditional farming systems of people in other parts of intertropical Africa are described. This section shows that the methods are still valid in widely varying conditions, and gives a basis for their more detailed use.

Perhaps some of Part 3, in the long chapter given over to hunters and food gatherers, could have been condensed, to make room for a more detailed treatment of the weight of information in Part 1. Part 3 also describes the pastoral systems and the relevance of population density estimates to this very significant section of African agriculture.

The final part of the book looks at the effect of change on the African farmer, the factors that have upset the balance of traditional systems, such as the introduction of cash crops, the imposition of peace, and improvement in health of both men and animals. Often the result is a higher standard of living for some, and increased population pressure and its accompanying problems for most. Change goes on, and Allan discusses the attempts being made to adopt systems of improved farming, and the demands that will be made on African agriculture by nations striving for economic development.

The African Husbandman is well written, with occasional delightful touches, such as the author's account (on p. 151) of the 'lawn' of Salvinia on Lake Kariba and the three kudu swimming out of it. He gives the impression of a man who was fascinated and excited with his work, who was also constructive and acute. The text is well documented, with a comprehensive bibliography, but for areas with which one is not familiar it would have been of considerable help to have more sketch-maps; that of Uganda is not particularly accurate.

It is worth noting that the work from which this book emerges was carried out by a team of four: Allan, the agriculturalist; M. Gluckman, the anthropologist; C. G. Trapnell, the ecologist; and D. U. Peters. This brings out the very significant contribution of Allan himself, his ability to view African agriculture in all its aspects, biological as well as economic, and, particularly, social. It is this which gives *The African Husbandman* its authority. Peasant farming systems in Africa are intricately bound up with the social customs and the way of life of the people. Any influence which brings change to the farming system will affect the social structure, and, further, any designs for improved farming are likely to be affected by long-established customs resistant to change. The developing countries are fortunate to have at their disposal a book which has consistently taken these factors into account.

The African Husbandman is a book to be used. Gluckman in his foreword, written from the anthropologist's point of view, says: "I dare affirm that it is equally essential to agricultural scientists". It is indeed equally essential. An agriculturalist would merely have said the reverse, that it was equally essential to the anthropologist. It is to be hoped that it will appear under both subjects in bookshops and catalogues.

H. L. Hutchinson

An Introduction to the Theory of Relativity

By Dr. W. G. V. Rosser. Pp. xiv + 516. (London: Butterworth and Co. (Publishers), Ltd , 1964.) 75*s*.

IN the past, books on special relativity have fallen into two distinct groups. There are some meant primarily for mathematicians which are exhaustive discussions in which the experiments take a back seat and the more abstract parts of the theory are fully developed. Now that the special theory is so well established, there is an important part for such books to play. The fact remains that undergraduates reading physics are not well catered for by these books, and those treatments of the subject which are more slanted towards physicists are all small light-weight accounts. Accordingly, *An Introduction to the Theory of Relativity* meets an important need and it does so magnificently. There can seldom have been such a complete justification for special relativity in terms of experimental results. Every important confirmation of the theory is fully dealt with and discussed in a simple and enlightening way.

The book begins with two chapters of historical introduction, one dealing with mechanics and one with the theories of the ether. The Lorentz transformations are then introduced on the basis of a constant velocity of light, and in the next two chapters their consequences in optics and mechanics are fully discussed. The author then goes over to the use of a four-dimensional representation and uses this to begin his discussion of electromagnetism, starting with the formula for a force between currents. After a fairly conventional treatment of Maxwell's equations, the author devotes a whole chapter to the clock paradox and criticism of special relativity. A concluding chapter gives an intuitive account of general relativity without going into the whole mathematical theory. There is an excellent bibliography and a very full index.

C. W. KILMISTER

Guide to Activation Analysis

Edited by William S. Lyon, Jun. Pp. xix + 186. (Princeton, N.J., and London: D. Van Nostrand Company, 1964.) 46*s*. 6*d*.

GUIDE to Activation Analysis describes the major aspects of activation analysis in eight chapters by different authors: two appendixes give calculated sensitivities and safety and licensing requirements. The first two chapters deal with basic nuclear properties and with reactor neutron flux characteristics, non-reactor neutron sources are then described and compared, and there is a short chapter on the subject of radiochemical separations. Various aspects of counting are considered in two further chapters, less widely applied techniques such as charged particle and γ-photon activation are then described by the Editor himself, and the final chapter is devoted to practical examples of activation analysis.

At a time when intact methods of analysis are becoming more widely applied, the chapter on scintillation counting techniques provides a useful introduction to instrumental methods, and the short section on semiconductor particle detectors is also most topical. It is unfortunate, however, that radiochemical separations, still a very important part of activation analysis, are not more fully discussed.

Valuable information is included in the second chapter on the measurement and application of epithermal and fast reactor neutron fluxes, and Appendix 1 contains a comprehensive bibliography of determinations carried out by activation analysis.

This is a useful book and when used in conjunction with the references quoted it will enable those interested in applying activation analysis to gain a satisfactory basis for their work.

T. B PIERCE

Matrices

Their Meaning and Manipulation. By Prof. W. G. Bickley and R. S. H. G. Thompson. (General Engineering Series.) Pp. xiii + 168. (London: The English Universities Press, Ltd., 1964.) 21*s*. net.

THIS book shows clearly how to present a difficult topic in a concise and interesting manner. The authors have used their great didactic skill in securing the co-operation of the reader, not only by appealing to his sense of purpose, but also by encouraging him to solve numerous problems which supplement the text.

Few would disagree that modern engineers ought to have a rudimentary knowledge of matrices. It is encouraging to see a text which strikes just the right balance between theory and application, includes numerical examples and gives a discussion of pitfalls awaiting the unwary. The book, appropriately enough, starts with a brief description of numerous present-day applications of matrices, both in engineering and in physics. This is followed by a brief exposition of matrix notation, matrix multiplication and its applications, and an introduction to the theory of determinants. The four final chapters cover linear equations (reciprocal matrices), eigen-values and eigen-vectors and a discussion of computational techniques appropriate to both topics. (Here the difficulties of presentation are by no means inconsiderable and it is very much to the credit of the authors that they managed to overcome them so successfully.)

The book, which can be recommended unreservedly as a basis of an excellent introductory course for third-year and postgraduate students, is well produced and very reasonable in price.

P. A. LINDSAY

Comparative Physiology and Pathology of the Skin

Edited by A. J. Rook and G. S. Walton. Pp. xiv + 794. (Oxford : Blackwell Scientific Publications, 1965.) 155*s*.

IT is all too easy for the physician to forget that species other than *Homo sapiens* contribute much more to medicine than their role as experimental animals for drug testing and similar procedures. Other species have their own physiology and pathological disorders, from which a very great deal of information of value to the study of human disease can be learnt, and this is as true of the skin as it is of other organs.

Schwartzman and Orkin's book, *Skin Diseases of Dog and Man*, has aroused a lot of interest since it was published in 1962, and now Rook and Walton have edited an excellent volume, *Comparative Physiology and Pathology of the Skin*, incorporating the proceedings of a symposium held in Cambridge in April 1964.

The contributors include a wide range of veterinarians, biologists, dermatologists and other scientists. The book is divided into sections dealing with comparative dermatology, hair, nutritional influences, porphyria and light sensitization, the mast cell, immunology and tumours of the skin.

The standard of the contributions in the various chapters is almost always quite outstandingly high; as indeed one might expect from the very distinguished contributors. Each appears to have given of his best. Individual readers will naturally find particular sections of special interest to themselves.

Of great merit is the style in which the text is written, a tribute to the authors and editors. It is entirely intelligible to the average reader not working in the specialized field, and so provides a good guide to the interests of other workers.

I find it impossible to give other than the highest praise to the contributors and editors of this book and to the School of Clinical Research and Postgraduate Medical Teaching, and to the Veterinary Medical School of the University of Cambridge who were responsible for arranging the symposium.

C D. CALNAN

ANTHROPOLOGY: ITS ACHIEVEMENTS AND FUTURE*

By Prof. CLAUDE LEVI-STRAUSS
Director of the Institut d'Ethnologie, University of Paris

AMONG the many cherished recollections that I have retained of the years I spent in the United States, one remains outstanding because it is associated with what, due to my inexperience, appeared to me as something of a discovery. Indeed, it embodies for me to this day the unfathomable wealth and mystery of the city of New York.

This apparent discovery took place quite casually one day on lower Broadway, when I stumbled on a bookstore which specialized in second-hand government publications and where could be bought, for two or three dollars apiece, most of the *Annual Reports of the Bureau of American Ethnology*.

I can scarcely describe my emotion at this find. That these sacrosanct volumes, in their original green and gold bindings, representing most of what will remain known about the American Indian, could actually be bought and privately owned was something I had never dreamed of. To my mind, they belonged rather to the same irredeemable past as the beliefs and customs of which they spoke. It was as though the civilization of the American Indian had suddenly come alive through the physical contact that these contemporary books established between me and their time. I felt somewhat akin to a sixteenth-century scholar who, finding in what must have corresponded to our second-hand bookstores old manuscript copies of the works of Homer, Plato or Virgil, is struck by the evidence that these great men had actually existed, since someone had seen and transcribed their written word. Although my financial resources were more than scant and three dollars represented all I had to spend on food for the same number of days, this sum seemed negligible when it could pay for one of these marvellous publications, more alluring to the eye than any costly art books, such as Mallery's *Pictographs*, Matthews's *Mountain Chant*, Fewkes's *Hopi Katcinas*, or such treasure troves of knowledge as Stevenson's *Zuni Indians*, Boas's *Tsimshian Mythology*, Roth's *Guiana Indians*, and Curtin and Hewitt's *Seneca Legends*.

Thus, it happened that, volume after volume, and at the cost of some privations, I built up an almost complete set (there is still one volume missing) of the *Annual Reports* 1–48, which belong to the 'great period' of the Bureau of American Ethnology. At that time, I was far from imagining that, a few months later, I would be invited by the Bureau to become a contributor to one of its major undertakings: the seven-volume *Handbook of South American Indians*.

Notwithstanding this close association and the years which have since elapsed, the work of the Bureau of American Ethnology has lost for me none of its glamour, and I still feel toward it an admiration and respect which are shared by innumerable scholars the world over. Since it so happens that, in the same year that marks the two-hundredth anniversary of James Smithson, the life of the Bureau has come to an end (though its activities are carried on under a new guise), the time may be fitting to pay tribute both to the memory of the founder of the Smithsonian Institution and to the Bureau which has been one of its greatest achievements and certainly a unique one of its kind.

Ever since it was founded in 1879 (which, incidentally, meant the emancipation of ethnology from geography and geology wherewith it had been merged so far), not only did the Bureau avail itself fully of the amazing opportunity provided by the presence of scores of native tribes at a few hours' or days' travel from the great cities, but also, as a distinguished anthropologist Dr. Godfrey Lienhardt puts it in a recent book: "The accounts of custom and culture published by the Bureau compare in thoroughness and quality of reporting with modern ethnographic studies" (*Social Anthropology*, 24, Oxford University Press, 1964). We are thus primarily indebted to the Bureau for instituting standards of scholarship that still guide us, even though we but rarely succeed in attaining them.

Above all, the collection of native texts and factual observations contained in the 48 major *Reports* and certain of the subsequent ones, in the 200 or so *Bulletins* and in the *Miscellaneous Publications*, is so impressive that, despite the use they have been put to for nearly a century, it is safe to say that only the surface has been scratched. This being the case, one can only wonder at the neglect in which this invaluable material has temporarily fallen; as if the far less rich material with which we must content ourselves concerning the beliefs and customs of Greece and Rome were not still laden with as yet unexploited and occasionally unnoted data. The day will come when the last primitive culture will have disappeared from the Earth, compelling us to realize only too late that the fundamentals of mankind are irretrievably lost. Then, and for centuries to come, as happened in the case of our own ancestral civilizations, hosts of scholars will devote themselves to reading, analysing and commenting on the publications of the Bureau of American Ethnology which preserve so much more than has been preserved of other bygone cultures (not to mention the unpublished manuscripts placed in the Bureau's custody): and, if ever we succeed in enlarging our narrow-minded humanism to make it include each and every expression of human nature, thereby perhaps ensuring to mankind a more harmonious future, it is to undertakings such as those of the Bureau of American Ethnology that we shall owe it. However, nothing could be farther from my mind than the notion that the work of the Bureau belongs to the past; I believe, on the contrary, that all of us, together with its legal successor the Office of Anthropology, should seek in these achievements a living inspiration for the scientific task ahead of us.

It has become the fashion in certain circles to speak of anthropology as a science on the wane, on account of the rapid disappearance of its traditional subject-matter: the so-called primitives. Or else it is claimed that, in order to survive, anthropology should abandon fundamental research and become an applied science, dealing with the problems of developing countries and pathological aspects of our own society. I should not want to minimize the obvious interest of these new researches; but I feel, nevertheless, that there is, and will remain for a long time to come, much to be done along the more traditional lines. It is precisely because the so-called primitive peoples are becoming extinct that now, more than ever, their study should be given absolute priority.

It is not too late for hundreds of anthropologists to set to work. As early as 1908, almost sixty years ago, Sir James Frazer, in his inaugural lecture in the University of Liverpool, stated that classical anthropology was nearing its end. What have we witnessed instead? Two great Wars, together with scientific development, have shaken the world and destroyed physically or morally a great many native cultures. But this process, however disastrous, has not been entirely one way. The First World War gave rise indirectly to Malinowski's new anthropology

* Substance of an address delivered in Washington at the Bicentennial Celebration held during September 16–18 commemorating the birth of James Smithson (see *Nature* of October 23, p. 321).

by obliging him to share the life of the Trobriand Islanders in a more durable and intimate manner than perhaps he would have done otherwise. As a consequence of the Second World War, anthropologists were given access to a new world: the New Guinea highlands, with a population of 600,000–800,000 souls whose specific institutions are changing presently our traditional outlook on many theoretical problems. Likewise, the establishment of the new federal capital of Brazil, the building of roads and aerodromes in remote parts of South America, have led to the discovery of small tribes in areas where no native life was thought to exist.

Of course, these opportunities will be the last, and there is no future ahead. Moreover, the compensation they afford is small indeed, compared with the high rate of extinction afflicting primitive tribes the world over. There are about 40,000 natives left in Australia as opposed to 250,000 at the beginning of the nineteenth century, most, if not all, of them hunger-stricken and disease-ridden, threatened in their deserts by mining plants, atom bomb test grounds and missile ranges. Between 1900 and 1950, more than 90 tribes have been wiped out in Brazil, where instead of a hundred there are now barely 30 still living in a state of relative isolation. During the same period, 15 South American languages have ceased to be spoken. Scores of similar examples could be given.

Yet, this is no reason to become discouraged. It is undoubtedly true that we have less and less material to work with. But progress in the meantime, we are able to compensate to some extent for this diminishing volume by putting it to better use, thanks to our greater theoretical and factual knowledge and more refined techniques of observation. If I may put it that way, we have not much left to work with, but we will manage to 'make it last'. We have learned how to look for the cultural 'niches' in which traditional lore finds refuge and where it offers the strongest resistance to the impact of civilization: language, kinship, ethnobotany, ethno-zoology and the like.

But although the physical disappearances of populations that remained faithful until the very end to their traditional way of life does, indeed, constitute a threat to anthropology, curiously enough a more immediate one comes from an evolution which has been taking place in such parts of the world as Asia, Africa and the American Andes, which used to be considered as falling also within the realm of anthropological studies. The demography of these regions was always high and it shows no sign of decreasing; quite the contrary. Physically speaking, the subject-matter is still there, as rich as ever, if not richer still. This new threat to our investigations is not then so much of a quantitative as of a qualitative nature, and this proves to be doubly true: in the first place, these large populations are changing fast, and their culture is resembling more and more that of the Western World. Like the latter, it tends to fall outside the field of anthropology. But this is not all, for the mere fact of being subjected to ethnographical investigation seems distasteful to these peoples, as though by studying the ways in which their old beliefs and customs differed from our own, we were granting these differences an absolute status and conferring on them a more enduring quality.

Contemporary anthropology thus finds itself in a paradoxical situation. For it is out of a deep feeling of respect toward cultures other than our own that the doctrine of cultural relativism was evolved; and it now appears that this doctrine is deemed unacceptable by the very people on whose behalf it was upheld, while those ethnologists who favour unilinear evolutionism find unexpected support from peoples who desire nothing more than to share in the benefits of industrialization, and prefer to look at themselves as temporarily backward rather than permanently different.

Hence the distrust in which traditional anthropology is held nowadays in some parts of Africa and Asia. Economists and sociologists are welcome, while anthropologists are tolerated at best, and from certain areas they are simply banned. Why perpetuate even in writing old usages and customs which are doomed to die? The less consideration and attention they receive, the faster they will disappear: and even should they not disappear, it is better not to mention them lest the outside world realize that one's culture is not as fully abreast with modern civilization as one deludes oneself in believing it to be. There have been periods in our own history when we too have yielded to the same delusion, only to find ourselves struggling to regain balance after eradicating so recklessly the roots that held us back to our past. Let us hope that this dire lesson will not be lost on others. The question is, in effect: what can we do to keep it from being lost? Is there a way of making people realize that they have a tremendous responsibility toward themselves and toward mankind as a whole, which is not to let perish before it has been fully recorded this past it is their unprecedented privilege to experience on a par with their incipient future? The suggestion has been made that in order to render anthropology less distasteful to its former human raw material, it will suffice to reverse the roles and occasionally allow ourselves to be 'ethnographized' by those for whom we were once solely the ethnographers. In this way, each in turn will get the upper hand. Since there will be no permanent privilege, nobody will have grounds to feel inferior to anybody else. At the same time, we shall get to know more about ourselves through the eyes of others, and human knowledge in general will derive an ever-growing profit from this reciprocity of perspective.

Well meant as it undoubtedly is, this solution appears naïve and unworkable to this speaker; as though the problems involved in the great confrontation now taking place between Western culture and the rest of the world were as simple and superficial as those of children unaccustomed to play together, whose quarrels can be settled by making them follow the elementary rule: "Let me play with your dolls and I shall let you play with mine". To arrive at an understanding between people who are not merely estranged from one another by their physical appearances and their peculiar ways of life but who also stand on an unequal footing to one another, is a different question altogether.

Anthropology is not a dispassionate science like astronomy, which springs from the contemplation of things at a distance. It is the outcome of a historical process which has made the larger part of mankind subservient to the other, and during which millions of innocent human beings have had their resources plundered, their institutions and beliefs destroyed, while they, themselves, were ruthlessly killed, thrown into bondage and contaminated by diseases they were unable to resist. Anthropology is daughter to this era of violence: its capacity to assess more objectively the facts pertaining to the human condition reflects, on the epistemological level, a state of affairs in which one part of mankind treated the other as an object.

A situation of this kind cannot be soon forgotten, even less erased. It is not on account of its mental endowments that only the Western world has given birth to anthropology, but rather as a consequence of the fact that exotic cultures, treated by us as mere things, could be studied, accordingly, as things. We did not feel concerned by them, whereas we cannot help their feeling concerned by us. Between our attitude toward them and their attitude toward us, there is and can be no parity.

Therefore, if native cultures are even to look at anthropology as a legitimate pursuit, and not as a sequel to the colonial era or that of economic domination, it cannot suffice for the players to simply change camps while the anthropological game remains the same. Anthropology itself must undergo a deep transformation in order to carry on its work among those cultures for the study of which it was intended because they lacked a written record of their past history. This, however, it will achieve in an

altogether different way, since instead of making up for this gap through the application of special methods, the aim will be to fill it in. Whenever practised by members of the culture which it endeavours to study, anthropology loses its specific nature and becomes rather akin to archaeology, history and philology. For inasmuch as anthropology is the science of culture as seen from the outside, it follows that the first concern of people made aware of their independent existence and of their originality could not be to observe from the outside the culture of their former masters while allowing the latter to observe their own culture also from the outside, but to claim the right to observe this culture of theirs themselves, and in the only way, that is, from the inside. The obvious conclusion is that anthropology will survive in a changing world by allowing itself to perish in order to be born again under a new guise.

Anthropology is thus confronted with two tasks which would prove contradictory only were they to be undertaken simultaneously in the same field. Wherever native cultures, though disappearing physically, have remained to some extent morally intact, anthropological research should be carried out along traditional lines and the means at its disposal increased to the utmost. Wherever the opposite situation prevails, that is, where populations have remained physically strong while their culture is rapidly veering toward our own, anthropology should accept to shift its goals and, while being taken over progressively by scholars from the land, adopt aims and methods similar to those which, from the Renaissance on, have proved fruitful for the study of our own culture.

From the very beginning, the Bureau of American Ethnology has had to face this two-fold necessity by reason of the peculiar situation of the American Indian who allied cultural remoteness and physical proximity, together with a tremendous will to live, at least among some tribes, despite all the ordeals they have been subjected to; thus the Bureau was compelled from the start to act both ways: on one hand, through ethnographical surveys, the precision and rigour of which have set up a standard that cannot be surpassed, and on the other, by encouraging the natives themselves to become their own linguists, philologists and historians. If the cultural riches of Africa, Asia and Oceania are to be saved, it will only be on the condition that, following this example, we succeed in raising dozens (and they themselves hundreds) of such men as Francis La Flesche, son of an Omaha chief, James Murie, a Skidi Pawnee, George Hunt, a Kwakiutl, and many others, some of whom, like La Flesche and Murie, were on the staff of the Bureau.

When we consider that this was taking place more than fifty years ago, we can but marvel at such maturity and foresight, and hope for the extension, on a world scale, of what a handful of resolute and enlightened men and women knew should be done in the wide field of American studies. Indeed, they did it in such an efficient way that through less than a century of effort on their part, a great deal more similar material to that which is already available. There remains such a considerable amount to be saved that the urgency of the task risks making us overlook the present evolution of anthropology, which as it increases in quantity is also changing in quality. Recognition of this fact should make us more confident in the future of our studies, and it can be verified in many ways. To begin with, new problems have arisen which can still be solved, even though they have received but scant attention thus far. It is worth noting, for example, with what disdain until recently anthropologists have neglected to study the relationship, in the case of each crop, between the amount of work involved and the yield, as well as the latter's elasticity. Yet one of the keys to the understanding of the social and religious importance of yams, which is so striking throughout Melanesia, can probably be found in the remarkable elasticity of the yield. The farmer who is exposed to harvesting far less than he actually needs must plant far more in order to be reasonably certain of having enough. Conversely, if the harvest is plentiful it can so widely exceed expectations that to consume it all becomes impossible; this leaves no other use for it than competitive display and social food presentation. In cases such as the foregoing, as in many others, we must learn to translate, in terms of several different codes, phenomena that we have been apprehending so far in terms of one or two codes only, and thus render the observed phenomena a great deal more significant.

A wide system of equivalents could then be established between the truths of anthropology and those of neighbouring sciences which have been progressing at a similar pace: I am thinking not only of economics, but also of biology, demography, sociology, psychology and logics, for it is through a number of such adjustments that the originality of our field will best appear.

There has been much question lately as to whether anthropology belonged among the humanities or among the natural sciences. In my opinion this is a false problem, since anthropology has the unique feature of not lending itself to such a distinction. It has the same subject-matter as history, but for lack of time perspective cannot use the same methods. Its own methods tend rather towards those of sciences not devoted to the study of man, though synchronically oriented like anthropology itself. As in every other scientific undertaking, these methods aim at discovering invariant properties beneath the apparent particularity and diversity of the observed phenomena.

Will this assignment deter anthropology from a humanistic and historical outlook? Quite the opposite is true. Of all the branches of our discipline, physical anthropology is probably the closest to the natural sciences. For this very reason, it is worth noting that, by refining its methods and techniques, it has been getting ever closer to, not farther from, a humanistic outlook.

For the physical anthropologist, to look for invariant properties traditionally meant to look for factors devoid of adaptative value and from the presence or absence of which something could be learned about the racial divisions of mankind. Our colleagues are less and less convinced, though, that any such factors really do exist. The sickle-cell gene, formerly held as one, can no longer be considered in that light if, as is now generally accepted, it carries a certain measure of immunity to malaria. However, as Dr. Frank B. Livingstone has brilliantly demonstrated (*Amer. Anthropologist*, 60, No. 3, 533; 1958), what appears as an irretrievable loss from the point of view of long-range conjectural history can be viewed as a definite gain from that of history as historians conceive it, that is, both concrete and at close range. For by reason of the adaptive value of the sickle-cell gene, a map showing its distribution throughout the African continent would make it possible for us to read, as it were, African history in the making, and the knowledge thus obtained could be correlated with those same invariant properties which have vanished at the superficial level where they were sought for originally, to reappear at a deeper functional level; and whereas they seem to grow less informative, they turn out to be more meaningful.

This remarkable process is actually taking place everywhere in our field. Dr. George M. Foster has recently given new life to what most of us held to be an exhausted question: the origin of the potter's wheel (*Southwestern J. Anthropol.*, 15, No. 2, 99). This he achieved by pointing out that an invention is neither simply a new mechanical device nor a material object that can be described objectively, but rather a manner of proceeding which may avail itself of a number of different devices, some of them crude and others more elaborate. In the field of social organization, I myself have tried to show that kinship systems should not be described by their external features such as the number of terms they use, or the way they classify, merge and distinguish all possible ties between

individuals. In so doing, all we can hope to obtain is a long list of types and sub-types which are there simply as meaningless objects, while if we try to find out how they work, that is, what kind of solidarity they help to establish within the group, their apparent multiplicity finds itself reduced to a few basic and meaningful principles.

Similarly, in the field of religion and mythology, an attempt to reach beyond external features, which can only be described and arbitrarily classified by each scholar according to preconceived ideas, shows that the bewildering diversity of mythical motifs can be reduced to a very few number of schemes, each of which appears endowed with a specific operational value. At the same time there emerge for each culture certain sets of transformation rules which make it possible to include in the same group myths previously held to be markedly different.

These examples, chosen among many others, tend to show that anthropology's traditional problems are assuming new forms while none of them can be said to be exhausted. The distinctive feature of anthropology, and that which singles it out among other human sciences, is to look at man from the very point where, at each period of history, it was considered that anything man-like had ceased to exist. Within a century or so, when the last native culture will have disappeared from the Earth and our only interlocutor will be the electronic computer, it will have become so remote that we may well doubt whether the same kind of approach will deserve to be called 'anthropology' any longer. Between these limits lies the only chance that man ever had or will have to look at himself in the flesh while still remaining a problem unto himself, though one he knows can be solved since it is already certain that the outer differences conceal a basic unity.

Let us suppose for a moment that astronomers should warn us that an unknown planet was nearing the Earth and would remain for twenty or thirty years at close range, afterwards to disappear for ever. In order to avail ourselves of this unique opportunity, neither effort nor money would be spared to build telescopes and satellites especially designed for the purpose. Should not the same be done at a time when one-half of mankind, only recently acknowledged as such, is still so near to the other half that except for men and money its study raises no problem, although it will soon become impossible for ever? If the future of anthropology could be seen in this light, no investigation would appear more urgent and no other could compete with it in importance. For native cultures are disintegrating faster than radioactive bodies, and the Moon, Mars and Venus will still be at the same distance from the Earth when that mirror which other civilizations still hold up to us will have so receded from our eyes that however costly and elaborate the instruments at our disposal we may never again be able to recognize and study this image of ourselves which will be lost and gone for ever.

ORIGIN OF PRECIOUS OPAL

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ELECTRON microscopy has shown[1] that the play of colours or 'fire' in precious opal is caused by diffraction effects from arrays of uniformly sized spheres of amorphous silica 1700–3500 Å in diameter. The spacing of the regular optical discontinuities in the arrays is determined by the diameter of the spheres and in turn determines the maximum wave-length which can be diffracted. With this relationship in mind we have examined many opal specimens from museums and private collections, studied the occurrence of the mineral in some Australian fields and synthesized opaline silica in the laboratory. We have reached certain tentative conclusions concerning the origin of precious opal and consider that some of these may be relevant in principle to the genesis of all opaline silica.

We find that the spheres in precious opal, and the common opal or the 'potch' with which it is associated, have an internal structure which suggests to us that these particles are secondary colloidal aggregates of primary particles up to 400 Å in diameter. We consider that the primary particles formed in ground-water which became concentrated by evaporation in partly filled cavities at shallow levels. Water evaporated from the free surfaces of liquid in the cavities at a rate limited by the vapour pressure gradient or the permeability of the cover rock, but the surfaces were kept at constant level by inflow of more solution from adjacent ground-water traps. The concentration of silica increased and, as primary particles formed, the contents of the cavities became thin gels. With increasing concentration, secondary aggregates developed to a critical size and settled through the gel, the consistency of the gel thus determining the maximum particle size. If the secondary particles were well-formed spheres and settled at an appropriate size, uniform close-packed arrays capable of diffracting visible light were generated and the material could become precious opal. If the rigidity of the parent medium prevented secondary particle growth, or inhibited the formation of regular spheres, or impeded selective settling of the larger particles only common opal or potch could be formed. In the final stage of cementation, desiccation of the environment caused the soft spheres and arrays to harden by loss of water and deposition of further silica. The quality of the 'fire' exhibited by the stone is influenced by the extent to which interstitial voids remained unfilled.

Field Environment of Opal

The main opal fields of Australia are situated in arid inland regions where the mineral occurs in a sub-horizontal sequence of Cretaceous claystones and thin bentonite beds. The claystones are composed of fine angular quartz and coarse kaolinite and are highly permeable. Siliceous duricrust up to 3 m in thickness covers much of these and is apparently of late Tertiary age[2]. Opal is found from depths as great as 60 m up to the surface, where it has been exposed by post-Tertiary erosion. We estimate that opal has been deposited under a cover of 5–40 m of rock during the Tertiary period, under climatic conditions not greatly different from those prevailing at present.

We consider that three features of the Australian fields have been particularly significant in the genesis of the opal.

(1) An abundant supply of readily soluble silica has existed in the host sediments. Detrital feldspars have been kaolinized *in situ* in the claystones, liberating silica in solution. Diagenetic changes associated with the benton-

ites could also have produced silica of the opaline variety giving the 'cristobalite' X-ray pattern known to be characteristic of such clays[3]. Amorphous silica micro-fossils and volcanic ash fragments have been recorded in some of the beds[4].

(2) The aridity of the climate has restricted the shallow ground-waters of the opal fields to sharply defined formational dams, such as the bentonite beds, for periods of millions of years. Except in the vicinity of such water traps, the rocks have dried out to depths of many metres. At present, ground-waters may be fresh where part of the drainage system, but where stagnant may contain up to 3 per cent of mixed sulphates and chlorides, 80 p.p.m. of silica and have slightly acid characteristics. Carbonated alkaline artesian waters exist at depths of 700–1,200 m in many fields but the present distribution of these shows no relation to the occurrence of opal.

(3) Opal typically occurs either as a cavity filling in the vicinity of junctions of bentonitic beds with overlying porous rocks or as slabs in joints and solution channels. Cavities have been formed throughout the host rocks by minor deformation and by the removal of fossils and minerals through decomposition or leaching. Many cavities remain unfilled and even within a favourable level the distribution of opal is very patchy. Most cavities along the top of a bentonite band may contain opal over an area of hundreds of square metres but immediately outside this area all similar spaces may be empty. Where cavities in porous rock have been filled with opal the rock pores are usually also filled, generally with opaline silica showing no 'fire' but occasionally with precious opal of the type called 'matrix'. Where masses of opal occur in bentonite the surrounding sediment is generally not silicified. Precious opal does not 'replace' other materials except by filling spaces left by extraction of the original substance. Veins of gypsum and alunite are commonly situated above opal levels along the upper surfaces of bentonite beds and opal may include or be included by these minerals.

Supply and Deposition of Silica

Since the sediments of the Australian opal fields have been rich sources of soluble silica and the amounts of ground-water present have been limited, we consider that water in formational traps could have dissolved up to 100 p.p.m. of silica from the host rocks at 20°–25° C under neutral or slightly acid conditions. Such solutions could have migrated laterally to places where evaporative loss of water through the rock cover concentrated the solutions to the point of deposition of colloidal silica. We have found no evidence to suggest that the solutions responsible for opal deposition were different from present-day ground-waters, and in fact we see no reason why the deposition of opal could not still be continuing in parts of these regions.

All field evidence is consistent with the suggestion that opal has been formed by slow evaporation of very limited and highly localized supplies of ground-water, a process similar in some respects to the formation of surface duricrust but occurring under a protective cover of rock sufficient to isolate the system from seasonal drying and flooding. Opal host cavities have characteristically been those situated in positions permitting steady-state concentration of ground-water by evaporation. Soluble salts have been removed by diffusion to zones, above the cavities, where crystallization occurred as capillary flow ceased. This separation of salts from colloidal silica particles has been an important factor enabling silica to be spatially concentrated in appreciable masses, sufficiently free from physical disruption to achieve the structure required for optical diffraction effects.

For purposes of illustration we may consider the typical situation of an isolated host cavity that has been situated in a wet zone near the top of a bentonite bed under a cover of porous claystone. In this zone capillaries were saturated, but a larger space, such as the host cavity, contained a free water surface. As water vapour left the free surface and migrated up to the atmosphere through the permeable cover rock, small primary colloidal silica particles formed as the residual solutions became concentrated. The level of the free liquid surface in the cavity was maintained by hydrostatic inflow of ground-water from points further along the formational dam. Particles grew to a diameter of 300–400 Å and settled as loose flocs, to give a zone with the consistency of very thin gel on the floor of the cavity. This zone of high population density of primary particles acted as the parent medium for the formation of secondary colloidal aggregates. Primary particles removed in the formation of the aggregates were continuously replaced by fresh particles diffusing down through the thin gel as evaporation proceeded.

It may be seen that the concentrating mechanism in our model is a free water surface from which vapour is progressively removed. Such surfaces may have existed in a host cavity, or in voids connected with the cavity by channels permeable to sol particles. If we consider a model in which the walls of a cavity were permeable only to solutions and assume that a free surface was continuously maintained in the cavity, the rate of deposition of opal may be estimated by calculating the rate at which a 100 p.p.m. silica solution could be concentrated in the cavity. This rate would be controlled either by the permeability of the cover rock to water vapour or the rate at which water could evaporate from the free surface and condense at the roof of the cavity under the influence of the thermal gradient in the surrounding rock. At deep opal levels the thermal gradient could be assumed constant at about 0·05° C per metre, the temperature decreasing towards the base of the zone of seasonal variation. This would give an evaporation flux across the cavity of the order of 10^{-10} g/cm²/sec. From measurements of the permeability of the cover rock at Andamooka we consider that this flux would readily have been sustained, even where thin bands of bentonite occur in the sequence. A spherical cavity losing water at this rate would concentrate silica solution at a rate sufficient to deposit a layer of opal up to 1 cm thick in 5 million years, even at a depth of 40 m. Opal at higher levels could have been deposited more rapidly, especially in cavities covered only by porous claystone, or at places connected with the ground surface through vertical open joints. At a depth of 10 m the maximum rate of deposition possible from a 100 p.p.m. silica solution would have been about 1 cm in 200,000 years, but this implies direct connexion to the atmosphere, an improbable condition in our view.

Rates of opal deposition calculated for an isolated cavity would not apply to a cavity connected to extensive areas of free surfaces in surrounding voids. Sol particles generated in such voids interconnected across an appreciable area of a formational dam could presumably be laterally segregated by fluid flow towards particular cavities which would therefore fill at higher rates. The large slabs of opal in joint systems and solution channels probably formed by this mechanism. Such material is generally potch, the best quality precious opal being found in small isolated cavities where the material has been formed by concentration of solution *in situ*. Opal from isolated cavities may show evidence of discontinuities in deposition, probably caused by periods of raised water level rather than desiccation. The quality of the stone then changes abruptly through a succession of sharply defined horizontal layers, the breaks in deposition being occasionally marked with films of clay. Textural evidence from masses of opal in open joints shows that such cavities have dried out on occasion, the existing opal having been cracked through shrinkage and the cracks filled in with later opal. The time required to cause such desiccation would not have been significant compared with the total time required to deposit the opal, but the observation supports the concept that the climate was arid when the opal was formed.

Development of Spheres

The growth of colloidal silica particles appears to be a complex phenomenon involving condensation reactions and aggregation effects. As solutions concentrate by evaporation macromolecules form by condensation of small polyanions in reactions liberating water. These macromolecules aggregate and become welded together by further condensation reactions to form sol particles, the distinction being made, by definition, when the particle diameter exceeds 10 Å. Particles are spherical because the substance is essentially amorphous and isotropic. Diameters of up to 300–400 Å are readily attained and the sols tend to become rather monodisperse, possibly because the systems are of the type in which particles, growing by the operation of a substantially linear growth-rate law, are being supplied with nutrient at a steady rate[5]. As the particle diameter increases, aggregation becomes the dominant growth mechanism, as small particles formed by condensation reactions become incorporated in the larger spheres before reaching any significant size. This change may be demonstrated by holding a sol for a long period with the nutrient supply cut off. Under such conditions a silica sol of particle size around 100 Å held for some months at room temperature becomes a suspension of secondary spherical aggregates 1500–2000 Å in diameter[6] as long as no flocculating ions are present to form a gel network which can severely impede aggregation. The term 'secondary' is used for convenience but has no absolute connotation in the silica system, where 'primary' particles may have formed by a similar aggregation mechanism on a smaller scale.

Electron micrographs of etched opal surfaces show that the large spheres are secondary aggregates of primary particles 300–400 Å in diameter. Australian opal generally shows the details of the internal structure of such aggregates better than opal that we have examined from other parts of the world. The structure has been found in Hungarian opal, but not in Mexican 'fire opal' nor in the black glassy variety from Nevada. In well-developed secondary aggregates a core is surrounded by 1–3 concentric shells of primary particles, the smaller aggregates having fewer shells (Fig. 1). Opal showing only green-blue-violet 'fire' usually has a sphere structure of 2 shells around a small core, or one shell around a larger core apparently formed by less regular aggregation of primary particles. Opal showing red 'fire' contains spheres larger than 2500 Å in diameter and these may have 3 shells around small cores.

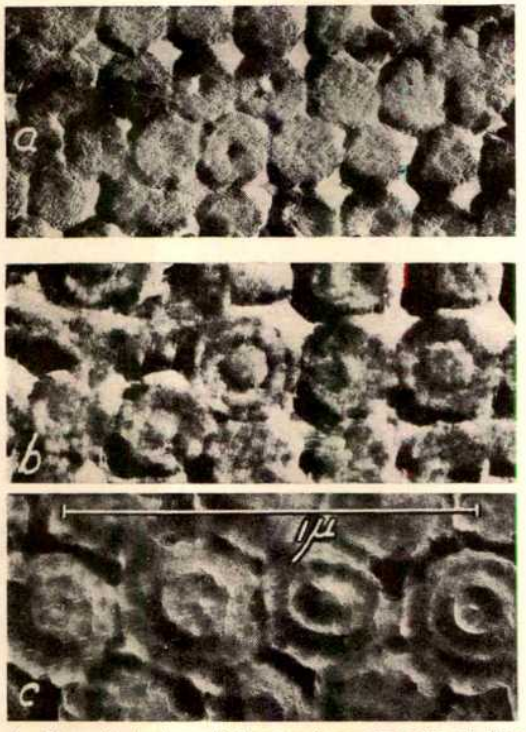


Fig. 1. Internal structure of the secondary aggregates of silica in Australian opals: (*a*) violet, (*b*) green and (*c*) red. Shadowed replica of surfaces etched in hydrofluoric acid (× 55,000)

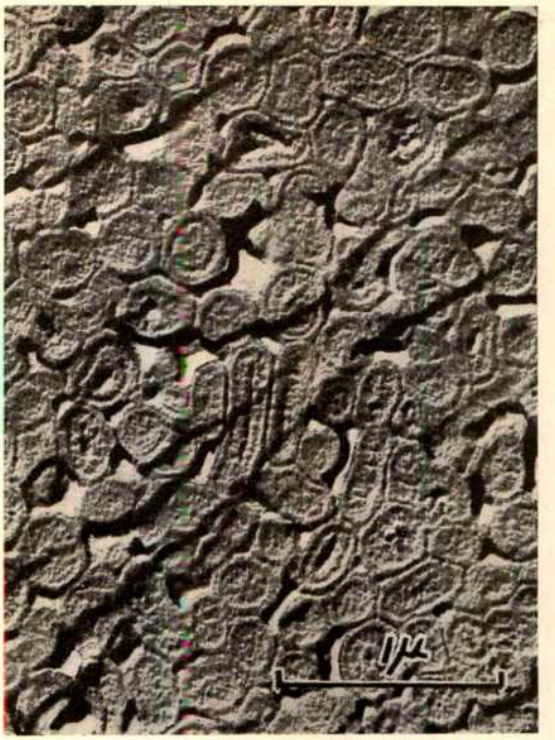


Fig. 2. Distorted cores and particle shapes in 'potch' opal from Andamooka. Shadowed replica of etched surface (× 30,000)

The perfection of shape of secondary aggregates is greatest in precious opal, where regular spheres have been formed and arranged in precise arrays of uniform particle size. In potch, secondary aggregates may be so malformed that arrangement in regular arrays has not taken place. Ellipsoidal aggregates formed around multiple cores, especially around straight chains of core particles, are common (Fig. 2). We consider that the consistency of the gel medium determined the perfection of shape of secondary aggregates. The more perfect spherical clusters probably developed in thin media which allowed greater freedom of movement of primary particles, while potch particles formed in thicker, more concentrated gels which inhibited the development of minimum free energy spherical clusters in the time available. This accords with the presence of the more perfectly shaped particles in opal deposited in small cavities by evaporative concentration of solution within the cavities. Secondary particles in other varieties of natural opaline silica examined to date are less than 1500 Å in diameter and are generally rather irregular in shape. Siliceous duricrust, wood opal, common opal and silica deposited in plants are all materials in this category.

Packing of Spheres

Iler has described the spontaneous formation of diffracting arrays of silica particles 1100 Å in diameter in aqueous suspensions left to stand for long periods[7]. Such small particles should diffract ultra-violet rather than visible wave-lengths and it seems likely that the colours he observed were produced by diffraction effects from irregularities in the arrangement of the spheres. We have grown spheres up to diameters of 2000–6000 Å by modifications of the techniques of Bechtold and Snyder[8], and we find that diffracting arrays may be prepared from these

within an hour by centrifuging. The colours can be preserved to some extent by drying and cementing the sedimented masses.

A special feature of the natural opal system is that spheres have been both formed and arranged in a still medium. This would not be possible in the laboratory systems that we have used, because large secondary particles sink into packed arrays within a few days in media having a viscosity near that of water. Primary particles 300 Å in diameter would not enter close-packed arrays of secondary spheres and aggregates would not continue to grow once settled into such arrays at a diameter of about 1000 Å. We therefore propose that secondary aggregates in opal grew in a gel medium of sufficient consistency to hold the particles suspended for long periods while growth by accretion of primary particles proceeded. When secondary aggregates became large enough to break free of the bonds holding the particles suspended in the gel network, sedimentation occurred, the particles joining regular arrays building up from the floor of the host cavity. In these close-packed arrays further growth of spheres by accretion of primary particles was impossible.

In our view the consistency of the parent gel has therefore determined the size to which spheres have grown. Very thin media allowed early settling of relatively small spheres, producing arrays on the scale characteristic of opal showing only violet 'fire'. Thicker media retained spheres in growth positions until the sizes characteristic of opal with green or red 'fire' were reached. Potch media were occasionally too thick to allow differential settling and the collections of particles that resulted were polydisperse, with a diameter range of 600–4000 Å in a single specimen.

According to our interpretation of the mechanism controlling particle-size distributions in precious opal, there was generally a systematic variation in the consistency of parent media during the deposition of the mineral. Arrays of spheres formed in the shape of vertical pseudo-crystals in most opal and such structures in specimens of known orientation that we have examined generally show a gradual transition in particle size, the spheres decreasing in diameter from the base of a pseudo-crystal to the top. The common gradation in the quality of the 'fire' exhibited is from red at the base to green at the top, suggesting that parent media became thinner as sedimentation of spheres proceeded. This supports the view that the spheres were formed by slow reorganization of the parent gel, the rate of supply of new primary particles being insufficient to maintain a gel network at constant consistency. The transition from larger to smaller spheres up the columnar arrays is not always shown, and we have in fact observed some instances of reversal, especially in thin vertical veins and in 'matrix' opal. In these, red-diffracting particles have been retained in the upper limits of vertical veins, possibly because the parent gels in such positions were drier and of thicker consistency than the media in the main opal levels below.

Macroscopic structures preserved in opal give some indication of the consistency of the parent gels. Loose flocs have been kept in suspension in some samples and the spheres developed in the flocs have been different from those in the surrounding gel. Gels have been unable to support sand-sized rock particles which have become detached from the roof of the host cavity, but flow lines have developed around the falling particles as existing arrays of spheres were disturbed. Such observations suggest that parent media had a consistency similar to that of fresh 0·1 per cent silica sol fully flocculated with salts.

Process of Cementation

When first formed, the secondary spheres in natural or synthetic opaline silica are soft, the component primary particles being held together by relatively weak bonds. Synthetic spheres can be deformed into polyhedra by excessive centrifuging or rapid drying and similar effects caused by compression during packing can be seen in electron micrographs of natural opal. Spheres become harder and denser on drying, especially if a little residual silica is present in the liquid being evaporated. In natural opal, arrays appear to have remained soft and uncemented for long periods before being hardened by desiccation, successive generations of different arrays having been deformed mechanically at the same time while still in a soft condition.

The consolidation of arrays by deposition of further silica and deformation of spheres during drying has given the degree of transparency required for the display of optical diffraction effects in opal, but the quality of the 'fire' is strongly affected by the extent to which the voids between the spheres have been filled. If the spheres were deformed and compressed into complete contact or if almost all the optical discontinuities were eliminated by deposition of interstitial silica, the intensity of the 'fire' is weak, as in jelly opal and Mexican opal. Complete elimination of discontinuities has produced glassy potch. If many irregularly arranged spaces have been left, general scattering of white light dilutes the 'fire', giving a milky variety.

Freshly formed synthetic opal gives a very diffuse X-ray pattern of the cristobalite type shown by precious opal. The pattern sharpens after several months' dehydration at 100° C, suggesting that variations in the patterns of different natural opals[9] may reflect the time and degree of exposure of the mineral to hot, dry conditions. We consider that the natural drying of opal in the Australian fields took place during Quaternary time, as erosion removed part of the rock cover and destroyed the continuity of shallow ground-water systems.

We thank the Australian Museum and the School of Mines, Kalgoorlie, for loans of specimens. We also thank the miners and cutters of Andamooka and Coober Pedy and the opal field wardens of the South Australian Department of Mines for their assistance.

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INTERNATIONAL GEOPHYSICAL CALENDAR FOR 1966

THE International Geophysical Calendar for 1966 has been drawn up by A. H. Shapley, chairman, and J. V. Lincoln, deputy secretary, of the Steering Committee of the International Ursigram and World Days Service, in close association with the International Geophysical Committee and the reporters and spokesmen for the various scientific disciplines, International Years of the Quiet Sun and the Committee of Space Research.

The Calendar designates days and intervals selected for special attention for geophysical observations, experiments, data interchange or analyses (Table 1). It is thus a framework for world-wide and interdisciplinary co-ordination in those programmes where it is not practical or meaningful to carry out the same work for each and every day. The Calendar serves mainly the branches of geophysics dealing with the Earth's atmosphere. A principal

use is for the co-ordination of the sampling of the many phenomena which vary significantly during the course of a year. The Calendar is prepared especially for the suggested scientific programmes recommended to be carried out for the days or intervals marked on the Calendar (see paragraphs 11–16). It is also common for individual geophysical stations or groups of stations to arrange some of their plans of observations according to the Calendar. Thus geophysicists can expect that their colleagues in other countries, in other laboratories and in other geophysical disciplines will tend to be making increased efforts for the days and intervals marked on the Calendar; the amount of geophysical data in existence, at the World Data Centres and elsewhere, will accordingly be greater for Calendar days.

(1) *Universal Time* (U.T.) is the standard of time for all world days on the Calendar, that is, each begins at 0000 U.T. and ends at 2400 U.T.

(2) *Regular Geophysical Days* (*RGD*) are each Wednesday throughout the year. This weekly sampling schedule is particularly designed for the purposes of the meteorological programme but has also been adopted for some other geophysical programmes.

(3) *Regular World Days* (*RWD*) are three consecutive days each month, always Tuesday, Wednesday, Thursday near the middle of the month. They are intended for observations, experiments or analyses which can or need be made for about 10 per cent of days and which should be spaced (in groups of three days) throughout the year.

(4) *Priority Regular World Days* (*PRWD*) are one day each month—the *RWD* which are also a *RGD* (Wednesdays). They are for work which can or needs to be done only one day each month throughout the year.

(5) *Quarterly World Days* (*QWD*) are one day in each quarter of the year. They are the *PRWD* which fall within the World Geophysical Intervals (*WGI*) and are also a *RGD* (Wednesday). The *QWD* serve to co-ordinate seasonal high-altitude rocket experiments.

(6) *World Geophysical Intervals* (*WGI*) during 1966 are fourteen consecutive days in each season, beginning on the second Monday of the selected months. They always include the three *RWD* of the month and the *QWD* for the season. The *WGI* are intended for intensified programmes aimed at the statistics of seasonal variations or the timing of seasonal changes. The schedule of *WGI* relative to the equinoxes and solstices is deliberately made different from year to year so that in due course a *WGI* will cover the time of seasonal change of each of various geophysical phenomena; variations from this principle will sometimes be made in order to include a solar eclipse within a *WGI*.

(7) *Solar Eclipses* are May 20 (annular) with central line extending from North Africa to Central Asia, and November 12 (total) with central line extending from the equator west of South America, to a point south of South Africa. Geophysical stations in the eclipse zones treat these days as world days and undertake special programmes to investigate eclipse effects on the Earth's atmosphere. (For maps of the eclipse zones and pertinent details see any standard astronomical ephemeris or yearbook.)

(8) *Meteor Showers* of special interest are also marked on the Calendar, including some of the important visual showers and also unusual showers observable mainly by radio and radar techniques. Attention is directed to these days (selected by P. Millman, Ottawa) in case ionization produced by meteors may account for unusual effects in other geophysical experiments. In 1966 the Leonid meteor shower, Nov. 16–17, may be stronger than in the past 25 years. Although the Leonids gave spectacular displays in 1833 and 1866–67, planetary perturbations prevented a repeat of these displays in 1900 and 1933.

Table 1. World Days marked on the Calendar

1966	*RWD*	*PRWD*	*QWD*	*RGD*	*WGI*	*Ecl.*	Meteors
Jan. 11, 12, 13	12	—	5, 12, 19, 26	—	—	3–4
Feb. 15, 16, 17	16	16	2, 9, 16, 23	7–20	—	—
Mar. 15, 16, 17	16	—	2, 9, 16, 23, 30	—	—	—
Apr. 12, 13, 14	13	—	6, 13, 20, 27	—	—	22
May 17, 18, 19	18	18	4, 11, 18, 25	9–22	20	4–6
June 14, 15, 16	15	—	1, 8, 15, 22, 29	—	—	6–10
July 12, 13, 14	13	—	6, 13, 20, 27	—	—	28–30
Aug. 16, 17, 18	17	17	3, 10, 17, 24, 31	8–21	—	10–14
Sep. 13, 14, 15	14	—	7, 14, 21, 28	—	—	—
Oct. 11, 12, 13	12	—	5, 12, 19, 26	—	—	20–21
Nov. 15, 16, 17	16	16	2, 9, 16, 23, 30	7–20	12	16–17
Dec. 13, 14, 15	14	—	7, 14, 21, 28	—	—	12–14 22–23

(9) *World Days not appearing on Calendar*. The occurrence of unusual solar or geophysical conditions is announced or forecast through various types of geophysical 'alerts' which are widely distributed by telegram and radio broadcast on a current schedule. The types of alerts are: magnetic storm (in telegrams *magstorm*), magnetic calm (*magcalme*), solar flare (*soflare*), solar activity (*solactivity*), cosmic ray event (*cosmic event*), and sudden and unusual stratospheric warmings (*stratwarm*). These alerts are issued by the IUWDS World Warning Agency or in certain circumstances by one of the solar-geophysical regional warning centres. The meteorological telecommunications network co-ordinated by the World Meteorological Organization carries the world-wide alerts once daily soon after 0400 U.T. Many geophysical stations in the various disciplines increase their programmes or carry on special experiments to take advantage of the special solar or geophysical conditions during the period of alert. Selections of *Retrospective World Intervals*, after a delay of a few months, are also announced by the World Warning Agency. An additional service of the regional warning centres is to notify geophysical and solar stations promptly (*Ursigrams*) with summary details of immediately significant geophysical observations and of major solar events which have important and sometimes long-lasting geophysical effects. The telegraphic addresses of the Regional Warning Centres are as follows:

Agiwarn Washington (U.S.A.); Dempa Tokyo (Japan); Nizmir Moscow (U.S.S.R.); Ionosphaere Darmstadt (G.F.R.) or Cnetagi Bagneux (France) or Agi Nederhorstdenberg (Netherlands). Associate Regional Warning Centres operate in some other localities.

(10) *The International Ursigram and World Days Service* (IUWDS) is a permanent scientific service of the International Union of Radio Science (URSI), with the participation of the International Astronomical Union and the International Union of Geodesy and Geophysics. IUWDS adheres to the Federation of Astronomical and Geophysical Services of the International Council of Scientific Unions. The IUWDS co-ordinates the international aspects of the world days programme and rapid data interchange, and also publishes subsequently an annual *Calendar Record* of solar and geophysical indices and events.

Recommended Scientific Programmes, Operational Edition

The following material was reviewed in 1965 by scientific working groups of the third Assembly of the IQSY Committee and the eighth Meeting of the Committee on Space Research as suitable for co-ordinated geophysical programmes in 1966.

(11) *Meteorology*. Particular efforts should be made to carry out an intensified programme on the *RGD*, each Wednesday, U.T. A desirable goal would be the scheduling of meteorological rocket sondes, ozone sondes and radiometer sondes on these days, together with maximum-altitude rawinsonde ascents at both 0000 and 1200 U.T.

During *WGI* and *Stratwarm* alert intervals, intensified programmes are also desirable, preferably by the implementation of *RGD*-type programmes on Mondays and Fridays, as well as on Wednesdays.

(12) *Geomagnetism*. It has always been a leading principle for geomagnetic observatories that operations should be as continuous as possible. Thus the great majority of stations taking part in the geomagnetic programme will

undertake the same programme without regard to the Calendar. The days marked on the Calendar will be of interest mainly to the following two types of geomagnetic stations: (*a*) stations recording quick-run micropulsations (with fast chart speeds) are requested to make such records on every *RGD* according to the following schedule: 1966 Jan. 5, from 1400 to 1800 U.T.; Jan. 12, from 1500 to 1900 U.T.; Jan. 19, from 1600 to 2000 U.T., etc. The observatories are not obliged to send their recordings to the World Data Centres except by special request (see IQSY *World Days Manual* under "Retrospective World Intervals on Micropulsations"). (*b*) Stations which, in addition to other activities, are equipped for making magnetic observations, but which cannot carry out such observations and reductions on a continuous schedule, are encouraged to carry out such work at least on *RWD* (and during times of *Magstorm* alert).

(13) *Ionosphere*. For the vertical incidence sounding programme, the summary recommendations are: (*a*) soundings to be made at 5-min intervals or less on *RWD* for stations normally making observations every 15 min or at more frequent intervals; all stations are recommended to make at least quarter-hourly observations on *RWD*; (*b*) *f*-plots are made for high-latitude stations and representative stations at lower latitudes for all days (that is, including *RWD* and *WGI*); (*c*) profile parameters *hc*, *qc* or recommended similar parameters to be determined and sent to *WDC* for *RWD* for all stations except those undertaking full profile programmes or producing monthly median profiles; (*d*) copies of hourly ionograms with appropriate scales for *PRWD* are to be sent to *WDC*; (*e*) stations in the eclipse zone should take continuous observations on solar eclipse days and special observations on adjacent days in accordance with detailed recommendations in *Ionosphere Manuals*. Continuous records of ionospheric parameters are acceptable in place of *f*-plots at temperate and low-latitude stations.

For the ionospheric drifts programme, observations are made at least on all *RWD* on all *WGI*, on every Wednesday (*RGD*) and on every Thursday (day following each *RGD*). Hourly tabulations for all days mentioned are sent to the *WDC*. It is essential that sufficient observations be made to provide representative values of the diurnal variations. Hourly tabulations for this purpose should also be sent to *WDC*.

For the ionospheric absorption programme, diurnal hourly observations are made on all *RWD* and hourly tabulations sent to *WDC*. Continuous observations are made on solar eclipse days, where possible, for stations in eclipse zone. Special efforts should be made to obtain additional absorption measurements at temperate latitude stations during the period of absorption winter anomaly, particularly on days of abnormally high or abnormally low absorption.

For back-scatter and forward-scatter programmes, observations should be made on all *RWD* at least.

For topside sounding experiments, it is recommended to send copies of records to *WDC* for all *RWD* at least.

It is recommended that more intensive observations in all programmes be considered on days of unusual meteor activity.

(14) *Solar Activity*. Observatories are invited to issue and send to the *WDC* of all disciplines special reports of their regular and any special observations on all solar eclipse days to assist in the interpretation of geophysical observations made in the eclipse zones. The total eclipse of November 12, 1966, although of relatively short duration, is of some special interest for astronomers and geophysicists since the path lies in low geomagnetic latitudes and near several long-established geophysical observatories.

(15) *Cosmic Rays, Aeronomy*. Research workers should take into account that observational effort in other disciplines tends to be intensified on the days marked on the Calendar, and schedule balloon and rocket experiments accordingly if there are no other geophysical reasons for choice.

(16) *Space Research*. In view of the variability of the *D* and *E* regions of the ionosphere, it is desirable to make rocket measurements of their characteristics on the same day at as many locations as possible. Where feasible, experimenters should endeavour to launch rockets on the Quarterly World Days (*QWD*) since these are also days when there will be maximum support from ground observations.

Copies of the Calendar are available on request to IUWDS, c/o Secretary General of URSI, 7 Place Danco, Brussels 18, or IUWDS, Deputy Secretary, Miss J. V. Lincoln, CRPL, National Bureau of Standards, Boulder, Colorado.

ANTARCTIC CREVASSE ACCIDENT

J. T. BAILEY, D. P. WILD and Dr. J. K. WILSON

ON October 12, 1965, a British Antarctic Survey tractor fell 150 ft. into a hidden crevasse when operating 250 miles inland from the coastal station at Halley Bay. In this tragic accident three men, J. T. Bailey, D. P. Wild and Dr. J. K. Wilson, lost their lives. A fourth man, J. Ross, a geologist, who was accompanying them with a dog team, brought the news to another field party which reported the accident by wireless.

Jeremy Bailey, son of Mr. and Mrs. A. Bailey, was twenty-four years old. After attending the Watford Grammar School, he read physics at the University of Bristol where he obtained his B.Sc. degree in 1963. He was president of the students' Physical Society during his final year at Bristol. His wide interests included music, scouting and other outdoor activities.

After graduating, he moved to the Scott Polar Research Institute in the University of Cambridge where he became a candidate for a Ph.D. degree and a member of Downing College. As a research student working on radio echo methods of sounding ice sheets, he took part in field trials in north-west Greenland during June–August 1964, as a member of the Scott Polar Research Institute group working in co-operation with United States scientists and personnel of the United States Army. For the latter part of these highly successful trials he had the sole responsibility for operating the equipment from the Scott Polar Research Institute. His natural enthusiasm and ability were evident, and greatly impressed his American colleagues. A preliminary note on this work appeared in *Nature* (204, 420; 1964).

From his arrival in Cambridge it had been planned that he should carry out a year's field research with the British Antarctic Survey. He sailed for Halley Bay in December 1964, since when radio reports have indicated that his work was progressing well. A 300-mile journey completed in April 1965 proved the effectiveness of recent improvements to the equipment and promised well for the inland trip in October, which ended so tragically. Publication of his results from Antarctica and Greenland will form an impressive memorial to the work which he enjoyed so much, but will not compensate for the loss of a young man so full of the promise of further achievement.

David Wild, son of Mr. and Mrs. Peter Wild, was twenty-four years old. He had been educated at St.

Asaph Grammar School and University College of Wales, Swansea, where he gained an honours degree in geography. All his life he had been a keen mountaineer besides being a Queen's Scout with much experience of camping and travelling. In 1961 he seized the opportunity to join his University expedition to Arctic Norway where his chief interest lay in the raised beaches and moraines. At the University he undertook additional courses to fit him for survey work in the Antarctic and finally joined the British Antarctic Survey as a topographical surveyor in 1963.

His evident qualities of leadership and his great good humour made him a popular member both on board ship and at his base. At the time of his death he was already in his second Antarctic year and would have returned in 1966 with an extensive coverage of the mountains at present being mapped. Unfortunately it is feared that most of this work was lost with him. Before leaving for the Antarctic he had presented a joint paper, with F. Solari, to the Photogrammetric Society entitled "Plane Table Photogrammetry with a Leica Camera in the Punjab Himalayas".

Dr. John Wilson, son of Dr. and Mrs. G. I. Wilson, was just twenty-nine. Born at Stafford he was educated at Bedales School and The Queen's College, Oxford, where he took his B.A. degree in animal physiology in 1959. The next three years were spent studying at Middlesex Hospital Medical School where he gained the degree of B.M., B.Ch.

Having qualified he successively held posts at Mount Vernon Hospital, Northwood, Middlesex Hospital and the Ipswich and East Suffolk Hospital.

In 1964 he volunteered for service in the Antarctic with the British Antarctic Survey. The next 5 months were spent at the Department of Human Physiology of the Medical Research Council, developing the research project he was to carry out at Halley Bay. This was chiefly concerned with variations in manual dexterity and sensitivity of touch in varying degrees of cold.

In December 1964 he sailed for Halley Bay, the most southerly of the British bases, where a ship can only pay a visit once a year. At the base he immediately became a popular and active member enthusiastically taking part in the general work besides following up his own research. Indeed, he became so keen that he applied to stay on for a second year, an application which was immediately accepted.

All these three men who were so tragically lost in their tractor were enthusiasts in their field and at the outset of careers which promised so well for the future. All young men who go to face the Antarctic require unusual strength of character, enthusiasm and dedication; these three excelled in them all.

V. E. FUCHS
G. DE Q. ROBIN

NEWS and VIEWS

Theoretical Astronomy in the University of Sussex

THE Science Research Council is to make a grant of up to £33,500 to enable the University of Sussex to establish a research group in theoretical astronomy. The group will be led by Prof. W. H. McCrea, at present professor of mathematics in the University of London, Royal Holloway College. The group will form part of a joint programme of teaching and research by the University of Sussex and the Royal Greenwich Observatory at Herstmonceux. In this connexion, the University has made the following appointments: *Visiting Professor*, Sir Richard van der Riet Woolley; *Visiting Readers*, Dr. D. Lynden-Bell and Dr. B. E. J. Pagel. The University is to make an appointment to a further chair in theoretical astronomy.

Biochemistry in the University of Liverpool: Prof. J. Glover

DR. J. GLOVER, Leverhulme reader in biochemistry in the University of Liverpool, has been appointed to the newly established second chair of biochemistry in the University. Dr. Glover is forty-six years old, and was educated at the Royal Belfast Academical Institution and The Queen's University, Belfast, where he gained a first degree in chemistry in 1940 and a master's degree in 1941. During 1941–45 he served as an experimental officer in the Chemical Inspection Department of the Ministry of Supply, and was in charge of the analytical laboratories at Chorley and later at Woolwich Arsenal. During 1945–47 he held the Musgrave research studentship and a senior research grant from the Government of Northern Ireland at the University of Liverpool, where he undertook research on vitamin A. He was awarded a Ph.D. in 1948 and a D.Sc. in 1961 by the University of Liverpool. In 1947 he was appointed assistant lecturer in biochemistry at the University of Liverpool and was promoted lecturer in 1951, Leverhulme lecturer in 1955, Leverhulme senior lecturer in 1958 and reader in 1964. In 1950 he was appointed a Commonwealth Fund Fellow and worked at Washington University, St. Louis, and Columbia University, New York. Dr. Glover has lectured in Switzerland, Washington, and New Jersey, and in Stockholm, and has attended many international conferences in the United States and in various countries in Europe. He is a member of the committee of the Biochemical Society.

Improved Research Reactors

THE *Pluto* research reactor at Harwell is now running at 20 MW—twice the power for which it was designed. The reactor has been running at 15 MW since 1962, when the first improved fuel elements were used; a new type of fuel element was recently introduced and the reactor operated, in a series of experimental runs, at 22·5 MW. Parallel tests are being conducted in the 'Materials Testing Reactor' (a *Pluto*-type reactor) at Dounreay. The reactor is now using the new type of fuel element and will be operating at increased power early in 1966. The basic reason for increasing the power of these reactors is to increase the neutron density in them and thus to enable research workers from the Atomic Energy Authority to increase the number of experiments carried out on them each year. The new fuel element largely responsible for the increase in power consists of four concentric fuel tubes instead of the ten-plate assembly previously used. It was developed by engineers at Harwell and Dounreay in conjunction with the Production Engineering Research Association and Marston Excelsior, Ltd. The Harwell work also included the addition of four vertical quick-acting control rods within the fuel element and this form of control will be used in both *Pluto* and the Dounreay 'Materials Testing Reactor'. It is believed that these improvements could be adapted to any of the existing reactors of similar type, namely, *Dido* at Harwell, *Hifar* in Australia, *DR 3* in Denmark, and *FRJ-2* at Jülich, Germany.

French Satellites in Orbit

ON November 26 the French satellite, *A*-1, was successfully launched from Hammaguir in the Sahara by a three-stage *Diamant* rocket. France thus became the third nation to launch a satellite unaided. The *Diamant*

rocket is 19 m long and has an initial weight of 18·4 tons. The first stage, called *Emeraude*, is a liquid-fuel rocket which burns for 93 sec and has a thrust of 30 tons. The second stage (*Topaze*) is a solid-fuel rocket giving a thrust of 15 tons for 44 sec. The third stage, which burns for 45 sec, is also a solid-propellent rocket. The *A*-1 satellite itself, which has a mass of about 40 kg, did not carry any scientific experiments. But it did have a radio transmitter operating on 136·53 Mc/s; these radio signals ceased after a short time in orbit. The satellite, which received the international designation 1965–96*A*, entered an orbit with the following parameters: inclination to the equator, 34·2°; orbital period, 108·5 min; perigee height, 530 km; apogee height, 1,800 km; eccentricity, 0·084. The predicted lifetime in orbit is about 100 years. Ten days later another French satellite, *FR*-1*A*, was launched by the United States National Aeronautics and Space Administration, under an international agreement similar to that for the British satellites, *Ariel* 1 and 2. The *FR*-1*A* satellite weighs 61 kg and has been designated 1965–101*A*. The main experiment, designed by the Centre National d'Etudes des Telecommunications, is concerned with the propagation of very low-frequency radio waves. A second experiment, provided by the University of Birmingham, consists of a probe to measure electron density. The *FR*-1*A* satellite is in a near-circular orbit at a height of about 750 km, the inclination to the equator being 75·8°.

Ministry of Technology: Regional Organization

In answer to a further question on December 7, the Minister of Technology, Mr. F. Cousins, said that he had established a strong regional organization, one of the main tasks of which was to promote the more vigorous application by industry of new scientific and technical knowledge resulting from research financed or sponsored by the Government. It was examining the limitation of the supply of information from the research associations to supporting firms and also the method by which information was disseminated through the Ministry's establishments. The establishment of the Ministry's regional offices was almost complete: new offices were opened during October in Birmingham, Bristol, Leeds, Manchester and Nottingham, and a further office for South-East England and East Anglia would be opened early next year. By the end of 1967, the Ministry would have set up a network of about seventy liaison centres, co-ordinating the activities of industry, education and the Government, and the regional officers would be represented on the regional planning bodies. At the end of June, the National Research Development Council had 95 development projects in hand; 17 new projects had since been authorized and a further 120 were being assessed. The Ministry's commitments on development projects exceeded £8 million.

Metric Screw Threads

In an answer in the House of Commons on December 7, the Minister of Technology, Mr. F. Cousins, stated that the International Organization for Standardization had reached agreement on recommendations for general-purpose screw threads comprising a system of metric threads and another of inch threads, which was the same as the existing Unified threads. These two systems had been published as British Standards *B.S.* 3643 and *B.S.*1580. Three general-purpose screw thread systems were used in Britain to-day, namely, Whitworth, B.S.F. and B.A. They were little used outside Britain, and to perpetuate their use would be to increase the costs of, and hamper, British export trade. The British Standards Institution had recently issued an important policy statement urging British industry to adopt the International Organization for Standardization metric system wherever possible in future designs. The statement had received strong support from industry, and the decision to recommend the use of metric screw threads had the full support of the Minister, although, with the British Standards Institution, he recognized that certain sectors of industry might find it necessary to continue to use the Unified Inch system for some time. The Institution's statement implied that the Whitworth, B.S.F. and B.A. threads were now to be regarded as obsolescent, although their replacement would be gradual and supplies of screws and tools to the obsolescent standards would be maintained for many years, for servicing designs and equipment already installed. He also supported the Institution's view that these systems should be superseded by metric threads in preference to an intermediate change to the standard inch threads. More than 80 per cent of the population of the world was in countries which had adopted or were adopting the metric system, and British trade with these countries was expanding faster than that with countries not using the metric system.

National Computing Centre

Again, in a statement to the House of Commons on December 7, Mr. F. Cousins said that a National Computing Centre was to be established. This should reduce wasteful duplication of programming by providing users of computers with information about programmes already available in its library and elsewhere. The Centre would also develop and sponsor the development of programmes designed to serve users having closely similar tasks. It would provide and encourage training in systems analysis, programming principles and applications of computers. It would promote research into methods of programming and operating computers and into the influence of these methods on the design of computers. These objectives would be pursued in close co-operation with the manufacturers of computers and with users in industry, commerce, administration, science and technology. The Centre would be established as an independent non-profit-making company, limited by guarantee. Mr. Cousins said he would be inviting representatives of manufacturers, users and of professional and other bodies to become members of the Centre and to serve on its Council. Prof. Gordon Black was being released by the Atomic Energy Authority to become the first director of the Centre, but he would retain his appointment as part-time professor of automatic data processing at the University of Manchester. Mr. Cousins hoped that the Centre would be built in Manchester on a site close to the University, and that it would be fully in operation by 1967. The Centre would probably require a staff of between 40 and 50, who would not be Civil Servants and would need to be experts. It was intended that the Centre should have a computer.

Dublin Institute for Advanced Studies

The Dublin Institute for Advanced Studies recently celebrated its silver jubilee. Established in 1940 by Act of the Irish Parliament, its aims, as laid down in the Act, are the furtherance of advanced study and the conduct of research in specialized branches of knowledge and the publication of the results of such study and research. These aims are carried out through its constituent schools, two of which, the School of Celtic Studies and the School of Theoretical Physics, were established in November 1940 and are specifically mentioned in the Act. A third School —the School of Cosmic Physics—was established in 1947. The School of Celtic Studies is now universally recognized as the foremost centre for research in all branches of that subject. The School of Theoretical Physics has had on its staff such eminent physicists as Dr. Erwin Schroedinger and Dr. Walter Heitler, and now has, as senior professors, Dr. J. L. Synge and Dr. Cornelius Lanczos. The first senior professors in the School of Cosmic Physics were the late Prof. L. W. Pollak, Dr. H. A. Brück (now Astronomer Royal for Scotland) and Dr. Lajos Janossy (now at Buda-

pest). The late Dr. M. A. Ellison succeeded Dr. Brück as senior professor in the Astronomical Section, and the present senior professor is Dr. P. A. Wayman, formerly of the Royal Greenwich Observatory. Dr. Janossy was succeeded by Dr. Cormac Ó Ceallaigh as senior professor in the Cosmic Ray Section, and Mr. Thomas Murphy succeeded Dr. Pollak as senior professor in the Geophysical Section. A duty laid down for all constituent schools is the training of advanced students in methods of modern research, and scholars from all five continents have studied at the Institute. The extent and quality of the research carried out in the Schools can be assessed by reference to the works published by members of the staff and the numerous papers by both staff and scholars which have been published in leading scientific journals and periodicals. An informal celebration of the silver jubilee was held in the Royal Irish Academy, Dublin, on November 16, at which the President of Ireland, Mr. de Valéra, who as Prime Minister was responsible for the setting up of the Institute, was the guest of honour.

Journal of Zoology

THE *Proceedings of the Zoological Society*, now published as the *Journal of Zoology*, being the Proceedings of the Zoological Society of London, publishes papers on all aspects of zoology, including systematics. In particular, it aims at publishing papers on the more general aspects of biology which do not fall within the province of the more specialized journals. The *Journal* has recently increased its number of pages and is able to accept papers of rather more than usual length, although, of course, it in no way encourages prolixity, and places quality before quantity. Traditionally the *Journal* specialized in systematic and morphological subjects; but it has now widened its scope to be a true journal of zoology, and a glance at the issues for recent years shows that ecological, physiological and ethological papers are now included, dealing with all phyla of the animal kingdom. Papers are published by workers in universities and research institutions from all parts of the world, provided they are of sufficient standard.

Journal of Cell Science

THE *Quarterly Journal of Microscopical Science*—one of the oldest British biological journals—is changing its title to *Journal of Cell Science*, with effect from 1966. It has for many years been publishing work on cells, being, in fact, one of the few journals in Britain to do so. The decision to concentrate exclusively on cytology was taken three years ago, and since that time the proportion of papers on histological and micro-anatomical topics, which at one time figured prominently in its pages, has gradually been reduced. The *Journal of Cell Science* will deal with the study of cell organization in all its aspects, publishing papers on the structure and functions of cells at all levels of analysis and including material on such topics as cell growth and division, cell movements and interactions, and cell genetics. Contributions on microorganisms and viruses will be accepted, in so far as they are relevant to an understanding of cell organization, and accounts of new techniques will be published. The journal is to have a new format and page size and will be published by the Cambridge University Press. The editors, Prof. H. G. Callan and Dr. A. V. Grimstone, will in future be assisted by an editorial board. The volume of research on cell biology in Britain is steadily increasing, and the need for a wide-ranging journal in this field has been felt for some time. It seems likely that the *Journal of Cell Science* will go far towards filling this need.

The Soils on Quaternary Beach Ridges in South Australia

CALCAREOUS beach sands are found in some thirty ridges about 50–100 ft. high, running sub-parallel to and up to 60 miles inland from the coast of the south-east of South Australia. The soils derived from these sands and from estuarine and lacustrine deposits are the subject of an interesting study by G. Blackburn, R. D. Bond and A. R. P. Clarke entitled *Soil Development Associated with Stranded Beach Ridges in South-east South Australia* (Australia: C.S.I.R.O. Soil Publication No. 22. With an Appendix by N. H. Ludbrook. Pp. 66 + 4 maps. Melbourne: C.S.I.R.O., 1965). The genetic status of the soil horizons has been established by the cumulative size-frequency distributions of the sand fractions (0·02–2·00 mm) and detailed consideration is given to the problem of accounting for the diversity of the main soil types in a region where the present climatic variation cannot be a major factor. The quaternary calcareous beach sands are not of aeolian origin but may occur as flat-bedded layers or as massive coastal dunes; the absolute ages of the ridges have not been determined, but it is probable that they have resulted from upwarping and from glacial–eustatic-sea-level changes. The four soil types described are: (1) podzols, characteristic of those in other parts of the world; (2) terra rossa which are not developed directly from hard limestone; (3) ground-water rendzinas which are variable in profile features; and (4) solodized solonetz which may display the morphological features commonly described but sometimes resemble the solonetz.

Coal Petrology

THE petrography of coal has had a long, chequered and controversial history of technical evolution, largely because different investigators over the years have adopted different techniques in examining, identifying, describing, and reducing to a universally accepted philosophical classification, optically distinguishable entities in coals. Most coals derive from plant accumulation in swamps; remnants of these plants are observed in coals, but were undoubtedly much altered during geological time by periods of compression and thermal conditions caused by blanketing under later sediments. Different parts of original plants have altered in different ways; thus, microscopical examination tends to show varying optical properties from point to point, often in the same thin coal section. With high magnifications, few areas of coal sections are seen to be homogeneous; with low magnifications, even visual examination, most coals reveal distinct layering of apparently similar materials. These are some of the practical difficulties encountered in petrographic investigations of coals, throughout the range of brown coal to anthracite. But despite this, in alliance with chemical, physical and technological examinations, petrological evaluations of coals have become an increasingly popular and valued tool both in coal research and industry. This is the theme of an excellent paper by C. F. K. Diessel and T. G. Callcott, entitled "Petrographic Features of New South Wales Coking Coals" (*The B.H.P. Technical Bulletin*, 23, 9, No. 2; August 1965. The Broken Hill Proprietary Co., Ltd., Melbourne, Australia). Although the purport of this paper is an appraisal of the petrographic and chemical composition of New South Wales coking coals, its value goes far beyond this, in that it examines contemporary systems of coal petrology now in vogue internationally and pin-points the still existing difficulties in the nomenclatures involved, despite the valuable publication of the *International Handbook of Coal Petrology, 1963*. This present paper includes a table of five different but co-related systems of nomenclature in coal petrology (macerals); these are: Thiessen–U.S. Bureau of Mines; B.H.P. System as used at present; Geological Institute of the Academy of Science, U.S.S.R.; Spackman System; and the Stopes–Heerlen System. As the authors remark, conversion from one of these systems to another is by no means a simple matter; apart from different techniques used in analysis, widely differing points of view prevail on the essentials of a descriptive basis for coals. The problems facing the International

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Committee on Coal Petrology in its attempts to standardize terminology have been, and still are, quite formidable. From a purely petrographical standpoint, although the optical identification of many components of coals has advanced considerably in recent years, compared with the days when 'clarain', 'durain', 'fusain' and 'vitrain' were the common labels (Stopes), there still remain problems of determining the ultrafine structure and molecular constitution of the now recognized components, and of relating these features to the physical properties of the many coal-types concerned.

Palaeontology in the U.S.S.R.

The recent volumes of the *Transactions of Palaeontological Institute (Moscow)* are: (103) *Corynexochoidea Trilobites and their Historical Development*, by N. P. Suvorova (1964); (104) *Tertiary Homoptera of Stavropol Region*, by E. E. Bekker-Migdisova (1964); (106) *Morphology, History of Development and the System of Ordovician and Silurian Bryozoa*, by G. G. Astrova (1965).

High-temperature Studies on Blast Furnace Slags

With the aid of differential thermal analysis and X-ray diffraction methods, the devitrification of solid glassy blast-furnace slag and crystallization of molted slag have been investigated in South Africa by J. E. Krüger, K. H. L. Sehlke and J. H. P. van Aardt, of the Materials Division, National Building Research Institute, C.S.I.R., Pretoria (*Reprint from Cement and Lime Manufacture*, 37, Nos. 4 and 5; July and September 1964. C.S.I.R. reference *R/Bou* 120). When vitreous blast-furnace slags are heated to 800°–900° C, devitrification takes place; the heat evolved during this process can be clearly seen on a differential thermal analysis curve, of which several examples are given in their paper, *High-Temperature Studies on Blast Furnace Slags*, by the authors concerned. It was found that for the ten samples of slag selected (seven of them South African), the appearance of the differential thermal analysis thermograms differed from slag to slag, even if the chemical compositions of the slags were similar; that when the slags were heated to temperatures corresponding to different points on the differential thermal analysis thermograms and subjected to X-ray diffraction analysis, analogous with the process of slow cooling of slag, different crystalline phases formed during devitrification; the latter are identified. Further, there is marked agreement between phases of a slag formed after complete devitrification and phases emerging after slow cooling of the molten slag to ambient temperatures. Two other significant conclusions arise from these investigations: slags possessing poor hydraulic properties would, on complete devitrification, give phases of low calcium content, such as pseudo-wollastonite and diopside, indicating a low calcium potential for such slags; and devitrification differential thermal analysis peaks persist even after the slags have been subjected to hydration for prolonged periods. This paper is worthy of study by all workers in the field of slag-mineral analysis, particularly as regards recognition and identification of mineral phases with change of state.

Handbook of Seed Health Testing

The *Handbook of Seed Health Testing*, issued by the International Seed Testing Association (I.S.T.A.), comprises four series of papers as follows: Series 1, general topics; Series 2, seed pathology of a particular crop or a group of organisms; Series 3, working sheets, each dealing with one seed-borne disease—the methods being based on co-operative work over the past 8 years by the Plant Disease Committee of I.S.T.A.; Series 4, description and illustrations of seed-borne fungi. The papers are first being published from time to time in the *Proceedings of I.S.T.A.*, and then reprinted so that they are available for general purchase. Thus, Chapter 1 of Series 1 is an annotated list of seed-borne pathogens published, in this case jointly, by the Commonwealth Mycological Institute, Kew, and I.S.T.A. in 1959; and Chapters 2–4 give information on seed weakness, pesticide treatment and a review of methods; of Series 3 the first 33 working sheets have been prepared (loose-leaf in binder), the host plants including wheat, rice and sesame; and, as noted in *Nature* (207, 579; 208, 319; 1965), the first papers of Series 4, descriptions and illustrations of 77 seed-borne fungi found mainly on oats and flax, have been compiled by Malone and Muskett. A colour transparency scheme is planned in connexion with Series 3 to help with recognition of seedling symptoms. The *Handbook* thus replaces the *Manual for the Determination of Seed-borne Diseases*, by Dr. L. C. Doyer, of the Wageningen Seed Testing Station, published in 1938 under the aegis of I.S.T.A. This, the only work of its kind, is now unobtainable. Those parts of the new *Handbook* already completed cost approximately 40 guilders (£3 sterling, 8.5 dollars) (half-price to members of I.S.T.A.), and are available at the I.S.T.A. Secretariat, Binnenhaven 1, Wageningen, Netherlands.

Aureomycin and the Cultured Pearl Industry

Japan to-day produces some 400 million cultured pearls per annum, of which 97 per cent are exported. It is a fact that the 'natural' pearls discovered now play a relatively insignificant part in the world market. It was towards the end of the nineteenth century that a Japanese, Kokichi Mikimoto, first hit on a successful technique for 'seeding' and caring for oysters, from which the idea of the cultured pearl gradually took shape; he patented his process and started to export this 'natural' product. Mikimoto was successful in a lawsuit that followed, in the course of which biologists at the time, who had tested both his and natural pearls, reported that there was no difference between them. Thus the cultured pearl industry grew apace. Japanese oyster farms are located in selected estuaries, where specially bred oysters are seeded with round beads manufactured from shells of what are called 'pigtoe mussels', most of the latter being derived from the Mississippi and Tennessee Rivers in the United States. Maturity of the pearls thus formed takes from three to five years, depending on the size of the pearls sought. But until comparatively recently, only three out of five oysters produced some sort of pearl, and only one out of every twenty pearls was considered up to top standard ('hanadama' or 'tears of the moon', as they are known in Japan). About 5 years ago the problem of improving yields and quality of these cultured pearls was investigated at the Fisheries School of Mie Prefecture in Japan, by a research team headed by Tetsuo Miyauchi. Starting with the theory that bacterial infections at seeding time were responsible for poor yield, misshapen or discoloured pearls, experiments were begun with applications of Lederle's broad-spectrum antibiotic, 'aureomycin chlortetracycline', in an effort to halt these infections. These trials were eminently successful. After four years of tests, this technique resulted in a 30 per cent increase in yield of top-quality 'hanadama' pearls and a significant increase in the total number of pearls obtained. This somewhat unusual application of a modern antibiotic is briefly described in an article, "Pearls: 'Tears of the Moon'" (*Cyanamid International Newsletter*, 4, No. 4. Cyanamid of Great Britain, Ltd., Bush House, Aldwych, London, W.C.2). The Japanese affiliate of Cyanamid, Ltd., Lederle (Japan), Ltd., has prepared a new formulation of 'Aureomycin' for the cultured pearl industry, known as 'Pearl-up' (chlortetracycline), designed to boost production on a world-wide scale.

Hospital Libraries

A pamphlet, *Hospital Libraries*, issued by the Library Association (*Hospital Libraries: Recommended Standards*

for Libraries in Hospitals. Pp. 16. London: Library Association, 1965. 5*s.*; L.A. members, 3*s.* 9*d.*) outlines recommended standards for libraries in hospitals. These cover planning libraries in hospitals, general library service for patients and staff, medical libraries in a regional board area, the functions of the library in a non-teaching hospital and libraries in training schools for nurses. There is a short list of books.

Commonwealth Universities

THE ninth edition, August 1965, of the *List of University Institutions in the Commonwealth*, first issued by the Association of Commonwealth Universities in 1949 (Pp. 12. London: The Association of Commonwealth Universities, 1965, *gratis*) is based on information collected in June 1965, and entries altered since the eighth edition (June 1964) are distinguished by a special mark. Full postal addresses are given. Schools of the University of London are listed in an appendix, while a second appendix lists universities in Eire and the Republic of South Africa.

Eye for Isotopes

THE Atomic Energy Authority's film, *Eye for Isotopes*, has been awarded a Special Diploma at the fourth International Festival of Scientific–Technical Films organized by the Yugoslav society "Nikola Tesla" in Belgrade. Some 200 films were presented at the Festival and only 13 awards were made. *Eye for Isotopes*, which runs for 27 min, was designed to encourage industrial audiences to consider how radioisotopes might be applied to their processes to save both time and money, by illustrating some of the now well-established techniques. Produced by Clifford Parris and directed by Derek Conrad, of World Wide Pictures, Ltd., and with Dr. John Putman, of the Authority's Wantage Research Laboratories, as technical adviser, *Eye for Isotopes* was awarded a first prize at the fourth International Film and Television Festival in New York in 1964 and also an Award of Merit at the International Science Film Festival in Toronto, also in 1964.

University News: Liverpool

PROF. T. W. GOODWIN, professor of agricultural chemistry in the University College of Wales, Aberystwyth, has been appointed to the Johnston chair of biochemistry as from October 1, 1966, on the retirement of Prof. R. A. Morton. A. Kirk has been appointed director of the Computer Laboratory. The following lecturers have also been appointed: Dr. H. J. Phillips (anatomy); H. Kinloch (mechanical engineering); Dr. K. N. Strafford (metallurgy).

London

PROF. W. R. MEAD, professor of geography at University College, has been appointed to the headship of the Department and chair of geography tenable at that College. The following professors have also been appointed: Dr. G. G. Meynell (Guinness chair of microbiology, tenable at the Lister Institute of Preventive Medicine); Prof. J. H. Westcott (control systems, tenable at the Imperial College of Science and Technology); Prof. P. Wheatley (geography, tenable at University College). The following titles have been conferred: *Professors*, Dr. M. J. Smith (biochemical pharmacology, in respect of his post at King's College Hospital Medical School); Dr. R. A. Shaw (chemistry, in respect of his post at Birkbeck College); *Readers*, Dr. J. F. A. P. Miller (experimental pathology, in respect of his post at the Institute of Cancer Research); Dr. P. A. Nasmyth (pharmacology, in respect of his post at St. Mary's Hospital Medical School); Dr. D. Wilkie (botany), in respect of his post at University College).

Newcastle upon Tyne

PROF. J. HOUSE has been appointed to the headship of the Department and the chair of geography on the retirement of Prof. G. H. J. Daysh. The following appointments have also been made: *Senior Lectureship*, Dr. M. G. McEntegart (microbiology); *Lectureships*, Dr. R. M. Keenlyside (physiology); J. D. Arthur (mechanical engineering).

Strathclyde

DR. D. W. A. SHARP, reader in inorganic chemistry in the Department of Pure and Applied Chemistry, has been appointed to the chair of inorganic chemistry from October 1.

Announcements

PROF. B. GREGORY has been appointed director general of CERN, the European Organization for Nuclear Research, in succession to Prof. V. F. Weisskopf, who has retired after 5 years' service and is returning to the Massachusetts Institute of Technology as Institute professor of physics.

DR. J. A. STRATTON is retiring as president of the Massachusetts Institute of Technology on June 30. He has been elected chairman of the board of trustees of the Ford Foundation as from January 1, on the retirement of Mr. J. J. McCloy.

DR. S. WORTMAN, formerly director of the Pineapple Research Institute, Hawaii, has been appointed director for agricultural sciences in the Rockefeller Foundation.

A SYMPOSIUM on "Recent Advances in Polymer Chemistry", arranged by the Bradford Chemical Society, will be held at the Bradford Institute of Technology on February 5. Further information can be obtained from Mr. W. A. Straw, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7.

A DISCUSSION on "Operating Experience with Deck Machinery", organized by the Power Division Professional Group on Road, Air and Sea Transportation of the Institution of Electrical Engineers, will be held at the Institution in January 5. Further information can be obtained from the Institution of Electrical Engineers, Savoy Place, London, W.C.2.

THE seventeenth Pittsburgh conference on "Analytical Chemistry and Applied Spectroscopy" will be held in Pittsburgh during February 21–25. The programme will include the following symposia: combined gas chromatographic–mass spectrometric techniques; fast reaction kinetics; electron spin resonance; electron microprobe; process control X-ray spectrometry, atomic absorption; Coblentz Society symposium; gas chromatography utilizing pyrolytic techniques; nuclear magnetic resonance spectra of polymeric materials; nuclear magnetic double resonance; magnetic optical rotatory dispersion; analytical instrumentation in the space programme; new instrumentation techniques. Further information can be obtained from Dr. R. E. Hein, Seventeenth Pittsburgh Conference, Inc., Mellon Institute, 4400 Fifth Avenue, Pittsburgh, Pennsylvania.

ADDENDUM. The publication, *Enzyme Nomenclature. Recommendations (1964) of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, together with their Units and the Symbols of Enzyme Kinetics*, referred to in ref. 1 of the article entitled "Enzyme Nomenclature", which appeared on p. 736 of the November 20 issue of *Nature*, has also been published as a paperback book and is available in Great Britain from the Elsevier Publishing Co., Ltd., at a price of 18*s.*

ERRATUM. In the article entitled "Precipitin Activity in the Sera of Patients with Deficiency of β-Lipoproteins (Acanthocytosis)", by Dr. R. F. Murray, jun., and Prof. B. S. Blumberg, which appeared on p. 357 of the October 23 issue of *Nature*, the asterisk placed by the name of Prof. Blumberg should have been placed by the name of Dr. R. F. Murray.

INHALED PARTICLES AND VAPOURS

FIVE years ago, at Oxford, the British Occupational Hygiene Society organized an international symposium on inhaled particles and vapours (*Nature*, 186, 524; 1960); a second meeting was held at St. John's College and the Engineering Laboratory, Cambridge, during September 28–October 1, 1965. Forty-seven papers were presented, of which only one dealt with a vapour. Some 300 scientists and doctors attended the symposium, about half of them coming from abroad.

The papers were issued as pre-prints and divided into seven sessions. Prof. B. Pernis of Milan gave the Earl King Memorial Lecture and spoke about immunological reactions and pulmonary dust disease; the lecture will be printed in *Annals of Occupational Hygiene*, 9, Part (ii).

Attention was directed, in the opening paper by A. Policard, A. Collet and C. Reuet-Normand, to the advantages of using cats for inhalation experiments; unlike those of the rodents commonly employed, the lungs of cats are similar to those of man in having only one or two orders of respiratory bronchioles followed by well-developed alveolar regions. Dust deposits form around the bronchioles and the vascular system, as in man, and dust nodules may develop. An apparatus for recording physiological data from experimental animals was described by S. Laham and G. F. Dowd.

E. G. Tombropoulos had prepared and centrifuged homogenates of the spongy tissue of animal lungs containing acetate-^{14}C. The labelled acetate was found to preponderate in the fraction rich in mitochondria, which are believed to transform short-chain fatty acids into long ones. The long-chain acids are incorporated in lung phospholipids which are responsible for maintaining the patency of the alveoli by concentrating at the surface of the alveoli.

An investigation of mucous flow in the human nose was reported by D. F. Proctor and H. N. Wagner; a small quantity of radioactive material was placed on the surface of the nasal mucosa and its movement plotted by a double collimated crystal detector; movement was towards the nasopharynx which was reached in about 8 min, clearance being completed in 10–30 min. Measurements of air flow in a model of the human nose showed that large areas of mucosal surface were only slightly ventilated and that turbulence was low.

The volumes of air inhaled by coal-miners engaged in various occupations had been measured by G. G. Hadden, C. O. Jones and D. C. Morgan. Minute volumes varied from 10 l. up to a maximum of 54 l. for a coal-filler using a hand pick. Some association was noted between peak activity and peak dust concentration on hand-worked faces but was not apparent in mechanized areas.

The second session, dealing with pulmonary response to dust, opened with an account of a quick-freeze method of locating changes in the size of airways caused by the inhalation of a histamine aerosol by cats. Constriction of terminal bronchioles and alveolar ducts was observed. Some reaction occurred in bronchioles as large as 0·4 mm diameter; deposition in these may have been due to the fine aerosol particles having increased in size by absorbing water vapour. This work was carried out by J. A. Nadel, M. Corn and associates.

The effects of textile dusts were described by A. Bouhuys and P. J. Nicholls, who incubated human lung tissue with various substances and measured the production of histamine. Cotton dusts were active with human but not with animal lung. The bracts of the cotton boll gave a potent extract. Compound 48/80 released histamine in human and animal lung tissue. Similar substances, when breathed as aerosols, produced respiratory reactions in some ways analogous to those produced in the culture experiments, again implicating cotton bracts as a cause of at least part of the symptoms of byssinotic cotton workers.

W. T. Ulmer, in a series of measurements of human respiratory resistance, showed that the response to dust inhalation was similar whether or not the subject had experienced occupational exposure.

Three papers dealt with silica dust. A. Swensson utilized intratracheal injection of saline suspension into rats to illustrate how the lung reactions to silica dusts differed, even between amorphous silicas. Reactions in both lungs and lymph nodes were graded histologically and, also, by collagen production. P. Gross and H. Brieger, also injecting rats, found that coesite dust and quartz dust caused inflammation of the alveolar membrane but not of the interstitium; lesions were mainly located in the proximal part of the racemus and might tend to trap the dust there. Finally, I. Webster, M. C. Path and associates injected silica suspensions intravenously into rabbits and guinea-pigs to produce silica shock; an increase in the kinins of the blood resulted. Silica shock could be prevented by the administration of anti-amine drugs, the most effective of which also depressed the solubility of silica. The non-lipoid fraction of alveolar macrophages, collected after the animal had breathed silica dust, produced a granuloma when inoculated into animals. This effect appeared to be associated with abnormal contents of histamine and of an unidentified reagent. It is suggested that silica may stimulate the release of a cellular product, similar to that produced by a carcinoid tumour, which causes fibrosis in the peripheral part of the silicotic nodule.

Observations of the cytotoxic action of silica on macrophages were described by A. C. Allison, J. S. Harington *et al.*, who also showed a remarkable time-lapse film to illustrate their conclusions. Particles engulfed by a macrophage are held in a phagosome to the membrane of which lysosomes adhere and release enzymes into the phagosome. Silica particles, perhaps by forming silicic acid, cause the phagosome membrane to become permeable so that the enzymes escape into the cytoplasm of the macrophage and destroy it. Polyvinylpyridine-*N*-oxide prevents this from happening by forming hydrogen bonds with silicic acid.

It has been claimed that calcium fluoride dust has high fibrogenic activity; A. J. de Villiers and P. Gross set out to check this by the intratracheal injection of rats. The dust was found to cause no significant pulmonary fibrosis. In some animals the chest was exposed to X-rays, which caused characteristic effects but had no influence on alveolar clearance when irradiation took place six weeks before the inhalation of dust.

F. J. Strecker experimented with rats inhaling various kinds of silica dust and also some inert dusts. This work suggested that there was a high mitosis rate of alveolar cells, which are turned over about once a week; the considerable surplus of cells is eliminated via the bronchial epithelium and is easily capable of phagocytosing a great number of dust particles. The inhalation of dust does not produce an extra proliferation of cells since the normal turnover is quite adequate to deal with such an emergency. Silicosis-producing dusts kill the cells and cause an increase in the mitosis rate in alveolar regions; there appears to be difficulty in eliminating cell debris via the bronchioles. This may mean that some cell debris penetrates the interstitium and attains the lymphatic vessels. Treatment of animals with polyvinylpyridine-*N*-oxide prevents cell necrosis due to silica. Different dusts and different species of animals yield differing elimination rates, but the over-riding rule that greater cytotoxicity causes greater translocation to the lymphatics seems to hold. Further

experiments using intraperitoneal injection supported this view.

A. Collet, J. C. Martin and colleagues described investigations of alveolar macrophages obtained by washing out animal lungs. The cells from different species and from man were similar in many respects and all had characteristics which were unique among pulmonary cells and indicated evolution from a small non-phagocytic lymphocyte. A non-pulmonary origin of macrophages is therefore a possibility, with lymphoid tissue as a likely alternative source. The ingestion of particles caused iron to accumulate in the cells.

A number of papers dealt with lung clearance in animals; after studying the deposition of dusts in rats by visual microscopy it was concluded, by P. Gross, E. A. Pfitzer and T. F. Hatch, that by about four days after exposure the peripheral region of the racemus is cleared of dust but that there is a build-up of deposit in the proximal region due partly to transport from the periphery and partly to initial deposition there. The proximal region comprises the respiratory bronchioles and alveolar ducts with directly associated or evaginating alveoli. The surface area of the proximal zone is only about one five-hundredth of that of the whole racemus, so that concentration of the dust, which was well distributed at initial deposition, is inevitable. It is the dust situated in the racemus that is responsible for the protracted clearance by the bronchial epithelium; the entire journey along the ciliated tract occupies only about 24 h, so that dust recovered from the bronchi and bronchioles after several days must have come from the deposits building up at the base of the mucous escalator in the necks of each racemus.

J. Ferin exposed rats to silica dust and analysed groups of lungs after 18 h for initial deposition and after 25 days to establish lung clearance. He demonstrated that the clearance of dust from the lungs was diminished if the animals were exposed to cold for long periods before and after inhaling dust, but that stress induced by injecting solutions of formaldehyde had no effect.

Short-term lung clearance in rabbits, after they had inhaled an aerosol consisting of radioactive plastic spheres 6μ and 3μ in diameter, was described by B. Holma. After exposure to the aerosol, the animals were scanned periodically from head to abdomen with an opposed pair of collimated crystal counters and the transfer of activity from thorax to abdomen was clearly shown. An initial rapid clearance from the thorax lasted up to 4 h from exposure, for the 6μ particles, but was much slower for the 3μ ones, presumably because more of them were deposited in alveoli, although this is not discussed. A secondary, much slower, clearance of residual activity followed. This had a half-life of about 70 h for the larger particles and 210 h for the smaller.

T. G. Morris, W. H. Roberts *et al.* compared the retention of asbestos and silica dusts in specific pathogen-free rats and normal rats. W. Klosterkötter and H. J. Einbrodt, also using rats, studied elimination, following the breathing of various dusts including highly dispersed forms of silica. Quartz dust was found to suppress the elimination of an inert dust. Aerosil, a hydrophilic colloidal silica, was eliminated rapidly, probably because of its high solubility, but hydrophobic forms of colloidal silica disappeared much more slowly and collected in the lymphatics.

Experiments on the removal of bacteria from the lungs of mice were discussed by G. Green and E. H. Kass. Using aerosols of radioactive bacteria and comparing the number of viable organisms with the quantity of radiation, it was established that killing of the bacteria occurred long before physical removal of the debris. A technique utilizing fluorescence made it possible to show that organisms were picked up by phagocytes in the alveoli; phagocytes could be found on the bronchial mucosa in the course of transportation out of the lungs several hours after administration. A lowering of the clearance rate when animals were stressed by hypoxia, ethyl alcohol starvation and cortisone was demonstrated. Different species of bacteria had different clearance rates.

Experiments aimed at elucidating prophylaxis against silicosis had been carried out by B. Rasche and W. T. Ulmer. After exposing guinea-pigs to dust, separable alveolar cells were washed out of the lungs with saline and incubated in culture media; the number of cells, their oxygen consumption and the production of lactic acid were measured. The breathing of quartz dust stimulated the production of macrophages, which was further enhanced if an aerosol of aluminium chloride was also inhaled. Aerosols of chlorides of iron, magnesium and calcium had no such effect, nor did aerosols of polyvinylpyridine-*N*-oxide the action of which, in inhibiting silicosis, cannot, therefore, be associated with elimination of dust.

W. J. Bair and J. V. Dilley failed to detect any cigarette smoke influence on the pulmonary clearance of radioactive dusts of iron oxide and chromium oxide from the lungs of rats and dogs. Deposited dust was assessed by scanning the animals with crystal counters.

Investigations of the lung burden of animals exposed in a field experiment were described by R. H. Wilson and J. L. Terry. The dust clouds contained a trace of plutonium and covered a very wide range of particle size, with mean mass diameters of 10–20μ. Initial deposition, following exposure to a bomb burst on a hard surface, was about the same in several species, but the lung burdens remaining after a lapse of several days were much less in sheep than in dogs. The short-term lung clearances had half-times of the order of several days, rather than the 24 h often quoted, and were followed by a long, slow decay extending beyond a year. The latter accounted for a residual 15 per cent of respirable material in dogs, 5 per cent in burros and only 1 per cent in sheep. The burro is assumed to be the best approximation to man.

When the dust of uranium ore is inhaled the secular radioactive equilibrium of the decay nuclides will not be maintained in the lungs if the various daughter products are subjected to differing biological rates of elimination. This was investigated by B. O. Stuart and T. M. Beasley, to see if the permissible level for pitchblende dust in the air of mines needed modifying, due to the building up of ^{230}Th in lung deposits on account of its relatively slow elimination compared with the parent radionuclide. Experiments with rats inhaling pitchblende dust over eight weeks revealed a gradual disproportionation of uranium and thorium, the proportion of the latter being higher, both in lung parenchyma and in the lymph nodes. Translocation to kidney, femur and liver ultimately led to 50 times more thorium being detected due to selective removal of uranium. The hazard from inhaling natural uranium, therefore, needs to be evaluated with separate allowance for the daughter products.

The fifth session, dealing with inhalation by man, was opened by the sole paper about vapour; this was by A. Morgan, D. J. Morgan and G. M. Arkell. They found that inhaled methyl iodide was retained from 53 per cent to 92 per cent, according to the tidal volume. Uptake by the blood was very rapid and metabolism was the same as for the iodide ion.

B. Altshuler, E. D. Palmes and N. Nelson used previous experimental data on total deposition of inhaled aerosol in man to calculate regional deposition. The method was to assume that part of the volume inhaled occupied the upper respiratory tract while the rest went into the alveolar spaces. The difference between measured total deposition and calculated upper tract deposition gives the lower tract figure. Deposition during inhalation, the respiratory pause and exhalation was calculated for the upper tract as if it were a continuous filter; allowance for mixing within the tidal air and between tidal air and residual air was based on data from previous wash-out experiments in which clean air was breathed after inhaling aerosol. The calculations were made for particles of diameter 0·14–3·2μ using a tidal volume of 500 cm^3 and

upper respiratory volumes of 100, 140 and 180 cm³. The results indicate that the diameter for maximal alveolar deposition exceeds 2μ, at which figure the alveolar deposition was between 20 per cent and 50 per cent, depending on the subject; the alveolar figure came out higher when the smaller upper respiratory volume was used. These curves for alveolar retention are at variance with previous work in that they suggest high alveolar deposition in the region of 3μ diameter.

E. D. Palmes, B. Altshuler and N. Nelson described experiments with an automatic apparatus for single-breath analysis. The human subject inhaled 625 cm³ of aerosol from a bag and exhaled twice this volume into another bag after a variable respiratory pause; the fraction of aerosol recovered decreased exponentially as the pause was lengthened.

Lung clearance in man was the subject of experimental work by P. E. Morrow and K. Gazioglu. Non-homogeneous radioactive aerosols were inhaled and the total deposition found by means of electrostatic precipitators. After washing out the mouth and pharynx the subject was measured in low-background surroundings by scintillation counters collimated to record both lung and tracheal activity. A continual decline in lung activity was observed, apart from two cases where a fluctuation was recorded in the first few hours. The validity of assuming a sequence of exponential decays is queried, but, on this basis, half-times of the order of 28 min, 80 min and 65 days could be deduced for an aerosol of manganese dioxide.

The sixth session was concerned with human lung dust. H. Leiteritz, H. J. Einbrodt and W. Klosterkötter reported extensive analysis of lung residues from coal-miners and compared them with the original mine dusts. Lung dust was separated by the formamide process and material from the Saar and Ruhr coalfields was examined. The quartz content of lung dust averaged 3–5 per cent and the peaks of the particle size distributions were in the 0·6–1·1μ diameter range for lung dust and more than 6·3μ, sometimes considerably, for mine dusts.

A discussion about the desirable size-selecting characteristics of sampling instruments in relation to the observed size frequency distribution of lung dust was presented by J. Cartwright. The average of many analyses of lung dust gave a mass frequency curve with a peak at 1μ diameter and 1 per cent of the peak value at 15μ. Selective sampling devices which accepted, on the basis of sedimentation rate, 50 per cent of the 5μ diameter particles and none greater than 7·1μ, would reject some 15 per cent by mass of the average lung dust in particles greater than 7·1μ, although the number of large particles in lung dust was very small. At the same time there was a great discrepancy between the number distribution curve of particle size in lung dust compared with that of the above-mentioned sampler, which collected a much greater proportion of the coarser particles in diameters up to 7·1μ. The net effects of these opposing tendencies was that the size-selected sample contained much more dust per unit volume of air than the lung residue and it had a completely different size distribution. In spite of these discrepancies, it was claimed, in this paper, that the relationship of sample weight to lung dust weight was not very sensitive to the variations of size distribution of airborne dust which are found in British coal-mines. The continued use of the demonstrably incorrect size selection was justified on the grounds that modification towards the true lung distribution would have caused inconveniently small samples to be collected, using the current times of sampling.

W. Stober, H. J. Einbrodt and W. Klosterkötter argued that, unless the elimination mechanism failed, it would be impossible for the quantity of dust retained in the lung to exceed a certain limit during exposure for an indefinite period. The equation which predicts this limit is in agreement with the results of animal experiments. Data from human lung residues were more uncertain, although there was some indication of the same pattern of retention. Human lung clearance appeared to be much slower than that of rats. There are indications that the human alveolar clearance mechanism breaks down after a certain dust load is attained, so that there is a marked increase in the rate of accumulation of lung dust.

The relationship between the chest X-ray picture and lung dust was the subject of a paper by C. E. Rossiter, D. Rivers and associates. A multiple regression analysis of likely factors led to the conclusion that the iron content of the lung was the best index of radiological category; the source and anatomical situation of this iron remain to be discovered.

L. Ulrich and A. Juck described analyses of the lungs of silicotic miners and noted that retention of fine particles apparently increases with size reduction, though it may be obscured by the relatively low frequency of occurrence of particles below 0·1μ in airborne dusts.

Four papers followed on the dose-response relationship in pneumoconiosis. Problems encountered in the Pneumoconiosis Field Research of the National Coal Board were explained by S. Rae. Evaluations of response by X-ray, pulmonary function or respiratory symptoms each have attendant difficulties; it is considered that the standardization of lung function tests is associated with less-severe drawbacks than the attainment of consistent X-ray pictures. Respiratory symptoms require, for their elucidation, a knowledge of the contribution of inhaled coal dust to chronic bronchitis.

D. G. Beadle spoke of silicosis in the South African goldfields. Groups of men engaged in eleven different occupations were examined and the associated dust exposures were evaluated. An average dust-level of 1,280 particles per cm³ was associated with 36 per cent certifications of silicosis after working 7,500 shifts and 700 particles per cm³ with 17 per cent certifications.

Routine recording of the monthly dust exposures of each miner in the West German coalmines made it possible for M. T. R. Reisner to show that the first pulmonary changes occurred earlier when the cumulative dust exposure was greater and the average dirt content of the respirable dust was lower. High dirt content delayed the onset of pulmonary changes within 7 years, possibly on account of a beneficial action of clay which has been shown in animals. The effect of peak concentrations over and above the integrated dosage is thought to be slight.

Another paper from the National Coal Board by J. M. Rogan, S. Rae and W. H. Walton reviewed the progress of the Pneumoconiosis Field Research which covers twenty-five selected collieries. A broad correlation has been found between the prevalence of pneumoconiosis and its progression, but the relationship of pneumoconiosis to dust concentration is vague, with some indication that certain collieries have more innocuous dust than others.

The practical difficulties associated with dust sampling by thermal precipitator have caused gravimetric sampling instruments to be regarded favourably, especially as the category of pneumoconiosis appears to be related to the weight and composition of the dust inhaled. It is hoped that gravimetric sampling will be more objective than particle counting. It is satisfactory that the prevalence of pneumoconiosis in British coal-mines has been materially reduced in the past five years, especially in the younger age groups.

H. E. Ayer and J. R. Lynch distinguished between motes and fibres of asbestos dust in the air of industrial plants. Most particles are motes, which resemble other industrial dust, but the proportion of fibres increases as the processing of raw asbestos advances. The health risk of asbestos dust is due to fibres, but whether the few long fibres are alone involved, or the more common fibres, 2–3μ in length, are important, has not been decided. Good indexes of exposure could be obtained by counting the concentration

of fibres using phase contrast microscopy or by using X-ray diffraction analysis.

A small cyclone size-separator was described by H. Breuer. This prevents coarse dust from reaching a sample collected on a filter and thus the size distribution matches that of dusts recovered from the lungs of coal-miners. The dust admitted to the filter is about 50 per cent of the airborne dust at 1·5μ diameter, rising to 80 per cent at 1μ; the acceptance curve is concave upwards and has, like the alveolar regions of the human lung, a small but definite acceptance beyond 7μ. The performance is insensitive to differences between quartz, coal and shale dusts.

The relationship between concentrations measured by mass and those measured by number was discussed by R. J. Hamilton, G. D. Morgan and W. H. Walton. The ratio of mass concentration (mg/m³) (measured after size selection by sedimentation with 50 per cent penetration to the sample at 5μ diameter), to number concentration, in thousands of particles more than 1μ diameter per cm³, ranged from 6 to 33; the spread was diminished when the size selection was made more rigorous, with a 50 per cent cut at 3·5μ, and this decreased the amount of dust collected by one-half to one-quarter. When the Breuer cyclone, described in the preceding paper, was used, the selected dust was about one-quarter that passing through the sedimentation instrument, which allowed a 50 per cent penetration at 5μ. Various gravimetric sampling devices agreed with one another within about 10 per cent.

In another paper, H. Breuer described measurements of size distribution of airborne coal-mine dust. The peak size frequency of dust during coal-getting occurs between 4μ and 8μ for coal dust, below 3μ for dirt and between 2μ and 6μ for quartz. The finer dusts, rich in dirt, are associated with pneumatic stowing and blasting. The composition of the fine, or respirable, fraction of dust depends on the method of selection.

W. Walkenhorst emphasized that, since knowledge of alveolar deposition of dust is so uncertain, it is desirable to analyse the composition of a series of fractions of dust in size ranges below 10μ, rather than taking a single arbitrary fraction of so-called respirable dust. He described a sedimentation classifier which distributed the particles, according to size, on a membrane filter. This allows analysis by optical microscopy, with determination of quartz content, in fractions down to 1μ.

The advantages of a personal dust sampler were stressed by R. I. Higgins and P. Dewell, who had made a small apparatus with a chest harness which weighed only 1·5 kg. Size selection was achieved by a cyclone with 50 per cent penetration for particles a little below 5μ in diameter, for density spheres, the sample being collected on a filter. Motive power was derived from rechargeable nickel–cadmium cells and an electric motor driving a diaphragm pump equipped with a pulsation damper.

The *Proceedings* of the symposium will again be published by Pergamon Press as a book; it is hoped that this will appear in June. C. N. Davies

CLEAN AIR

CLEAN air, in the true sense of these words, does not imply simply smokeless air; there is very much more to it than this. There are other aggressive agents of air pollution the control and ultimate elimination of which are of as great importance to the health of the community as the elimination of smoke, although the latter may often be the vehicle of their dissemination. These observations, and many other matters pertaining to the effects of the Clean Air Act and its impetus on progress in solving the problems of "the fog and filthy air", were well reviewed at the recent Clean Air Conference convened at Eastbourne during October 26–29, 1965, by the National Society for Clean Air. Pre-prints of papers presented at this Conference, together with summaries released at the time, are now available (Pp. 155. National Society for Clean Air, Field House, Breams Buildings, London, E.C.4, 1965. 15*s*.). The subject of smokeless air has been briefly discussed in these columns before (*Nature*, 204, 835; 1964); much has happened since then, particularly in respect to other hazards, notably sulphur dioxide, carbon monoxide, hydrocarbon exhaust fumes, grit and dust. In this connexion, a tightening up of the Clean Air Act (1956) would seem to be a practical possibility in the foreseeable future, particularly if coupled with the formulation of a sound national fuel policy, now receiving the attention of Mr. F. Lee, Minister of Power, according to *Smokeless Air* (35, No. 134; Summer 1965. Pp. 255. National Society for Clean Air. 2*s*. 6*d*.).

The presidential address to the Conference was delivered by Sir Alan Wilson. His main points were: a survey was carried out in fifteen pairs of housing estates of similar character except that one estate of each pair was subject to smoke control and the other was not; it was found that over 18 months, in the smoke-controlled areas, average concentration of smoke was 40 per cent less than in the areas not subject to smoke control (actual figures, 100 μg/m³ compared with 140 μg/m³). Although total smoke pollution had been considerably reduced, diminution in domestic smoke had been disappointingly slow; in London, however, during the period when the relevant air-pollution measurements were made (1952–62), smoke was reduced by about 60 per cent—much better than the national average of only 35 per cent. Against this, sulphur dioxide emission had increased in the London region by about 80 per cent as compared with slightly less than 25 per cent in Britain as a whole. Sir Alan explained this by the fact that much more sulphur dioxide was dispersed into the upper atmosphere through high chimneys than smoke produced near ground-level. He went on to say: "Since one ton of low-temperature coke contains only about 75–85 per cent of the sulphur in one ton of coal, and since 15 cwt. of coke produces as much heat as one ton of coal, replacement of coal by coke reduces the sulphur dioxide by about 40 per cent". Further, "Since there is no reasonable prospect . . . of reducing the total emission of sulphur dioxide throughout the country to negligible proportions, there is only one solution to the problem of smog, namely, town planning accompanied by the control of emissions by the regulation of fuel usage". This, of course, invokes the thorny problem of a national fuel policy with which, as already stated, the Ministry of Power is now grappling.

Most industrialized towns need not be dirty towns. This was the theme of three papers describing successes with smoke control. One of these is Manchester, the history of "some form of smoke control" of which extends over 120 years, according to Mr. J. Richards, senior public health inspector of that city. At the moment, 41·5 per cent of the total area of Manchester is subject to smoke control orders; it is hoped to make most of the city smokeless by 1970. Another city is Sheffield, discussed by Mr. J. W. Batey, chief smoke inspector; as a result of the City Council's efforts, air pollution measurements between 1956 and 1964 reveal that smoke nuisance has been halved and the amount of sulphur dioxide in the air considerably reduced. The third 'success story' is Scunthorpe, where Mr. G. O. Allen, chief public health inspector, stated that although the town had not reached

a "prosperous termination" of their desired end of air pollution, they were well on the way to their ultimate goal of "blue skies and pink lungs". Incidental to the problems faced is the question of solid fuel distribution and supplies; this was discussed by Mr. E. Otty, head of Special Projects Branch, National Coal Board, and Mr. W. H. Bourne, manager, National Federation of Coke Distributors' Associations.

One session of the Conference was devoted to the subject of air pollution and mortality. Mr. A. J. Wicken, head of Health Surveys, AGB Research, Ltd., stated that the findings of a recent survey in north-east England indicated that both lung cancer and bronchitis mortality are associated with air pollution. Speaking of odour control by catalytic combustion, Mr. E. Betz directed attention to the fact that many industrial effluents were not, as yet, covered by real legislation, it being left to local authorities to try to enforce production of clean and odour-free air; he claimed that, of available methods of removing organic fume nuisances, catalytic combustion was the most efficient; this could solve odour problems in the chemical industry, in nitric acid manufacture and in the oven industry among many other applications. The Belgian method for dealing with road vehicle pollution was described by Mr. E. J. Coucke, director, Fund for Study and Research on Road Safety, Brussels. This method is based on a closely integrated system of technical inspection of motor vehicles, involving fifty inspection stations, each equipped with a smoke-meter to measure diesel smoke density. Mr. Coucke went on to describe an interesting experiment designed to reduce rate of emission of carbon monoxide by petrol engines, which was conducted with vehicles used by the Paris Prefecture of Police; this is essentially a matter of precise adjustment of carburettors aimed at the systematic reduction of the production of this toxic gas while the motor is idling. His paper on Belgian practice in the matter of pollution of air by road vehicles is comprehensive and worthy of study.

It was perhaps inevitable that in such a Conference as this, new horizons in domestic heating should command attention, and the now familiar propaganda of what each of the competitive fuel industries has to offer in various heating systems, and what the domestic consumer in the United Kingdom may look forward to in the near future to realize efficient, economical and completely smoke-free warmth in the home, was characteristically conveyed by Mr. H. R. Brewster (Shell-Mex and B.P., Ltd.) representing oil interests; by Mr. R. N. Bruce (chairman, South Eastern Gas Board), concerned with gas (not excluding North Sea prospects); and by Dr. W. Reid (chairman, Northumberland and Durham Division, National Coal Board) in discussing steps being taken to promote higher standards of home heating apparatus, hence clean air, by production of adequate supplies of smokeless fuels, by stimulating design and production of new and, as near as possible, automatic appliances, and by district heating. The case for electricity was ably put by Mr. C. T. Melling (deputy chairman, the Electricity Council).

Mr. J. Hodgson spoke on industrial aspects of dust collection and selection of proper equipment to deal with the particular factory product or plant involved; his paper dealt, *inter alia*, with methods of settling chambers, inertial collectors (cyclones), fabric collectors, wet collectors and electrostatic precipitators. On the controversial subject of chimney heights, Mr. S. C. Beaumont (chief smoke inspector, Birmingham) referred to the *Memorandum of Chimney Heights* issued by the Ministry of Housing and Local Government which, although welcomed, did not provide all the answers to problems raised by conflicting interests in this matter, and which even the Clean Air Act failed to satisfy; he claimed that ". . . for reducing pollution from sulphur dioxide, tall chimneys apparently were the present answer, but the best remedy would be the use of low-sulphur content fuels". The Conference concluded by considering some unresolved problems in air pollution. In this connexion there was an important contribution by Mr. F. E. Ireland, chief alkali, etc., works inspector of England and Wales, who described some of the difficulties still with us concerning nuisances relating to sulphuric acid, nitric acid, ceramic industries, iron and steel, non-ferrous metals, metal recovery, and lime works; he added: "The search for solutions and improvements to industrial emissions continues unabated, both nationally and internationally".

The publicity afforded to the vital subject of clean air is in itself a healthy sign. The work of this Eastbourne Conference is a credit to all concerned. Britain is facing up to the problems involved in combating air pollution, from whatever source it may derive, and it is encouraging to know that so many other countries are doing the same. The first congress of the newly formed International Union of Air Pollution Prevention Associations will be held in London during October 3–7, 1966; some details of this desirable function are given in a recent number of *Smokeless Air* (36, No. 135; Autumn 1965. Pp. 13, 18. London: National Society for Clean Air. 3*s*.).

REACTIVITY OF PHOTO-EXCITED ORGANIC MOLECULES

THE Solvay Institute for Chemistry was founded in 1913, at a time when international conferences in science were comparatively rare. Since that time, as with other aspects of the rising tide of scientific knowledge, methods of communication have become much more numerous. Discussion meetings, conferences and symposia are being organized all over the world, to promote the advancement of knowledge on broad or narrow fronts of science. At the present time, those who are concerned with such organizing have to decide a number of questions, which it is useful to keep in mind when considering the outcome of any meeting of more than purely local appeal. When the primary object is to bring together and integrate related advances in different directions, as has always been the aim of Solvay conferences, the timing of the meeting, and the choice of those eligible to attend it, need to be planned with much greater care than is needed for more omnibus gatherings, at which the main object is to promote binary and ternary collisions of minds more or less at random, but in a favourable environment. If an integrating theme is aimed at, its selection must now also pay some regard to any related discussions at an international level, held or due to be held over a period which may extend over several years. Finally, the decision whether to publish *Proceedings* of a conference, to be of real service to a much wider circle of readers than those who actually attended it, calls for responsible judgment. This may not be easy, in view of the flood of scientific literature already available.

In the broad range of possible kinds of conferences, the pattern chosen by the Solvay Institute is one of the highest selectiveness in the choice of those invited to take part in the week's discussions. Only one or two pre-printed contributions were considered each day. In the present instance these were by C. A. Coulson, R. Daudel, G. S. Hammond, E. Havinga, G. Porter, G. O. Schenk, G. M. J. Schmidt and N. C. Yang. The printed texts were not restricted in length, in order to

permit each author to indicate the present problems of his own speciality, on an extensive perimeter. In addition to the authors and the members of the Scientific Council of the Solvay Institute, a limited number of leading scientists concerned with this field were also invited to attend. On this rather exclusive basis, those present found it feasible to discuss and define their difficulties, as well as to communicate their progress, without undue pressure from other speakers more anxious to communicate, than to attempt in common any Socratic assessment of ignorance. As in the case of other recent meetings of the Solvay Institute, on inorganic structural chemistry (1956), on nucleoproteins (1959) and on energy transfer in gases (1962), the invited papers and considered discussion contributions resulting from the compact team-work of the present meeting will be published in full, as soon as possible.

At this meeting some highly suggestive theoretical considerations about photo-excited molecules attracted much discussion. Advances in theoretical chemistry indicate that interactions between excited molecules may differ markedly from the situation described by the more familiar force fields and potential energy surfaces for molecules in the ground state. Compared with these, even the analogues of van der Waals' forces are likely to be of considerably longer range between excited species. In a molecular encounter, at the stage near what would be the transition state for unexcited molecules, more intense interactions likewise call for profound modifications in their description when they refer to electronically excited species. A general enlargement of the 'molecule volume' may be anticipated, within which the distribution of electronic charge can affect the course of chemical reaction between excited species.

This general inference points to a number of novel directions for theoretical and experimental research. Quite generally, even when apparently non-bonded, the influence of neighbouring molecules on a specific molecular reaction can be much more profound, when this involves photo-excited species, than for molecules in the ground state. A particular instance of the controlling influence of neighbours, which was extensively discussed at the meeting, but for which there still remains much to be clarified, refers to photo-chemical reactions in the crystalline state, or in solid solution.

Striking solvent effects on reactions between excited molecules may be closely related to this general principle, and several instances were discussed. Again, effects of pressure in determining the relative role of alternative chemical reactions should be much more prominent and distinctive, in favourable cases, than can normally be expected for reactions in the ground state.

The complexity of molecules the photo-chemical reactions of which can now be studied experimentally has increased in recent years to a notable extent, through the development of new methods of photo-excitation, and the use of various refined methods of chemical identification of the products. This has given new impetus to highly suggestive researches on problems such as the transmission of excitation energy within a polyatomic molecule, resulting from primary absorption of photons, or from irradiation by γ-rays or particles. Regions of a photo-excited molecule that may undergo quite diverse reaction can be examined on a selective basis. In addition to the great scientific interest of researches in this general direction, the technological mastery of economic production by photo-chemical means of quite complex molecules offers suggestive possibilities; though these were not discussed at any length at the meeting, they seem likely to attract more attention to this field in the future.

Calculations of the distribution of electron densities in molecules in their ground state can now be made with considerable confidence in favourable instances. However, there was some discussion about the extent to which such density distribution can guide assessments of reactivity of excited states. Novel ideas about charge transfer between excited molecules, put forward at the meeting by R. S. Mulliken, provided further fascinating glimpses of important future work in a field which promises to grow rapidly.

A. R. UBBELOHDE

SCHISTOSOMIASIS

MALARIA has been aptly called 'public enemy No. 1' and the title 'public enemy No. 2' might well be given to schistosomiasis, a lingering and often fatal disease of man and other animals including farm stock. The disease is caused by parasitic trematode flatworms belonging to the genus *Schistosoma* or *Bilharzia*. The latter name commemorates Dr. Bilharz, who first discovered the eggs of the parasite in Egypt and who was a pioneer in research on this trematode and on the disease it causes. Species of this genus, unlike other parasitic trematodes, are unisexual and live, not in the digestive tracts of their hosts as do many parasitic trematodes, but in the blood vessels: for this reason they are often called 'blood flukes'.

It is indeed good news that the Rockefeller Foundation, continuing its policy, so fruitful in the past, of giving financial aid to studies of the grave diseases that plague the world, has decided to undertake field investigations of schistosomiasis. The Foundation has been interested in schistosomiasis for 40 years. These investigations will be carried out on the small island of Santa Lucia in the Windward Islands; the Government of the Islands has also decided to give financial aid to these investigations. Schistosomiasis causes incalculable suffering and economic loss. It is estimated that some two hundred million people suffer from it in Egypt, Africa, South America, the Caribbean Islands, India and the Near and Far East. It has crippled large human populations and also agricultural progress, for some species of these trematodes infect farm stock. Egypt has been called the 'cradle' of the disease, because formerly it was prevalent there (the eggs of these trematodes have been found in Egyptian mummies dating from 1,000 B.C.).

Schistosomiasis is a disease that especially affects agricultural peoples who work on irrigated land or irrigation systems. Man is infected by larval forms of the trematodes (cercariae) which develop in certain species of aquatic snails living in streams, ponds, lakes and irrigation systems in which he may work or bathe. The cercariae are able to penetrate human skin and enter the blood stream, which carries them to the blood vessels of the abdomen. Here they settle down and become sexually mature and lay spined eggs which work their way out of the infected person through the wall of the intestine or bladder into the faeces or urine. In so doing they cause bleeding from, and other forms of damage to, the walls of the bladder or intestine. Human faeces and urine contaminated with the eggs of the trematodes are frequently discharged into local waters. The eggs liberate larvae into the water which penetrate into snails living under water and develop inside the snails into cercarial larvae which leave the snails and can subsequently infect man.

It will be clear, therefore, that the disease is, in this sense, man-made.

Control of the disease aims at: (1) Protection of the human skin from the cercariae by wearing suitable clothing or by avoiding infected waters. (2) Destruction of the species of snails involved by means of chemical sub-

stances (molluscicides) and by measures which will make the environments of the snails unfavourable (clearing of vegetation, etc.). (3) Education of the people in the life-history of the parasite and the mode of infection, and in the need for better methods of disposal of their excreta.

Much research is needed on molluscicides and on methods of applying them, and on the habits and biology of the species of snail involved. Investigation of methods of treating infected people and on drugs suitable for this purpose is also necessary. Unfortunately the drugs so far discovered that will kill the adult trematodes in the blood vessels are compounds of arsenic or antimony, which may have serious effects on the patients treated with them. The World Health Organization* is also sponsoring work on this disease, and Prof. George Macdonald has given up his directorship of the Ross Institute of the London School of Tropical Medicine and Hygiene to lead a team based at Makerere Medical School, Kampala, Nigeria, which will work on schistosomiasis. In 1961 the Rockefeller Foundation gave a grant of 142,500 dollars to aid this work. The team will work in co-operation with the staff of the East African Medical Research Council on the disease in Africans in the area of Lake Victoria.

G. Lapage

* WHO Technical Report Series, No. 299 (price 5s., from H.M.S.O., P.O. Box 569, London, S.E.1) and *Snail Control in the Prevention of Bilharziasis* (price 30s., from the same address).

HYDATID DISEASE

A NUMBER of publications recently issued by the National Hydatids Council of New Zealand will be of interest to anyone concerned with public health.

One of these, entitled *Hydatid Disease in New Zealand**, describes the events which led to the establishment of the National Hydatids Council. It gives statistics about the prevalence of the hydatid tapeworm in New Zealand dogs and the incidence of hydatid cysts, which are the larval (bladderworm) stages of this tapeworm, in cattle, sheep, pigs and man, together with figures of the incidence of the cysts of another tapeworm of the dog, *Taenia hydatigena*. It has been estimated that hydatid disease is more common in New Zealand than in any other country, and that as a cause of death it is three times more prevalent in New Zealand than in Australia and twenty times more than in England and Wales. It is five times more prevalent in Maoris than in Europeans, and at the present time more than 20,000 New Zealanders may be infected with hydatid cysts. This is ascribed to the fact that the range and degree of moisture in New Zealand favour the survival of the eggs of the tapeworm after they have been passed out of the dog in its excreta. Pertinent is the fact that New Zealand farmers feed their dogs on uncooked offal containing the hydatid cysts, from which the adult tapeworms develop in the food canal of the dog. The pamphlet also describes early and subsequent attempts at control of the infection in man. It discusses methods of educating the public in the ways in which infection with hydatid cysts may be acquired, spread of knowledge of this kind and its effects, the general awakening of public conscience about the disease, and the formation of the National Hydatids Council.

The function of the Council is to exercise central control of measures used for the eradication of the tapeworm and of the disease caused by its hydatid cysts. It is now an offence to fail to register a dog or to bring it to one of the control centres for dosing with a drug that will kill the tapeworm. Furthermore, it is illegal to feed a dog on raw offal or to allow it to have access to such. As a result, the infection of dogs with the hydatid tapeworm has been reduced in about seven-eighths of New Zealand to 2 per cent, and in half the country to 1 per cent or less. Control of *T. hydatigena* has been less satisfactory, but there has been a considerable reduction of the rate of infection of dogs with this species. It is, however, hoped that it will be controlled by the measures taken to eradicate *E. granulosus*—the life histories of these two species being similar.

Further information about the work of the National Hydatids Council is given in the fifth annual report and statement of accounts of the Council for the year ended March 31, 1965†. The Council has also issued a valuable pamphlet entitled *Hydatids Eradication in Iceland*‡, which describes the remarkable achievement of that country, in which, during 1860–70, some 15,000–20,000 dogs heavily infected with *E. granulosus* were kept by a total population of 70,000 people, most of whom were sheep farmers living under relatively primitive conditions. In 1840 the Chief Medical Officer estimated that one person in every seven was infected with hydatid cysts. Later evidence showed that one in every four or five was infected. Methods used to control the disease included: (1) education of the public in the life history of the tapeworm and the parts played in it by man, dog and sheep; (2) taxation of dogs in order to limit their numbers—at present there is a tax of £7 10*s*. on each pet dog and 15*s*. on each working dog, and in some towns the local authorities are empowered to forbid the keeping of dogs; (3) compulsory annual treatment of all dogs with an anti-tapeworm drug at special centres; (4) enforcement of the law of 1890 forbidding the feeding of dogs on any entrails of sheep or cattle and enacting the deep burial or burning of the internal organs of sheep infected with hydatids; (5) improvements in the general hygiene of the people and in certain farm practices likely to favour the infection of man with the cysts.

A table demonstrates the dramatic decline in the incidence of human infections with hydatid cysts that followed these energetic measures. In 1904 it was possible to conclude that hydatid disease was practically extinct in Iceland and there was a corresponding decrease in the incidence of the cysts in sheep and also of the tapeworm dogs. Although *E. granulosus* and its hydatid cysts thus appear to have been eradicated from man in Iceland, *T. hydatigena* and its cysts in sheep have not been eradicated; however, their incidence has been reduced.

For readers who are not parasitologists but who require a clear and accurate account of the structure and life history of the hydatid tapeworm *E. granulosus*, the National Hydatids Council has issued a publication entitled *Parasitic Animals, with Special Reference to Hydatid Disease*§. It is well illustrated and gives an excellent account of the characteristics and life history of *E. granulosus*. Also dealt with are the relationship of this parasite with other tapeworms and the epidemiology of hydatid disease, its incidence in New Zealand and the measures designed to prevent this disease. Another table gives a comparison of the appearance, lengths and hosts of the seven species of tapeworms found in dogs in New Zealand.

G. Lapage

* *Hydatid Disease in New Zealand: An Account of the Events leading to the Establishment of the National Hydatids Council, and the Methods adopted to eradicate the Disease.* By F. S. Maclean. Pp. 48. (Wellington: The National Hydatids Council, 1965.)

† National Hydatids Council, Wellington, New Zealand. Fifth Annual Report and Statement of Accounts, year ended 31st March, 1965. Pp. 40. (Wellington: The National Hydatids Council, 1965.)

‡ *Information Bulletin* No. 15: *Hydatids Eradication in Iceland.* Pp. 11. (Wellington: The National Hydatids Council, 1965.)

§ *Parasitic Animals, with Special Reference to Hydatid Disease and its Prevention.* (Reprinted from *Biology for Sixth Forms.* Teach and Test Publications, Ltd., Otahuhu, New Zealand.) R. M. Miller and B. M. B. Harris. Pp. 36. (Wellington: The National Hydatids Council 1965.)

INACTIVATION AND INDUCTION OF ELECTRON SPIN RESONANCE-CENTRES IN TRYPSIN BY IONIZING RADIATION AND BY ULTRA-VIOLET LIGHT

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IN an investigation of inactivation on RNase by cobalt-60 γ-rays, Hunt and Williams[1] correlated the yield of one class of radicals with the changes in the radiosensitivity of the enzyme irradiated in vacuum and in oxygen, and after post-irradiation treatment with oxygen. The rate of production of "sulphur-type" radicals was correlated with the yield of free SH-groups measured by titration.

Henriksen[2] has shown that the total yields of electron spin resonance (ESR) centres in trypsin, exposed at various temperatures to different types of radiation, parallel those for enzyme inactivation under the same conditions[3]. Similar investigations have not been conducted with non-ionizing ultra-violet light. However, Kock and Mönig[4] found that ESR-centres were induced in bovine serum albumin when exposed to ultra-violet light of wave-length 254 mμ, and furthermore, that the shape of the ESR-signal of centres induced by X-irradiation in this compound changed on subsequent exposure to ultra-violet light of wave-length exceeding 300 mμ, without concomitant change of the total number of radicals.

I have carried out investigations with the view of making a comparison between enzyme inactivation and the number of ESR-centres, induced by γ-rays and ultra-violet light separately.

Twice-crystallized trypsin was obtained from Worthington Biochemical Corporation, United States. About 10 mg was placed in each of a number of quartz tubes, evacuated for 24 h and sealed off under a vacuum of about 5×10^{-6} mm mercury. One set of samples was subjected to γ-rays from a 5,000 c. cobalt-60 therapy unit (type 'Gammatron 3'), manufactured by Siemens, Germany. The dose-rate was about $2{\cdot}5 \times 10^4$ rads/min and the temperature of the samples during irradiation was kept at 0° C.

Another set of samples was subjected to ultra-violet light from a medium pressure mercury lamp (type *UVS* 500) made by Engelhard Hanovia, England. Samples subjected to ultra-violet light were irradiated with the proteins evenly distributed in the tubes, and shaken every 3 min during the exposure, in order to obtain a more uniform dose distribution in the sample material. During the exposure to ultra-violet light, the tubes were kept immersed in distilled water at a temperature of approximately 10° C. After the exposure, the ESR-signals were determined in an X-band spectrometer with transmission cavity and 110-kc/s field modulation. The spectra are recorded after phase-sensitive detection and represent the first derivatives of the absorption curve. Before carrying out the analysis in the ESR-spectrometer, the sample material was transferred to one end of the quartz tube and immersed in liquid nitrogen while the other end of the tube was heated to eliminate the ESR-centres in the quartz tube itself. The number of ESR-centres was obtained by double integration of the spectra and comparison with diphenylpicrylhydrazyl (DPPH) standards, according to the method described by Henriksen[5]. After completion of the ESR-analysis, the tubes were broken in air and enzyme activity determined according to the method described by Schwert and Takenaka[6].

It is seen in Fig. 1 that the dose-effect curves are exponential when trypsin is subjected to γ-rays, but this is not so when exposed to ultra-violet light. This is probably due to heterogeneous dose distribution within the sample material in the latter case.

It is seen in Fig. 2 that the shape of the ESR-spectrum of radicals induced by ultra-violet light has many features in common with that of the γ-ray-induced centres. The peaks in the spectra occur at the same magnetic field strengths, but they appear to be somewhat better resolved in the latter case.

Fig. 3 shows that there is a linear relationship between the number of ESR-centres induced by ultra-violet light and the corresponding inactivation, throughout the inactivation range studied. When exposed to γ-rays, on the other hand, the number of radicals per gram increases rapidly with increasing inactivation, but it would seem that a saturation is approached after prolonged exposure. Since the molecular weight of trypsin is about 23,000, the saturation value corresponds on the average to slightly less than one spin per enzyme molecule. Thus, the spins induced by ionizing radiation have a different dose-dependence from the inactivation. There may be an approximate linear relationship between the number of spins per gram and the corresponding inactivation when the latter is very small, as indicated by the dashed line in Fig. 3. Of particular importance is the fact that within the linear portions of the curves shown in Fig. 3, approximately five molecules are inactivated per spin induced by ultra-violet light, whereas only 0·25 molecules are

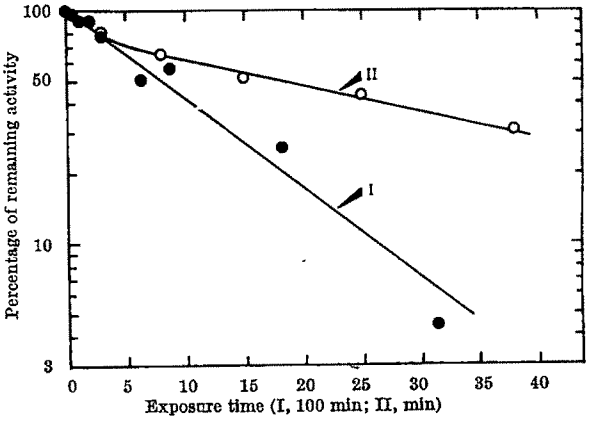


Fig. 1. Dose-effect curves of trypsin exposed to: I, cobalt-60 γ-rays; II, ultra-violet light

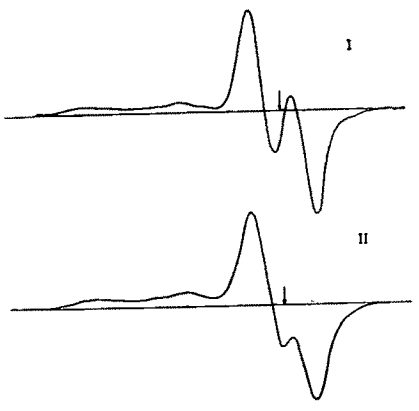


Fig. 2. Electron spin resonance spectra of trypsin irradiated with. I, cobalt-60 γ-rays; II, ultra-violet light. The arrow indicates the position of the DPPH signal

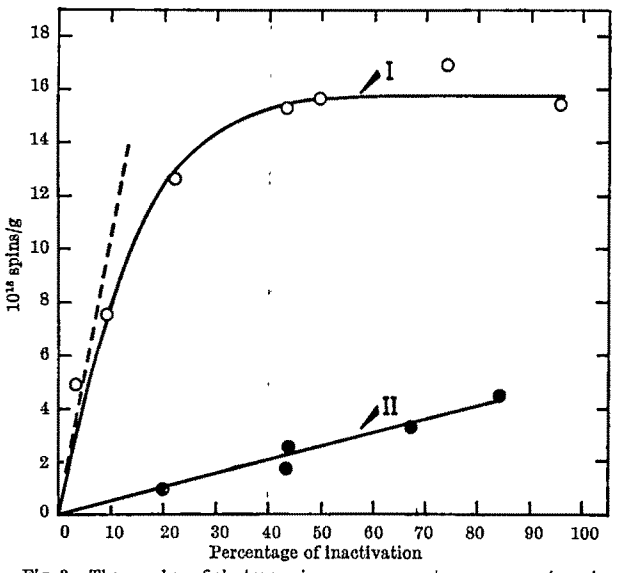


Fig 3. The number of electron spin resonance centres per gram trypsin as a function of the degree of injury, expressed as per cent inactivations: I, cobalt-60 γ-rays; II, ultra-violet light

inactivated per spin induced by γ-irradiation. This indicates that the ESR-centres induced by ultra-violet light and γ-irradiation play different parts in the processes leading to enzyme inactivation. It is possible, however, that enzyme inactivation may be induced by ultra-violet light of certain wave-lengths without simultaneous induction of spins, and/or that the spins detected at room temperature merely represent a late step in a long chain of reactions initiated by the primary absorption event, and that the inactivation is related to an earlier step in this sequence of events.

In the present investigation it has been shown that ESR-centres are induced in dry trypsin by ultra-violet light. Although the shape of the ESR-signal induced by ultra-violet light resembles that induced by ionizing radiation, it is important to note that they are not identical. A linear relationship was found between the number of spins induced by ultra-violet light and the degree of inactivation. A similar relationship does not seem to hold for ionizing radiation, except when the inactivation is quite small, where the injury per spin induced by ultra-violet light may exceed that per spin induced by γ-irradiation by more than an order of magnitude. The number of observed γ-induced radicals (X) increases with the exposure time (t) in a manner which may be described by the kinetic equation:

$$\frac{dX}{dt} = I - kX^2$$

$$X = \sqrt{\frac{I}{k}} \tanh \sqrt{kI}\, t$$

where I is the number of radicals induced per unit time by the radiation and k is the rate constant for reaction between two radicals. The present findings may have a bearing on the question of the relative roles of ionization and excitation processes in the radiation inactivation of enzymes[7].

I thank the Norwegian Cancer Society for grants, and Nansenfondet and Stangs Legat for technical equipment.

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CEMENTED FORAMINIFERAL OOZES FROM THE MID-ATLANTIC RIDGE

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DURING a recent investigation[1] of the mid-Atlantic Ridge about 22° N., several pieces of late Tertiary foraminiferal limestone were recovered. These represent at least two and probably three distinct ages, indicated by their assemblages of planktonic Foraminifera. The oldest is probably late Miocene, correlative with the assemblage associated with basalt dredged in this same area[2] on an earlier cruise. Another assemblage is somewhat younger, probably early Pliocene, and a third is possibly still younger, approaching middle Pliocene.

The present collection of cemented oozes came from a peak on the western flank of the Ridge. Several pieces of dry, buff-coloured cemented ooze, showing only traces of manganiferous coating, were collected in a free corer[3] dropped on top of the peak, at 3,000 m depth. Although the core liner was filled to 10 cm with lumps of ooze, it contained no unconsolidated material, suggesting that the cemented ooze was here exposed on the sea floor. Although there were no apparent differences among the pieces, they were individually labelled and several examined separately, in an effort to discern a stratigraphical sequence.

Three other distinct pieces were obtained in a pipe dredge, dragged from 3,300 m to almost 3,100 m depth, up the southern slope of the same peak: *Dr* 10–*P*1—a lump of cemented ooze; *Dr* 10–*P*2—a lump of cemented ooze with many inclusions of orange altered palagonite; and *Dr* 10–*P*3—a chip of fresh basalt, coated on one side with cemented ooze. Each of these pieces showed a heavy manganiferous coating. The Foraminifera from each piece were examined separately.

The species assemblages of the various samples, and the ages assigned, are shown in Table 1. The fact that all the samples are late Tertiary can confidently be established, as can be most of the relative ages; there may be some question whether the *FC* 24 samples are certainly younger than *Dr* 10–*P*1. The relative age determinations have been based on the succession of planktonic Foraminifera established for the Caribbean[4,5], and on the recently proposed Neogene zonation of Blow and Banner[6]. Considerable uncertainty still exists, however, as to how these foraminiferal successions correlate with the standard European Stages and Series boundaries. Therefore, we use here the terms late Miocene, lower Pliocene and middle Pliocene as units of convenience; these provisional age designations can be adjusted later if necessary.

The oldest samples, *Dr* 10–*P*2 and *Dr* 10–*P*3, contain similar assemblages and are essentially contemporaneous. They are correlative with the cemented ooze previously dredged in the vicinity and, therefore, are late Tertiary, probably late Miocene, in age[2]. Diagnostic species include: *Sphaeroidinella seminulina, Globoquadrina dehiscens, G. altispira, Globigerina nepenthes, Globorotalia*

miocenica and *G. tumida sensu lato*. Although the ranges of these species are not yet completely established in the published literature, their association indicates an age within the *Sphaeroidinella seminulina* zone of Blow[5] and Zone *N*-18 of Blow and Banner[6]. As proposed, both these zones are believed to represent latest Miocene[5,7].

Specimens referred to here as *Globorotalia miocenica* and *G. tumida sensu lato* are identical with those previously designated *G. fohsi robusta*[2]. This change is based on the recent revision and clarification of the *G. fohsi* and *G. tumida* groups[7,8].

The *Globigerinoides bisphaericus* group homoeomorphs include specimens which, in general form and chamber arrangement, are indistinguishable from *G. bisphaericus*, *G. glomerosus*-and *G. transitorius*, parts of a lineage that provide important index markers in the middle Tertiary. According to W. H. Blow (personal communication), however, our mid-Atlantic Ridge specimens can be distinguished by their lack of slit-like apertures, and usually less deeply incised sutures. Such forms, apparently, are not uncommon in late Tertiary samples (Blow, personal communication); therefore, their occurrences here are stratigraphically compatible with the rest of the assemblage.

Dr 10–*P*1 is characterized by a younger assemblage, although having much in common with the preceding samples, including *Globigerina nepenthes*. Absent here, however, are *Globoquadrina dehiscens*, *G. altispira* and *Sphaeroidinella seminulina*. All the *Sphaeroidinella* in *Dr* 10–*P*1 have well-developed secondary apertures and are referable to *S. dehiscens*, a species that is younger, and descendant from *S. seminulina*. Also occurring in *Dr* 10–*P*1, indicating an age younger than *Dr* 10–*P*2 and *Dr* 10–*P*3, is a keeled form of *Globorotalia punctulata*.

The *FC* 24 samples differ from *Dr* 10–*P*1 mainly in the absence of *Globigerina nepenthes*. Replacing this species is the similar, but distinct form, *G. decoraperta*. The records[9] of *G. decoraperta* thus far are from Japan, where it is associated with and ranges higher than *G. nepenthes*; thus, the presence of *G. decoraperta* along with the absence of *G. nepenthes* is suggestive of a younger age for the *FC* 24 samples.

We are aware of four additional recoveries of cemented ooze from dredges elsewhere on the Ridge. Two of these, one from the east slope of the central valley floor (45° 44′ N., 27° 43′ W.) and one from a peak near the central valley (46° 51′ N., 27° 39′ W.), consisted of several cobbles of "foraminiferal limestone"[10]. No reports of the other two recoveries have been published. One (Case 3) was dredged at 36° 31·5′ N., 33° 34′ W. between 1,800 and 1,650 m depth, on the south side at the top of a ridge on the south side of the median valley. The other (Case 4) consisted of specimens dredged at 42° 39′ N., 28° 59′ W. about 1,700 m deep and at 42° 41′ N., 28° 02′ W. about 1,850 m deep; these dredges are described (Chase, personal communication) as having been on opposite sides of a north-trending ridge that here forms the eastern side of the median valley. In each case the Foraminifera have been examined (cases 1, 2—Funnel, personal communication; cases 3, 4—Todd, personal communication) and assigned to typical Quaternary assemblages.

Table 1

	Late Miocene		Lower Pliocene	Middle Pliocene		
	Dr 10 *P*2	*Dr* 10 *P*3	*Dr* 10 *P*1	*FC* 24 1	*FC* 24 3*b*	*FC* 24 5
Globigerina bulloides d'Orbigny				×		
Globigerina decoraperta Takayanagi and Saito				×	×	×
Globigerina nepenthes Todd	×	×	×			
Globigerinella aequilateralis (Brady)			×	×	-	
Globigerinita glutinata (Egger)				×		
Globigerinoides bisphaericus Todd homoeomorphs	×			×	×	×
Globigerinoides bolli Blow	×					
Globigerinoides conglobatus (Brady)			×	×	×	×
Globigerinoides elongatus (d'Orbigny)	×		×	×	×	×
Globigerinoides obliquus Bolli	×		×	×		×
Globigerinoides ruber (d'Orbigny)	×		×	×	×	×
Globigerinoides trilobus (Reuss)	×	×	×	×	×	×
Globoquadrina altispira (Cushman and Jarvis) *sensu lato*	×	×				
Globoquadrina dehiscens (Chapman, Parr, Collins)	×	×				
Globorotalia hirsuta (d'Orbigny)			×	×	×	×
Globorotalia miocenica (Palmer)	×	×	×	×	×	×
Globorotalia tumida (Brady) *sensu lato*	×	×	×	×	×	×
Globorotalia punctulata (d'Orbigny)				×	×	×
Orbulina universa d'Orbigny	×	×	×	×	×	×
Sphaeroidinella dehiscens (Parker and Jones)			×	×	×	×
Sphaeroidinella seminulina (Schwager)	×	×				

Case 1, 2—Funnel, personal communication; cases 3, 4—Todd, personal communication.

In assessing the significance of these cemented oozes and of their relative ages, it is important to have a hypothesis of the mechanism of consolidation. Although it is frequently assumed that the only mechanisms available require sub-aerial exposure, this is not geologically correct; in the case of the mid-Atlantic Ridge dredges, such exposure seems very improbable. One alternative has recently been described[2] in the form of silicic cementation noted in material dredged from a valley near the location of the collections described here; this silicic cementation was postulated as resulting from volcanism in the area.

More generally, the sort of slight cementation with calcium carbonate, observed in the cases noted here, may be expected to result simply from uplift of an ooze to a depth 2,000 m or so less than that at which its interstitial water had become equilibrated. The sedimentological and geochemical evidence for high-pressure dissolution of carbonate sediments has recently been summarized[11]. The physical chemistry of calcium carbonate dissolution in the interstitial waters of deep ocean sediments is not yet well understood. Analytical data on such solutions[12], together with recent investigations of the effects of pressure[13] and temperature[14] on calcium carbonate solubility, allow us to estimate that in an ooze initially close to equilibrium with its interstitial water, the reduction in hydrostatic pressure and increase in temperature consequent on uplifting by about 2,000 m would permit local reprecipitation of as much as 200 mg calcite per litre of sediment. Such an amount seems not unreasonable to provide the very friable aggregations of ooze observed. As Siever[12] has summarized, saturometer[15] measurements on surface sea water everywhere show super-saturation with calcite; by contrast, interstitial waters, even when expressed from foraminiferal oozes of considerable palaeontological age, are very frequently undersaturated. Siever suggested[12] that the waters are, in fact, in equilibrium, and that the observations may indicate that the low-magnesium calcite of foraminiferal tests is less soluble than the reagent-grade calcite used in the saturometer as standard. Lacking conclusive evidence, it seems to us best to accept the sedimentological evidence for the low-temperature, high-pressure dissolution of foraminiferal oozes, and consequent reprecipitation when uplifted.

Other hypotheses may be considered: one is that the spectrum of biogenic carbonates reaching the ocean floor ranges from low-solubility, low-magnesium calcites to high-solubility, high-magnesium calcites[16], and that as the solution of the latter supersaturates interstitial waters with respect to the former, cementation occurs by grain-growth at the margins of the low-solubility tests. Such a mechanism, we believe, would result in relatively rapid cementation of oozes buried anywhere on the ocean floor; in fact, as shown by the relative infrequency of their finding, cemented oozes are not common in marine sediments, even when high rates of sedimentation would appear to favour their production. Analytical data[17] have shown consistently low magnesium content for the tests of planktonic Foraminifera. The depletion in magnesium generally shown by interstitial waters[12] also argues against

this mechanism. Finally, work in progress on the mineralogy of oozes from the mid-Atlantic Ridge in the area about 23° N. has consistently shown a well-crystallized calcite with only one mole per cent $MgCO_3$ in solid solution.

Similar arguments seem to obtain against alternative precipitations of silica or carbonate in low or high *p*H regions of a sediment[18]; if operative, this should give local cementation rather commonly in oozes; also, considering the relative abundances in plankton of siliceous and carbonate skeletons[19], silica cementation should be roughly as common as is calcitic, whereas the former appears much rarer. The one case of siliceous cementation we have observed[2] presents a combination of silica and calcite cement which could not be produced by the mechanism proposed.

We therefore still consider, then, that mixed calcite–silica cementation offers an indication of volcanism, and that the so far more typical purely carbonate cementation of oozes indicates uplift, after a significant equilibration time at water depths of 5,000 m or more. On this basis, it seems of real interest that foraminiferal limestones of Tertiary age have been recovered on the flanks of the Ridge, from relief argued[1] from other evidence to be relatively old, whereas similar rocks but of recent age have repeatedly been collected near the crest of the Ridge, from relief which appears from other evidence[1] to be much younger. It is equally interesting that in the one case where, even marginally, an age-progression can be suggested (*Dr* 10), the older limestones are those associated with the basalt surface. Should the hypothesis posed here prove generally useful, palaeontological dating of cemented oozes would provide a valuable way of setting limits to the timing of tectonic processes in the deep ocean. Careful search for other occurrences of this sort is an important part of our planning for forthcoming cruises; we urge its importance on our colleagues.

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STRAIN IN CRYSTALS DETECTED BY X-RAY

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IT is useful to know whether the crystals forming a rigid polycrystalline body are strained, especially when such bodies are formed by compaction of the powdered microcrystalline material at moderate pressures. Residual internal stress in the crystals may have an influence on its mechanical properties, particularly its stability, when subjected to cyclical conditions.

X-ray diffraction transmission Laue photographs of deformed polycrystalline materials often show asterism, the radial distortion of the spots[1,2]. This article discusses the use of this technique to detect strains in samples of hydrated cement, $Ca(OH)_2$, and analytical reagent grade NaCl in the form of compacts, powders, and wafers.

In this technique, unfiltered copper X-radiation was employed and the diffraction patterns were recorded on film held either by a flat-plate holder mounted at a distance of 4 cm from the sample or by a cylindrical cassette of 5·73 cm diameter. The samples were also examined on the petrographic microscope.

A normal portland cement was used which was hydrated by two procedures. In the first the cement was hydrated essentially to completion in an excess of water by continuous rotation in a bottle on a wheel. The hydrated cement was recovered as a powder. In the second the cement was hydrated in the normal fashion as a paste with a water/cement ratio of 0·5 by weight.

Two varieties of $Ca(OH)_2$ were investigated. One variety formed as a hydration product of cement. When hydration was carried out in an excess of water the crystals reached a size of about 1 mm. They were separated by sieving from the hydrated cement powder. The second variety of $Ca(OH)_2$ crystals was grown by the diffusion technique of Johnston[3] using $CaCl_2$ and NaOH.

The compacts were made in a mould 1·25 in. in diameter and the quantity of powder was adjusted so that after compaction the sample had a thickness of 0·25–0·5 mm when pressures in the range of 5,000–100,000 lb./in.^{2} were employed. Some compacts of NaCl were annealed in a muffle furnace at a temperature of 500° C. Wafers of dimensions similar to the compacts were cut from a cylinder of normally hydrated cement by means of a diamond saw. Powder samples were gently pressed by hand into a flat-plate holder and held in place by admixture with a small quantity of gum.

Laue photographs obtained from wafers of the cement hydrated as a paste showed marked asterism (Fig. 1*a*). This was also present on Laue photographs of the compacted and uncompacted samples of cement powder hydrated in an excess of water. A Laue photograph of the uncompacted $Ca(OH)_2$ separated by sieving from the cement hydrated in an excess of water also showed asterism (Fig. 1*b*). The film was remarkably similar in appearance to that obtained from the cement powder hydrated in an excess of water, suggesting that in this case the observed diffraction effects resulted largely from the $Ca(OH)_2$ crystals. The radial distortion of spots was even more marked on a photograph obtained from this material after it had been compacted at 20,000 lb./in.^{2}. The powder sample of $Ca(OH)_2$ crystals grown by the slow diffusion process gave Laue photographs which showed very little asterism. Laue photographs of this material after compaction at 20,000 lb./in.^{2} showed a similar amount of asterism to that observed on films of the uncompacted $Ca(OH)_2$ separated from the cement powder by sieving. A single crystal of the $Ca(OH)_2$ separated from the hydrated cement powder showed distorted spots on

discontinuous Debye–Scherrer powder rings; Laue spots showed asterism.

The NaCl (reagent grade) uncompacted powder sample gave a Laue photograph which showed some asterism. Compaction at 800, 6,000 and 20,000 lb./in.^{2} was accompanied by a progressive increase in the amount of asterism shown on the Laue photographs. Annealing the compact for 1 h at 500° C removed a large amount of the asterism from the corresponding Laue photograph. Annealing for 42 h was required before the Laue photograph of the compact showed no asterism. There was only a very slow decrease with time in the amount of asterism present on Laue photographs of an NaCl compact conditioned in a desiccator held at 50 per cent relative humidity. This result is in accord with that of Lewis and Pearson[4], who studied X-ray diffraction-line broadening in the alkali halides.

X-ray photographs of the polycrystalline samples examined showed discontinuous Debye–Scherrer rings, but these were sometimes only visible in the back reflexion region. Their absence in the front reflexion region on some photographs was attributed to absorption in somewhat thicker or denser samples. Spots were commonly distorted around the circumference of the circle. After annealing the circumferential streaks on the photographs from the NaCl compacts were replaced by small sharp spots, the extent of the replacement depending on the length of annealing time. Photographs of the $Ca(OH)_2$ separated by sieving from the cement powder showed this effect though no attempt was made to anneal the sample. The Debye–Scherrer rings were generally more continuous on photographs of compacted samples than on photographs of uncompacted samples and the size of the spots was often reduced.

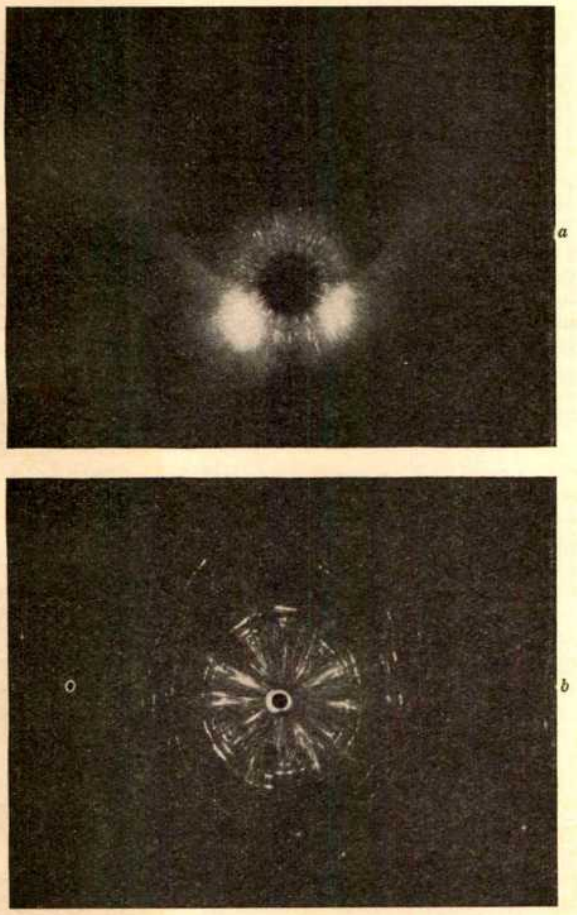


Fig. 1. X-ray diffraction photographs showing asterism. *a*, Cement wafer hydrated as a paste; *b*, $Ca(OH)_2$ sieved from cement hydrated in excess water

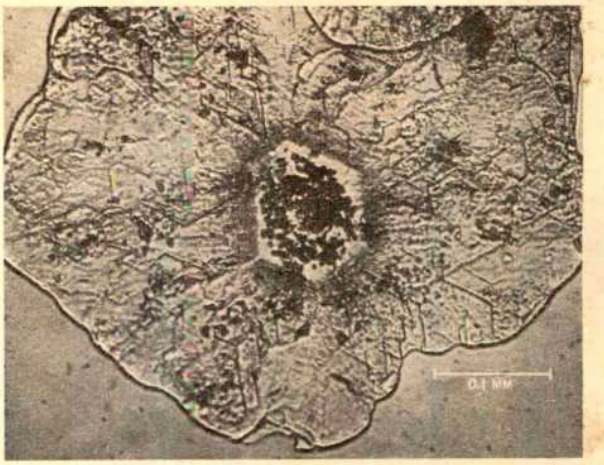


Fig. 2. Photomicrograph of oriented inclusions in $Ca(OH)_2$ separated from cement hydrated in excess water

Most of the samples were examined under low power magnification under the binocular and at higher magnification under the petrographic microscope. The $Ca(OH)_2$ crystals which grew as a product when the cement was hydrated in an excess of water were plate-like. Those grown by the diffusion technique of Johnston[3] were of different morphology. They were elongated normal to the base (0001) in the form of hexagonal prisms which were sometimes hollow. Differences in shape of $Ca(OH)_2$ crystals have been discussed by Hedin[5] in terms of differences in growth rate on different faces. He suggested that the presence of a very low concentration of impurities which absorb on growth steps such as screw dislocations may affect the growth mechanism[5]. He found that growth of prism faces began at a lower concentration of solution than did growth on the prism bases. Once growth had started, however, an increase in solution concentration caused a more rapid increase in growth rate on the basal surface than on the edges of the crystals. Predominant growth on basal surfaces favours long rod-like crystals whereas, if the growth is mainly on the edges, plates are produced. Differences in concentration between the $CaCl_2$-NaOH and hydrating cement system and much higher concentration in the latter of impurities with possible growth-interfering properties may have influenced the difference in morphology of the two types of $Ca(OH)_2$. The $Ca(OH)_2$ sieved out from the cement powder contained small inclusions symmetrically arranged in hexagonal form. Some of the inclusions within the hexagons appeared to show a spiral arrangement (Fig. 2). This is suggestive of rotation during growth and may result from a screw dislocation type of growth mechanism. Alternatively, the method of preparation may be responsible as this involves continuous rotation on a wheel. A few of the $Ca(OH)_2$ crystals contained randomly oriented lath-shaped inclusions of larger size than the symmetrical inclusions. The $Ca(OH)_2$ showed undulatory extinction on rotation of the microscope stage under crossed polars.

The classical interpretation of asterism is that it is due to diffraction from crystals in which the lattice is bent. Annealing removes the distortion and the diffraction streaks are replaced by sharp spots characteristic of diffraction from an undeformed crystal. The separation of the streaks into a number of sharp spots is attributed to polygonization or the fragmentation of the larger distorted crystal into a number of well-defined sub-grains which differ slightly in orientation.

On the basis of this view it appears that there is distortion in the crystal lattice of the $Ca(OH)_2$ formed during hydration of cement in an excess of water. This conclusion is supported by the undulatory extinction observed under crossed polars on the petrographic microscope. The similar effects observed on the X-ray photographs of the

cement hydrated as a paste suggest that distorted $Ca(OH)_2$ was also formed during the paste hydration process. The polygonization in the separated unannealed crystals implies that partial recrystallization also took place when an excess of water was present. Very little lattice distortion was present in the crystals of $Ca(OH)_2$ grown by slow diffusion. Compaction resulted in deformation and a reduction in the size of the crystals. Similar conclusions apply to the results obtained with the NaCl powders and compacts.

The evidence of distortion in the crystal lattice leads to the conclusion that the crystal was subjected to some form of stress at some stage in its formation. This was certainly the case during compaction, but such stress is difficult to account for when crystals grow from solution in a free medium, as was the case for the $Ca(OH)_2$ formed by hydration of cement in a rotating bottle. The fact that inclusions were observed in the crystals of $Ca(OH)_2$ (Fig. 2) may account for the presence of strain. When cement hydrates as a paste it is acceptable to consider that stress is applied on the growing crystal, because the products must accommodate to the spaces formed by the structure of the unhydrated particles as well as to the primary hydration products.

The important question is whether distortion in crystals is evidence for the presence of residual internal stress. If so, one would anticipate that such crystals would be more reactive, might recrystallize (age) more readily, and would be less stable dimensionally especially under cyclical conditions of temperature or moisture change, than crystals of the same size having no residual internal stress.

Certainly in the case of NaCl, the lattice distortion tended to disappear after annealing, indicating that the distortion resulted from residual internal stress. Residual internal stress in crystals may have a significant influence on the mechanical properties of a rigid polycrystalline body.

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NECROGENIC ACTION OF CARBON TETRACHLORIDE IN THE RAT: A SPECULATIVE MECHANISM BASED ON ACTIVATION

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'NECROSIS is the death of a group of cells as a result of injurious influences while still in contact with the living body[1].'' The group of cells may be very small in number or of grave and catastrophic proportions, as in acute yellow atrophy of the liver, where over 90 per cent of the parenchymal cells are destroyed.

Biochemical and histopathological studies aimed at a successful interpretation of the mechanisms culminating in necrosis have been legion. In general, attention has been concentrated on the response of experimental animals (in particular, the rat) to a relatively few poisonous substances which are readily available and which produce a consistent injury. One such substance studied *in extenso* has been carbon tetrachloride[2].

Administration of a suitable dose* (0·125 ml./100 g body-weight) of carbon tetrachloride to rats (and to many other species, though not to new-born rats or chickens) results in a severe centrilobular type of necrosis and fatty degeneration of the liver[3]. These processes induced by carbon tetrachloride, and which lead to necrosis on one hand, or to fatty accumulation on the other, appear to utilize different metabolic pathways which, to a large extent, can be dissociated from one another, at least in their terminal regions[4].

Recent investigations of the effects of carbon tetrachloride on the synthesis and secretion of hepatic lipoproteins have outlined in broad detail the mechanisms of this part of the overall disturbance[5]. However, despite such encouraging signs of advance along the one pathway, little progress has been made in unravelling the associated perturbations proceeding to necrosis. It is the purpose of this communication to consider the latter problem and to suggest a hypothesis accounting for the early damaging actions of carbon tetrachloride on the liver, actions which ultimately result in the appearance of necrosis. Obviously, during the later stages of the injury, the interplay of factors affecting the final result is complex in the extreme (for example vascular changes, hormonal influences, cell–cell interactions). These complications are not included here, where only the very early direct actions of carbon tetrachloride on liver parenchyma are considered. Any hypothesis embracing such effects should supply satisfactory answers to certain basic questions, the more important of which are formulated below and later discussed.

Basic questions concerning the necrogenic activity of carbon tetrachloride. (1) Are the toxic properties of carbon tetrachloride on rat liver due to carbon tetrachloride itself, or is a metabolite, or metabolites, of this substance the active agent? (2) Is the action in the rat direct on the liver or do effects at distant sites have significant importance? (3) Why is the liver necrosis central in nature? (4) If the effect of carbon tetrachloride on the liver is a direct action on the parenchymal cells, what is the intracellular locus of the primary disturbance? Is it diffuse, involving several intracellular components concurrently, or sharply localized on one? (5) Why are new-born rats and certain animal species (for example, chickens) much less sensitive to carbon tetrachloride than adult rats? (6) What are the mechanisms involved in the protective action of the wide variety of substances and treatment shown to be active in diminishing the extent of necrosis following carbon tetrachloride administration?

Several difficulties arise in attempting to answer these questions so long as the hypothesis of the mechanism of action of carbon tetrachloride is based on the belief that its action is solely that of a lipophilic solvent. For example, (*a*) the central location of necrosis while the highest concentrations of carbon tetrachloride in blood reaching the liver enter the portal tracts first; (*b*) the much greater damage in the first few hours to the endoplasmic reticulum, whereas other intracellular particles, also bounded by lipid membranes, apparently suffer much less early disturbance; (*c*) the resistance of the new-born rat although carbon tetrachloride has been shown to reach the liver; (*d*) the restriction of severe necrosis to the liver whereas much higher concentrations of carbon tetrachloride accumulate in other tissues; (*e*) the dissociation of the necrosis and of the accumulation of fat brought about by various experimental procedures. It is suggested in this communication that such difficulties can be overcome by a mechanism based on the concept that, before carbon tetrachloride can manifest its latent necrogenic properties, it has to undergo an activation step.

* Within certain limits the degree of necrosis produced by carbon tetrachloride is proportional to the amount administered either orally or by ventilation. In our colony increasing the oral dose above 0·125 ml. carbon tetrachloride/100 g body-weight has little effect on the final extent of central necrosis, and we shall refer to this level as the standard dose.

Metabolism of carbon tetrachloride. Several reports[6,7] in the past few years have indicated that, after being fed to rats, carbon tetrachloride is metabolized to a limited extent. Following administration of ^{14}C-carbon tetrachloride, ^{14}C-label can be detected in expired air as carbon-14 dioxide and as ^{14}C-chloroform. There is evidence that carbon tetrachloride (or its metabolites) is quickly bound to material in the liver. Following administration of ^{14}C-carbon tetrachloride to rats, material containing ^{14}C-label becomes tightly bound to liver protein. The amount was equivalent to 1 μmole carbon tetrachloride/g wet-weight liver protein[8]. Since only a small proportion (about 2 per cent) of administered carbon tetrachloride enters the liver, this suggests that virtually all this becomes combined with liver material and is not present simply as a dissolved solute in fatty material.

However, none of these data provides any information as to whether the metabolism of carbon tetrachloride is important for its role as a liver toxin, or whether it is a side reaction of little relevance to the problem under consideration. This is obviously a key question, to be answered before one can hope to clarify the reactions involved in the necrogenic activity of carbon tetrachloride.

One way of investigating this question in an indirect manner is to consider the relative necrogenic activities of the series of halogenated methanes in relation to their known ease of chemical decomposition which, in such a closely related series, and in a particular animal species, may be expected to be related to the likelihood of their metabolism *in vivo*.

The carbon–fluorine bond in aliphatic hydrocarbons has a higher bond dissociation energy than a carbon–chlorine bond present in a similar structure[9]. In carbon tetrachloride the bond dissociation energy for a C–Cl bond is 68 ± 3 kcal/mole whereas in carbon tetrafluoride the C–F bond dissociation energy is 121 ± 4 kcal/mole. In $CClF_3$ the C–Cl bond dissociation energy is 83 ± 3 kcal/mole, a substantial increase compared with carbon tetrachloride itself. No values are apparently available for trichlorofluormethane but it can be expected that, even here, the C–Cl bond is less easily broken than in carbon tetrachloride. As a consequence it can be expected that trichlorofluormethane should be rather less easily decomposed (metabolized) *in vivo* than carbon tetrachloride.

Trichlorofluormethane is not appreciably toxic to rats. A large dose was found to give no decrease in liver $NADPH_2$ and no increase in the activity of certain serum enzymes[10]: changes characteristic of carbon tetrachloride intoxication. Rats maintained in an atmosphere of 50,000 p.p.m. trichlorofluormethane for 30 min did not develop liver necrosis[11].

What significance do these observations have in relation to the mechanism of action of carbon tetrachloride? One obvious interpretation is that at least some of the administered carbon tetrachloride has to undergo a chemical transformation before its necrogenic potentialities are fully revealed. The increased stability† of the trichlorofluormethane molecule appears to prohibit this possibility to any significant extent. Further indications that carbon tetrachloride alone is inadequate for the development of necrosis are mentioned later. Of course, the necessity for an activation step does not mean that the ensuing damage is not modified nor even greatly extended by the presence of the non-metabolized carbon tetrachloride itself.

The idea that some form of transformation is necessary to explain the hepatotoxicity of carbon tetrachloride has occurred independently to several other investigators, but the full implications of such a reaction to the problems associated with the necrogenic action of carbon tetrachloride on the liver do not appear to have been discussed. Butler[7] suggested that conversion to an ionized form, possibly a free radical, was important. Glynn[12], Fassett[13] and Reynolds[53] have suggested the probability that some form of metabolism is necessary. At present, however, no direct evidence is available concerning the mechanism by which carbon tetrachloride is activated; it need not, however, be a heterolytic process as suggested for certain lachrymatory halogeno-compounds[14]. In fact, if 'activation' occurs in a lipid-rich membrane, in conjunction with an endogenous free radical-producing process, a homolytic, non-ionic, bond scission to produce a free-radical product would appear the more favoured.

In connexion with the suggested activation stage it is important to note that high concentrations of carbon tetrachloride can be detected shortly after administration in such tissues as bone marrow, brain and adipose tissue; significant amounts are found in liver, kidney and muscle[8]. In the rat, however, extensive necrosis occurs only in the liver. If the toxic properties of carbon tetrachloride were due solely to its lipophilic nature and, as a consequence, to unspecific damage to lipid-rich structures, it is strange that those tissues which accumulate it most readily do not suffer the most severe damage. Clearly the indications are that some more specific interaction is necessary for necrosis to develop. The simplest explanation is that carbon tetrachloride is converted to an active form only in those tissues which afterwards become extensively necrotic.

Direct action on the liver. The question arises: are the effects on the liver due to a primary effect of carbon tetrachloride on that organ, or is the carbon tetrachloride provoking a response in some other tissue which then induces the liver changes observed?

The development of satisfactory isolated-liver perfusion techniques[15] has enabled direct tests to be made concerning the effects of carbon tetrachloride and chloroform on the liver (the relationship in chemical structure and properties, and the close similarity of the fully developed hepatic lesions, strongly suggest that carbon tetrachloride and chloroform must act by way of very similar, if not identical, primary mechanisms). Although experiments have not been sufficiently prolonged after infusing carbon tetrachloride or chloroform to demonstrate central necrosis, there is adequate evidence that in such preparations similar changes to those occurring in the intact living animal are occurring in the isolated liver. Decreased cytoplasmic basophilia, disorganization of the endoplasmic reticulum, disturbances in lipoprotein secretion and in protein synthesis have all been demonstrated[16]. It would appear justified, therefore, to conclude that many of the effects of carbon tetrachloride on the liver reflect a primary action of the poison on this organ. It is likely, however, that such changes are themselves modified later by disturbances set into motion elsewhere.

Centrilobular distribution of necrosis. The central location of necrosis has been ascribed to the rapid development of a central ischaemic anoxia[17]. However, although this attractive hypothesis is probably of considerable relevance in later stages of the injury, direct measurements of hepatic blood flow in the first few hours after poisoning have failed to detect significant changes[18]. Further, in an elegant experiment, Brauer *et al.*[19] showed that reversing the course of blood flow through an isolated rat liver preparation (for example, afferent by way of the central vein; efferent through the portal vein) did not affect the central location of early damage caused by chloroform. This last-mentioned finding is of considerable importance, and, moreover, indicates that the 'protective' action of hyperoxic conditions[20] (and the aggravating effect of hypoxia) on the necrosis induced by chloroform and by carbon tetrachloride may be related more to the electronic structure of oxygen (for example its biradical structure) than to its partial pressure in the afferent blood. In a recent

† Until quantitative data on bond strengths, etc., and results with isolated liver preparations are available for trichlorofluormethane, it is impossible to be sure that the increased stability alone is sufficient to account for the relative non-toxicity. Other factors may be of importance *in vivo*, such as an increased rate of elimination; a decreased central uptake; and different solubility properties in intracellular lipids.

discussion of the role of vascular changes in the primary phase of liver injury caused by carbon tetrachloride, Brauer[21] wrote: "Taken together, the circulatory observations, the biochemical data, and the isolated organ experiments indicate clearly that the primary liver lesion resulting from exposure to chloroform or to carbon tetrachloride is independent of any vascular changes, and indeed precedes these in time by several hours, if indeed haemodynamic changes are at all a regular accompaniment of this type of liver injury".

Several other factors may contribute to the central location of the developing necrosis. First, carbon tetrachloride may be preferentially removed from the blood primarily at the centre of the lobules, as are several dyes. In an important investigation, using autoradiography following dosing with ^{14}C-carbon tetrachloride, Rogué and Fèdorko[22] have shown that preferential central accumulation of carbon tetrachloride does in fact occur.

It has already been suggested that an important part of the reactions leading to necrosis is an 'activation' or transformation of carbon tetrachloride. If this reaction were also concentrated in the centrilobular region the effect in terms of cell damage would be synergistic with a preferential central uptake of the poison.

There are numerous examples in the literature of a lobular heterogeneity of enzyme activity obtained by both histochemical and micro-dissection techniques[23]. As a particular example, flavin-linked reactions appear to be concentrated in the central region of the liver lobule. Flavin-coenzymes are known[24] to undergo free radical stages during enzyme-catalysed dehydrogenation and could conceivably act as nidus points for the homolytic cleavage of the C–Cl bond.

Intracellular localization of initial damage. Investigations using ^{14}C-carbon tetrachloride have shown that the ^{14}C-label quickly reaches a high concentration (expressed as mg carbon tetrachloride/mg protein) in the mitochondria and in the endoplasmic reticulum[25]. Thus there is evidence of early accumulation of the poison in lipoprotein-rich membrane systems.

Electron microscopical examination of liver sections from poisoned rats revealed early swelling, disorganization and degranulation of the endoplasmic reticulum but no equivalent damage to the mitochondria, particularly in the central zone, has been observed[26,27]. Biochemical studies have likewise shown that early alterations in the microsome fraction take place. Appreciable changes in the ribosomal population occur 30–60 min after oral dosing with carbon tetrachloride[27]. During this period there is a large decrease in microsomal glucose-6-phosphatase activity[28] and in drug detoxication enzyme systems[29]. Early changes in microsomal NAD(P)-neotetrazolium reductases and in inorganic pyrophosphatase activity have also been found[30].

No similar early biochemical changes have been reported to occur in mitochondrial components (excepting a transient calcium influx)[26] despite the presence of appreciable concentrations of carbon tetrachloride in these organelles. Mitochondria retain citrate[31] and nucleotides[30] until relatively late in the injury, and liver temperature shows no early rise as would be expected from a generalized uncoupling of oxidative phosphorylation[18]. It is worth noting at this point that the early mitochondrial damage reported in histological studies is mid-zonal[32] and not in the central region where necrosis ultimately develops. It is also important to remember, when evaluating results obtained on homogenates, that the data represent a summation of the values pertaining throughout the lobule. What may be a large change in a few cells around the central vein may be hidden by there being no change (or even the opposite tendency) in the remainder of the cells in that lobule.

Apparently the earliest changes‡ produced by carbon tetrachloride in liver cells occur in the microsomal fraction. If activation of carbon tetrachloride is required for the overall injury, then it would appear likely that this process occurs in the endoplasmic reticulum. If the activation stage were a homolytic fission of a C–Cl bond, then systems in the membrane phase involving the controlled production and utilization of free radicals could act as initiators of a new, uncontrolled and powerfully deleterious free radical chain. There are several such natural processes which are believed to occur by way of a free radical stage in the microsomes and which could so act. (For example, the NAD-ascorbate oxidase system[33]; enzyme reactions involving flavins[24]; and the $NADPH_2$–ADP–iron catalysed lipid peroxidation sequence supposedly closely related to the detoxication enzyme systems of the endoplasmic reticulum[34].) It is suggestive that inhibitors of this latter reaction also reduce the extent of central necrosis and/or other toxic manifestations following the administration of carbon tetrachloride: for example, dimethyl-*p*-phenylene diamine[35]; *SKF 525A*[30]; vitamin E[35]. Further, the $NADPH_2$–lipid peroxidation system is only weakly active in liver from new-born rats[30] which are markedly resistant to liver damage induced by carbon tetrachloride[41].

In an interesting report, Tappel and colleagues[36] have shown that intracellular particles bounded by a double lipid membrane (mitochondria) are far less susceptible to disorganization induced by free radicals than are systems bounded by a single lipid membrane (lysosomes). In fact, for equal areas of membrane, approximately 500 times more radicals were required in the first instance than in the latter. It has already been mentioned that carbon tetrachloride is quickly concentrated both in the mitochondria and in the endoplasmic reticulum but that mitochondrial damage is apparently far less severe in the early stages. It is difficult to understand this difference in the degree of damage if carbon tetrachloride is the active agent. However, activation to a free radical form with a subsequent damage can be seen from the results of Tappel *et al.* primarily to favour damage to the endoplasmic reticulum which is limited by a single membrane structure. No oxidative enzyme systems involving free-radical intermediates have been detected in lysosomes, and as a consequence activation of carbon tetrachloride presumably does not occur in these particles. This could provide an explanation of two findings; first, that little damage to the lysosomes occurs during the early phase of carbon tetrachloride intoxication[37]; and secondly, that low concentrations of carbon tetrachloride added *in vitro* to purified lysosomal preparations do not induce rupture, unlike the case with homogenates, where activation can occur in neighbouring mitochondria and microsomal particles[38]. An activation mechanism can thus offer an explanation as to why different types of intracellular lipid-bounded particles are damaged in differing degrees.

It is of interest in view of the proposed free-radical mechanism to note that $NADPH_2$ is very unstable in solution when exposed to a radical-producing system[39]. This might have some relevance to the rapid drop in $NADPH_2$ which occurs after carbon tetrachloride dosing[40].

Resistance of new-born rats. Carbon tetrachloride apparently produces little or no signs of classical necrosis if fed to rats less than about 6 days old[41]. This suggests that new-born rat liver is deficient in some property vital for the toxicity of carbon tetrachloride, and points yet again to the conclusion that the mere physical presence of carbon tetrachloride is not enough to provoke necrosis. On the basis of previous discussion, the lack of toxicity could be due to a lack of activating enzyme resulting in a failure in the formation of the metabolite of carbon tetrachloride which is the active structure responsible

‡ In general, it would be wrong simply to equate earliness of effect with importance of result. Early (possibly reversible) changes may under certain conditions be innocuous compared with some later and irreversible process. With carbon tetrachloride, however, the early damage to the endoplasmic reticulum is very extensive, and many of the changes are prevented by the administration of 'Phenergan', so that it is reasonable to believe that the processes culminating in necrosis have their origin in these early changes in the endoplasmic reticulum.

for the onset of necrosis. There are considerable precedents for such a suggestion. Many enzymes are missing or at most are present to a very limited extent during the first few days of life; in many cases enzyme activity increases rapidly some 2–10 days after birth. It seems plausible that the onset of the necrogenic property of carbon tetrachloride is connected with the rapid appearance of an enzyme activity provoking its activation.

Protectors. It has long been known that various substances which, by themselves, are relatively innocuous, when given together with carbon tetrachloride, either profoundly increase the severity of the ensuing lesion or will so modify the course of the intoxication that liver cell necrosis is greatly diminished[42].

A representative list of protective agents§ is included in Table 1; it can be seen that the substances fall into several groups. In group (*A*) Rees and colleagues[44] have shown that there is a close relationship between protection against necrosis and the physico-chemical properties of the substances concerned. Only those substances which are amphipathic cations at neutral *p*H were effective in reducing the extent of necrosis. Such a relationship does not, however, hold for substances in other groups of Table 1; for example, selenium dioxide, colloidal carbon and molecular oxygen. Clearly the inference must be that if all these substances with such widely disparate chemical structures really do inhibit the development of necrosis other than by secondary mechanisms such as a lowering in body temperature[43], then they are not acting on one locus of disturbance: several mechanisms of protection must in fact be operative.

Table 1. Treatments and Substances Reported to Protect Against Liver Necrosis or other Toxic Manifestations Induced by the Administration of Carbon Tetrachloride

Treatment	Refs.
A. Amphipathic cations	
for example, phenothiazines	44
cetab	
nupercaine	
B. Non-specific inflammation?	
for example, sub-cutaneous injections of carbon, xanthine, etc.	55
C. Lowering in body temperature	
for example, cordotomy	43
adrenalectomy	
D. Antioxidants, chain terminators	
for example, vitamin E	
dimethyl-*p*-phenylene diamine	35
selenium dioxide	
oxygen?	20
cysteamine	30
E. Inhibition of microsomal activation step?	
for example, phenothiazines, *SKF*-525*A*	30,34,44

Such a conclusion is only to be expected if the previous discussion were correctly based. For instance, if the location of necrosis is dependent on the co-operative action of central uptake and central activation, inhibition of either process will afford some measure of protection. Further, since each process is probably the sum of several events, it is presumably possible to interfere with each in more than one manner. This can best be illustrated in relation to the proposed mechanisms for carbon tetrachloride activation.

Fig. 1 gives a scheme which follows the views already propounded. Carbon tetrachloride is 'activated' to a free radical form (assumed to be $CCl_3\cdot$ for the sake of argument) by interaction with a normally occurring homolytic process under metabolic control. The free radicals so formed then interact with neighbouring lipid-rich material causing disturbance in structure and function. Protection against the overall process could be obtained by blocking the endogenous radical system; by removal of the $CCl_3\cdot$ radicals preferentially by combination with excess added acceptor (for example, the protective action of antioxidants); by providing a competitor for carbon tetrachloride in its reaction with the endogenous radical chain yielding a less-toxic product; by promoting the removal of $Y\cdot$ through an increase in the rate of the endogenous reaction (oxygen effect ?); or by unspecific action as in the case of cordotomy where a general decrease in body temperature slows down metabolism overall[43]. The properties required of substances in these five types of interaction could clearly be different.

It can be seen that the acceptance of an activation stage as a necessary event in the development of liver necrosis following the administration of carbon tetrachloride enables a scheme of the above type to be formulated in which a plurality of mechanisms can be envisaged for protective agents. So long as carbon tetrachloride was believed to exert its effects by way of an unspecific lipophilic action, it was difficult to visualize how such a wide range of protective agents could all be effective. Activation provides a key to understanding such a complex sequence of events.

One characteristic of chain reactions is that following initiation there is a lag phase which is greatly prolonged by the addition of chain terminators; ultimately there is a very rapid increase in reaction rate. This behaviour has similarities with the histological development of liver necrosis induced by carbon tetrachloride, which, in its later stages (12–18 h), develops with an explosive violence. This necrogenic reaction can be temporarily inhibited by a suitable dose of a phenothiazine drug such as 'Phenergan'[45]. The phenothiazines are known to be capable of forming free radicals[46] and possess the —S—C = C—N— grouping which has powerful quenching properties[47]. 'Phenergan' inhibits flavin-linked enzymes[48], probably by way of a free-radical stage, and inhibits very strongly the $NADPH_2$–lipid peroxidation process present in liver microsomes (50 per cent inhibition at 30 μM final concentration, *in vitro*[30]). Although part of the long-term protective influence of phenothiazines on rats treated with carbon tetrachloride is no doubt due to an effect in lowering body temperature[49], there appears ample evidence that more direct protective actions are operative in the early stages. The most likely is an interference with free radical producing systems in a lipid interface.

The preceding discussion has led to the suggestion that an important aspect in the necrogenic activity of carbon tetrachloride on rat liver is a so-called activation stage in which either carbon tetrachloride itself is metabolized to a powerful destructive free radical form or, by its presence, induces the formation of other and equally deleterious radicals. The intracellular production of significant quantities of extraneous free radicals (that is, radicals not associated with a controlled metabolic sequence) can be expected to produce severe damage near the site of formation. In the presence of lipid-rich mem-

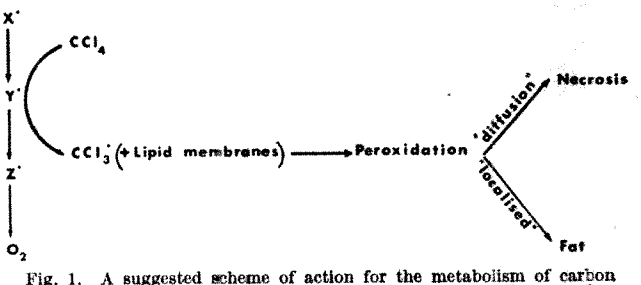


Fig. 1. A suggested scheme of action for the metabolism of carbon tetrachloride *in vivo* via an interaction with a naturally occurring homolytic chain reaction ($X\cdot$, $Y\cdot$, $Z\cdot$) in the endoplasmic reticulum. As a result of such an interaction, extraneous radicals are produced (shown here as CCl_3) which, in the presence of lipid-rich materials and oxygen, result in peroxidative damage to intracellular membranes. At some point in this reaction the processes leading to necrosis and to the accumulation of fat probably diverge. The possibility that one mechanism for such a divergence of effects can be based on the extent of diffusion of the free radicals produced by the metabolism of carbon tetrachloride is speculative. Furthermore, it is not intended to suggest (see text) that an activation process, such as depicted in Fig. 1, is sufficient to produce the full consequences of carbon tetrachloride intoxication, but only that an activation step is a necessary feature of the injury

§ The criteria used in assessing protection have varied widely[42]. In some cases it has been effectiveness in reducing death rate; in others, the effectiveness in preventing rises in serum enzyme-levels; in others, the effect on the histologically assessed parameters of fat and necrosis. Since the demonstration[43] that a decreased body temperature can effectively inhibit liver damage caused by carbon tetrachloride, it is evident that many early reports claiming protection for a variety of materials will have to be checked for possible variations in body temperature.

branes and of oxygen, lipid peroxides could be formed with a consequent loss of structural function and organization. Damage to mitochondria[50], lysosomes[51] and the endoplasmic reticulum[34] has been reported following the experimental initiation of lipid peroxidation. This type of consequence appears the most likely following the initiation of extraneous free radical production in the cytoplasm. It has already been mentioned that the probable sites of extraneous free radical formation following carbon tetrachloride administration are the mitochondria and the endoplasmic reticulum. The results of Tappel *et al.*[36] indicate that a structure bounded by a single lipoprotein membrane (for example, the endoplasmic reticulum) would be far more sensitive to damage induced by free radical attack than a double-layered membrane as in the mitochondria. A further and probably important factor favouring preferential early damage to the endoplasmic reticulum is the concentration of endogenous antioxidant material (for example, tocopherol and ubiquinone) in the mitochondria[52]. This can be expected to stabilize these particles against the type of mechanism suggested here. Finally, it is worth mentioning that, in agreement with speculations made here, and since this manuscript was prepared, Recknagel and Ghoshal[54] have reported an increased lipid peroxidation in liver microsomal fractions isolated from rats poisoned with carbon tetrachloride.

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AGGREGATION OF TUMOUR CELLS

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Moskowitz[1,2] and Moskowitz and Amborski[3] have investigated the formation of aggregates by various mammalian tumour cells in tissue culture. They found that agitation by shaker or roller drum induced aggregation in the cell lines investigated, as did the use of a protein-free medium or the addition of equine β- and γ-globulin fractions to stationary cultures. These aggregates grew to as much as 5 mm in diameter, when the surface became grooved and the aggregates divided. Because these aggregates have a differentiated surface membrane and an inner cell mass of less oriented cells, Moskowitz[2] suggested that they be called 'aggregens', which he states "fulfil all the criteria which are applied to the description of an independently living organism. From another point of view, they can be regarded as free-living tumours". Moskowitz distinguishes between

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'clumps', which are accidentally formed groups of cells, and 'aggregates' or 'aggregens', which represent at least a partial expression of the developmental potential of the cells. The purpose of this article is to report experiments on aggregation of several more lines of tumour cells.

Six cell lines from the RPMI collection were examined: RPMI Nos. 2650, 2650-*LN*-2, 2650-*R*, *T*-3-1, 6410, and *WI*-38. The first three are all derived from a pleural effusion of a patient with squamous-cell carcinoma. Chromosome investigations[4] show the normal diploid count of 46, although human tumour-cell lines are usually aneuploid, most commonly near-triploid. Reasons for considering the cells of this line to be tumour cells, rather than normal cells selected from a mixed population by the culturing technique, were summarized by Moore and Sandberg[4]. Briefly, they include the following: successful heterotransplantation to conditioned mice; resemblance of the heterotransplants to the original tumour; injection into volunteer patients and resemblance of the resulting growth to the original tumour; and, finally, successful culturing through many passages. The line is now in its seventy-fourth passage, and it has been maintained in culture for three years.

Hayflick and Moorhead[5], in an exhaustive investigation of human diploid cell strains, found that they could not be maintained for more than a limited (though perhaps large) number of passages, nor for much more than a year. They consider the alteration of a cell strain to form a potentially immortal cell line to involve mutation to aneuploidy. Although RPMI 2650 is diploid, the genotype may have been significantly altered in other ways. In view of the age of this cell line, it seems probable that it could be maintained indefinitely. Collectively, the reasons cited comprise a strong presumptive case for its malignant character.

RPMI 2650 is the unmodified cell line. In stationary cultures, it forms a good monolayer; and in suspension cultures, the cells are well dispersed, with only occasional small clumps. RPMI 2650-*R* was X-irradiated four times, the total dosage being 7,000 r. It grows more slowly than 2650, and is much more difficult to maintain. In stationary cultures, the cells grow in clumps or aggregates of a few cells to several hundred cells (Fig. 1). These have very little tendency to spread over the glass. In suspension cultures, they form aggregates that tend to fall out of suspension. Viability tests show that almost all the dissociated cells are non-viable. The chromosomes again number 46, but one of the smallest chromosomes (21 ?) is deficient and resembles the Philadelphia chromosome.

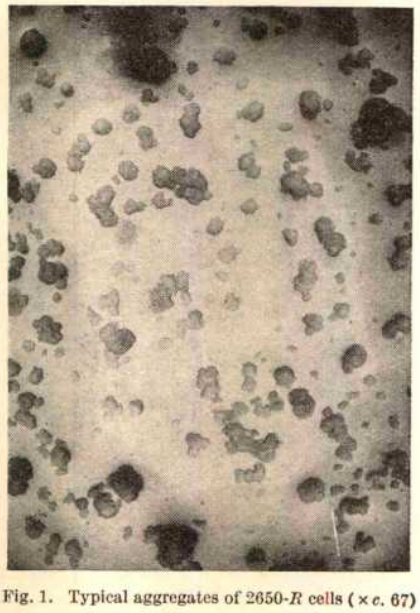


Fig. 1. Typical aggregates of 2650-*R* cells (×*c.* 67)

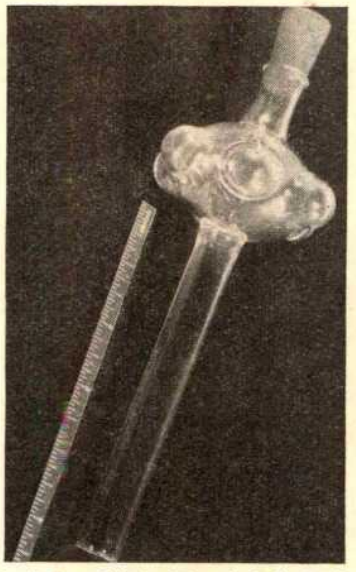


Fig. 2. A Steward flask. The ruler indicates the size

RPMI 2650-*LN*-2 is a spontaneous mutant of 2650, one that suggests 2650-*R* in some of its characteristics. In stationary cultures, it adheres to the glass in a reticular pattern, interrupted by occasional aggregates, which, however, have a more regular appearance than do those of 2650-*R*. In suspension cultures, it forms many aggregates that tend to settle out. RPMI *T*-3-1 and 6410 are both myeloid leukaemia cell lines that tend to form aggregates spontaneously in stationary cultures. Finally, *WI*-38 is a line of normal human embryo fibroblasts.

Media used include the following: RPMI 906 with foetal calf serum varying from 0 to 30 per cent; McCoy's medium 5*A* with either 10 or 20 per cent serum; RPMI 1629 with 30 per cent serum; RPMI 1630 with 5 or 10 per cent serum: or RPMI 1595 with 5 per cent serum. Stationary cultures were grown in *T*-flasks of 30-ml. capacity with 6 ml. of medium, or of 60-ml. capacity with 12 ml. of medium. Some of these were agitated by placing them on a Clay-Adams rotary shaker set for its lowest speed (90 r.p.m.). Others were grown in a new type of vessel consisting of a 50-ml. bulb with six small wells around the circumference (Fig. 2). The bulb is fused to a glass tube, by which it is mounted on a roller drum. As the drum rotates (about 1 r.p.m.), the medium is poured from one well to the next, thus maintaining constant, mild agitation. The volume of medium used was 5 ml., sufficient to fill two wells at a time. Because these vessels are based on similar, though much larger, flasks used in the laboratory of Prof. F. C. Steward for culturing plant cells, we call them 'Steward flasks'.

After aggregates were well established, the strength of cell adhesions was tested by putting them on a Boerner shaker, either in the growth medium, in EDTA, or in trypsin, and trying to disrupt them. Cell counts in the supernatant served as an index of dissociation of the aggregates.

Because of the importance of the part played by mesoderm in the organization of the embryo, mixed cultures of *WI*-38 (fibroblasts) and 2650 (epithelial cells) were made. A similar test was made with another fibroblast line (RPMI 4269) obtained from a tumour.

The effects of various concentrations of protein in the medium on aggregation were determined by growing cultures in medium 906 with foetal calf serum added in concentrations of 0, 5, 10, 20 and 30 per cent. The cell lines examined were 2650, 2650-*R* and *WI*-38. The medium was not changed during the course of the experiment; but cultures were fed by the addition of glucose solution.

WI-38 cells, a fibroblastic line, were badly damaged by culturing on the protein-free medium. After five days, the

cultures were sparse, the cells looked moribund, and there was no tendency toward formation of aggregates. After 10 days, the cells were much shrunken, and the protoplasm appeared scant. Cells tended to draw into cords, which formed a network on the glass. Many sloughed off into the medium, and these were all dead, as indicated by uptake of trypan blue. The five cultures in this series were discarded after 10 days. Controls on McCoy's medium with 10 per cent foetal calf serum grew vigorously throughout the experiment.

In a preliminary experiment to test the effect of protein concentration on cells of line 2650, five cultures of 2650 were set up in medium 906 without serum and five more in 906 with 5 per cent foetal calf serum. Similarly two cultures of 2650-*R* were set up in protein-free 906, and three in 906 with 5 per cent foetal calf serum.

After one week, the 2650-*R* controls were well established and showed the growth pattern typical of the line. They continued good development throughout the experiment. The experimentals, however, showed insufficient growth to establish the typical pattern. After 10 days, they were becoming sparse; and after two weeks, the cultures were dead. The 2650 controls grew rapidly and became heavy during the experiment. Those on the protein-free medium remained light. Some aggregates formed and sloughed off into the medium, but they did not grow. They were discarded after 18 days of 'growth'.

In another experiment with 2650, three cultures each were grown in medium 906 with foetal calf serum supplements of 0, 5, 20 and 30 per cent. Those in the protein-free medium followed the course already described. Clumps of cells were formed, but no aggregates. After eight days, these were discarded. Meanwhile, the controls in 906 with 5 per cent of foetal calf serum had formed a good monolayer, which was maintained throughout the experiment, although excess cells sloughed and died. Within 5 days, the cultures with 20 per cent foetal calf serum showed many cells in the medium, including clumps, but no aggregates. After a week, there were perhaps more cells in the medium than on the glass, and yet most of these were dissociated cells or clumps, with very few aggregates. Most of the cells in the medium proved to be non-viable when stained with trypan blue. After 10 days, many of the cells remaining on the glass were arranged in small clumps that resembled small aggregates of 2650-*R*. Many of the cells were vacuolated, pale, and more broadly spread out than normal cells. These cultures were maintained for nearly three weeks, but further changes were mainly degenerative.

The cultures in 30 per cent serum were similar to those in 20 per cent, but they left the glass more rapidly and more extensively. The viability of cells in the medium was very low. Cells which remained on the glass were commonly arranged in groups suggestive of the smaller aggregates of 2650-*R*, and hence it may be that a high serum level in the medium does promote aggregation of cells of line 2650.

Efforts were also made to promote aggregation by placing cultures in T-flasks on a rotary shaker. Cultures were put on the shaker at its minimal speed (60 r.p.m. according to the dial, but actually 90 r.p.m.). After one week, there were many cells of 2650 in the supernatant. These formed many clumps, but no aggregates. The cells that remained on the glass were not evenly distributed; they were arranged in clusters suggestive of the aggregates of 2650-*R*, but they never formed clear-cut aggregates.

When cultures of 2650-*R* were similarly treated, aggregates of 64 cells or more came off the glass within two days. Within four days, almost all cells were in the medium, and some aggregates were as much as 5 mm in diameter. Some were sheet-like, others more string-like in form. Within six days, most of the cells had died.

In heavy cultures of *WI*-38 cells, macroscopic fibrous structures, looking like fragments of white thread up to 2 mm long, appeared. Microscopic examination showed these to be tightly aggregated cells, all oriented lengthwise with the thread, and suggesting early stages in the formation of connective tissue. Between these heavy, macroscopic concentrations were lighter ones, often in the form of a network. Such cultures were placed on the rotary shaker. Within two days, all the cells appeared to be concentrated in a single mass about 3 mm in diameter and less than 1 mm thick. The trypan blue test showed that most of the cells were viable. Two days later, however, this cell mass stained intensely, showing that most of the cells had died.

The first experiments with Steward flasks were run with a very slowly revolving drum, which made one complete revolution in five minutes. Later experiments were run at 1 r.p.m., but the results were similar. RPMI 2650 cells soon settled on the glass, especially in the wells. There they became arranged in streaks running in the direction of the flow of the fluid. These streaks tended to become confluent, but there was no tendency to form aggregates. When inoculated into siliconized Steward flasks, however, the cells did sometimes form aggregates; and, contrary to expectation, these quickly settled out on the siliconized glass. These aggregates were 0·5–1·0 mm wide, and they were elongated in the direction of flow. In some areas, they formed extensive networks. After eight days, counts of cells in the supernatant were made. The average was 9 viable cells per mm^3. Most of these were dissociated, but twos and threes were seen.

After shaking in the medium for 10 min on the Boerner shaker, no increase in turbidity was noticed. The cell count had risen only to 12 per mm^3, and all cell masses were still on the glass. The medium was then replaced with EDTA, and the flask was returned to the shaker. Fragments began to break off almost immediately. After shaking for 10 min, the count in the supernatant was 62 per mm^3. After 20 min, all the masses were off the glass, and the count in the supernatant averaged 594 per mm^3. Only 10 per cent of the cells were fully dissociated, however, the rest still being in aggregates, many of them too large to permit counting. After 30 min, the viable cell count had risen to 1,500 per mm^3. Results with trypsin were similar, but dissociation was slower in early stages.

RPMI 2650-*LN*-2 cells tended to remain in suspension in the Steward flasks. They formed numerous small aggregates less than 1 mm in diameter, and an occasional larger one, with a diameter up to a maximum of 4 mm. These cells were easily dissociated, for after shaking for 10 min in EDTA, no true aggregates were left, although there were still some clumps. For example, a culture which was set up with an inoculum of 300,000 cells was gathered after one week. Before shaking in EDTA, a count showed 50 viable cells per mm^3, in the supernatant, when it had an abundance of small aggregates. After 10 min of shaking, there were 726 cells per mm^3, or 3,630,000 in the culture. Results were more variable with 2650-*LN*-2, however, than with 2650 or 2650-*R*.

RPMI 2650-*R* cells aggregated very rapidly in the Steward flasks. Overnight, numerous small aggregates, 1 mm or less in diameter, settled on the glass of the wells. A few larger aggregates were formed as quickly. For example, one was observed which was about 4 mm long by 2·5 mm wide (Fig. 3). Such aggregates were generally elongated in the direction of flow. Within a week these would grow and anastomose to form an extensive network over the surface of the wells. A single strand might extend as much as 2 cm, and the breadth of strands varied from 0·5 mm to 5–6 mm.

Typically, all the viable cells were confined to the aggregates. They were very firmly bound, for shaking in the medium did not break up the aggregates or free them from the glass. When the cultures were shaken in EDTA, pieces began to break off the glass almost immediately, but fairly large aggregates remained on the glass after shaking for 10 min. After 20 min, the largest mass was reduced to

about half its initial size, and the supernatant contained many small aggregates. After 30 min, there were many small aggregates in the supernatant, and the largest were about 2 mm in diameter. Even after 40 min, there were still many small aggregates and clumps, although there were also many dissociated cells. In another run, they broke down more quickly. After 10 min of shaking in EDTA, a count of 1,352 viable cells per mm³ was obtained, although many of these were in small aggregates and clumps. After 20 min, all cells were either dissociated or in small clumps, most commonly of two or three cells. There were not aggregates. On shaking in trypsin, similar results were obtained, but the early stages of the break-up of the aggregates went more slowly.

T-3-1 cells were also examined in Steward flasks. These cells formed abundant small aggregates, 1 mm or less in diameter, within a few days. Within a week, a very few larger aggregates, up to 5 mm in diameter, were formed. These larger aggregates tended to become perforated and rather lacy in appearance, and then they broke up into many small ones. Most of these remained in the medium, but a few adhered lightly to the glass. After two weeks, the cultures appeared to be dying, and they were discarded.

Experiments with RPMI 6410 and *WI*-38 in Steward flasks were less productive. RPMI 6410 cells grew very satisfactorily in the Steward flasks, but they occurred almost entirely as dissociated cells. A few small clumps of 2–4 cells were seen, but no aggregates. It is interesting to note that these cells are derived from myeloid blood cells, which, as dissociated cells, form an important part of the circulating blood *in vivo*. When grown in stationary cultures, they form large aggregates spontaneously; but in mildly agitated Steward flasks, they are again almost completely dissociated. *WI*-38 cells failed to grow at all in the Steward flasks, and the cultures soon died.

Because of the importance of mesodermal tissues in embryonic induction, mixed cultures of epithelial and fibroblast cells were set up. The principal combinations used were *WI*-38 (fibroblasts) added to established cultures of 2650 and 2650-*R*, 2650 added to established *WI*-38, 2650-*R* added to established *WI*-38, and *WI*-38 and 2650-*R* inoculated simultaneously. In addition, 2650 and 2650-*R* were added to another line of fibroblasts, RPMI 4269, which was derived from an epithelial tumour. The fibroblasts, however, had taken over the cultures from this tumour.

The most productive experiment was the addition of 2650-*R* cells to established cultures of *WI*-38. Within a few days, it appeared likely that the 2650-*R* aggregates were elongating along the *WI*-38 cells as guides. After a week, this was clearly the case. After two weeks' growth, the 2650-*R* aggregates had assumed bizarre shapes never suggested in the pure cultures, and those shapes always corresponded to the distribution of the *WI*-38 cells. The typical 2650-*R* aggregate is a compact round-to-oval cluster, looking superficially much like a grain of sand (Fig. 1). In the mixed cultures, they were elongated, often branched, and sometimes took on turtle-like and other highly modified configurations, conforming to the distribution of the underlying *WI*-38 cells. After about three weeks, the cultures were very heavy, and the largest aggregates began to disorganize.

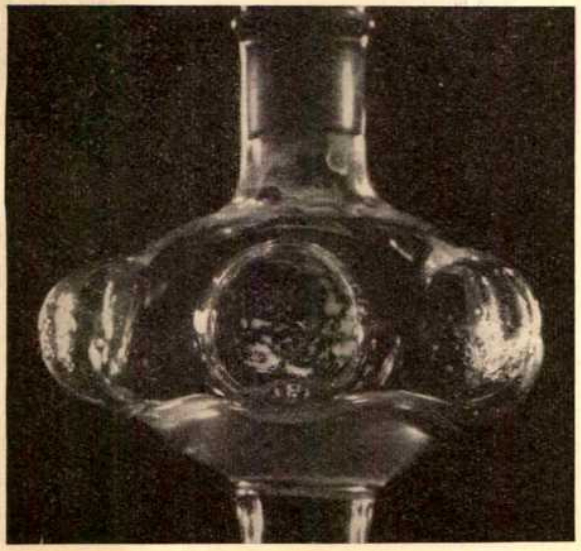


Fig. 3. Recently established aggregates of 2650-*R* cells growing in a Steward flask

The reciprocal experiment, addition of *WI*-38 cells to established cultures of 2650-*R*, was much less productive. After 9 days, a suggestion of elongation of some of the 2650-*R* aggregates along the fibroblasts was seen. Although the cultures were maintained for more than a month, the elongation never developed much beyond a suggestion. Much the same thing was true when 2650-*R* and *WI*-38 were simultaneously inoculated into new cultures. Apparently the capacity of established *WI*-38 cultures to direct the organization of newly forming 2650-*R* aggregates does not extend to other combinations of the two cell types.

RPMI 2650 cells did not appear to be influenced by *WI*-38 cells in any combination. Cultures were maintained for four weeks, but neither cell type ever showed any tendency to interact with the other or to orient its growth in any way different than the pure cultures.

Epithelial cells were also added to established cultures of fibroblasts derived from biopsy of a cancer patient, RPMI 4269. These fibroblasts grow vigorously, in typical fibroblast fashion. To each of a series of well-established cultures in T-30 flasks, 300,000 RPMI 2650 cells were added. Within 3 days, all of the RPMI 2650 cells had disappeared, a fact that may be related to the failure of the epithelial cells in the original culture (and perhaps to the death of the patient). The cultures were then re-inoculated with 400,000 cells each. The following day, the RPMI 2650 cells again seemed to be dying out, except in one culture, in which the fibroblasts had been sparse from the start. After two weeks, however, some epithelial cells were still recognizable, although the fibroblasts were strongly predominant in the cultures. After two and a half weeks, no 2650 cells at all were identifiable in one culture, and they formed no more than a small minority in any of the others.

When 2650-*R* cells were added to 4269 fibroblasts, the results were quite different. Initially, the epithelial cells regressed, and the individual aggregates had a rather constricted appearance. They were confined to spaces between the sheets of fibroblasts, and they seemed to regress. After 10 days, however, some growth was evident. They were still confined to spaces between fibroblasts, where they might grow out in long, narrow tongues, quite unlike their normal pattern or their pattern of growth on *WI*-38 cells. After two weeks, this pattern was prominent; and after two and a half weeks, the aggregates looked heavy enough to suggest the possibility that they might soon overwhelm the restrictive influence of the fibroblasts.

The suggestion made by Moskowitz[2] that cultured aggregates of tumour cells "can be regarded as free-living tumours" is plausible. If it is correct, then the examination of such aggregates should be even more *à propos* to the problem of cancer than the study of the same cells in monolayers.

Both the conditions of culturing and the inherent characteristics of the cell influence the aggregation of cells. No conditions were found under which 2650-*R* did not form aggregates, but these aggregates seldom reached large size. The largest in stationary cultures were about 2 mm in diameter. The centres of these would break out, leaving a ring that fragmented into many smaller aggregates. In Steward flasks, larger aggregates were formed because of the tendency of the aggregates to elongate in

the direction of flow. In extreme cases, these might reach a length of 2 cm and a maximum breadth of 3–4 mm, but their outlines were always highly irregular. The tenacity of the cell adhesions is shown by their resistance to separation on the Boerner shaker. Prolonged shaking in the growth medium did little or no damage. Shaking in EDTA or trypsin did break the cells apart, but some aggregates generally remained after shaking for 20 min, and small clumps remained even after half an hour.

In contrast, it was very difficult to induce aggregation of RPMI 2650 cells. They did show a very limited tendency to aggregate in cultures with high content of foetal calf serum, or in siliconized Steward flasks. Aggregation in the latter was a sporadic event, and its causes have yet to be determined. When aggregated, however, the adhesion was quite tenacious: shaking in the medium had no effect, and shaking in EDTA or trypsin required about 30 min to reduce the aggregates to dissociated cells.

Line 2650-*LN*-2 shares characteristics of its sister lines. In stationary cultures, the cells formed small, very regular, oval aggregates, but it was difficult to increase the size of the aggregates by experimental techniques. Larger aggregates developed sporadically in Steward flasks, and this behaviour remains to be investigated.

Cells of 6410, a myeloid leukaemia line, formed large aggregates spontaneously in stationary cultures. The largest was nearly 4·5 cm long and had the form of a tongue worm, with the ovoid 'body' about 2 cm long and the narrow 'tongue' about 2·5 cm long. The borders of these large aggregates were not sharp, as Moskowitz believes they should be, yet I believe that such clusters are true aggregates in Moskowitz's sense of the term, for several reasons. Their formation under the conditions of culture was invariable. Almost all the cells in the supernatant were dead, whereas almost all those in the aggregates were living. Finally, even the general form was fairly constant—always an irregular ribbon. In view of these facts, it seems clear that these are not accidental assemblages of cells. They are true aggregates, resulting from the developmental potential inherent in the cells. These aggregates, however, are not stable. It is difficult to handle the culture dishes without dispersing the aggregates to some extent: and as gently as the cultures were agitated in Steward flasks, the aggregates were completely broken up in them, and only infrequent clumps of 2–4 cells were seen.

T-3-1 cells form large aggregates (4–5 mm in diameter) spontaneously in stationary cultures, yet these are so weakly bound that the gentle agitation of the Steward flasks breaks them down to a much smaller size. Finally, *WI*-38 cells did form small fibres spontaneously in stationary cultures, and they did form a single large mass when cultures were kept on a rotary shaker; but the cells soon died after forming such a mass.

To summarize in embryological terms, competence to respond to the conditions for aggregate formation varies widely among the cell lines studied, from a maximum in the case of 2650-*R* to a minimum in the case of 2650. *WI*-38 cells have a very low competence for aggregation, but they do display some potency for guiding the aggregation of 2650-*R* cells. That this potency should characterize an embryonic line of fibroblasts rather than the adult line which was also tested is hardly surprising.

I thank Dr. George E. Moore for the facilities of his laboratory and his advice. This work was supported by research grant 1–S01–*FR*–05250–01 of the U.S. Public Health Service, awarded to Dr. G. E. Moore.

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A NEW GENETIC VARIANT OF β-THALASSAEMIA

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THE heterogeneity of thalassaemia is well recognized; but the marked diversity of its manifestations has complicated the many efforts[1–7] for classification. General agreement exists about a primary sub-division into α- and β-thalassaemias, following the suggestion of Ingram and Stretton[8]. A further sub-division of β-thalassaemias is difficult since genetic or environmental modifiers may be involved.

We wish to present a family with a hitherto undescribed form of β-thalassaemia, in which the unusual findings must be explained on the basis of a single gene defect with very constant phenotypic expression.

In Fig. 1 the pedigree of this family is reproduced. It can be seen that the presence of homozygotes in this family is a result of the fact that the parents are second cousins and both carry the abnormal gene. The family was traced back as far as 1720 and seems to be of pure Dutch stock.

The two homozygotes in this family (*V*-27 and *V*-28), both about forty years old, are unusual in various respects. They have reached adult life without any serious illnesses; only occasional bouts of jaundice were reported to have occurred in *V*-28. A very marked increase of erythropoietic activity—also resulting in considerable radiological abnormalities of the skeleton—leading to a well-compensated anaemia with haemoglobin-levels of 12·1 and 14·3 g/100 ml. is present in the two brothers. The morphology of the red cells is, although not at all classical for thalassaemia major, nevertheless rather abnormal (Fig. 2). The presence of numerous nucleated red cells in the index case dates from 1953, when he underwent a splenectomy.

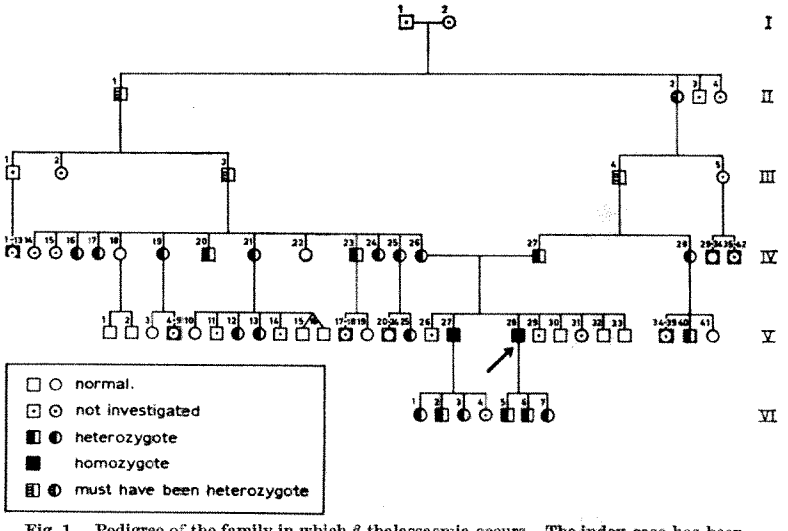


Fig. 1. Pedigree of the family in which β-thalassaemia occurs. The index case has been indicated by an arrow

The haemoglobins of the homozygotes were investigated on various occasions by different electrophoretic techniques and by chromatographic studies on 'Amberlite *IRC* 50' and DEAE–cellulose. Neither with these techniques nor with an immunological analysis using anti-Hb A serum could any Hb A be detected. The only haemoglobin components present were Hb F, which varied in concentration between 98 and 98·8 per cent, and Hb A_2 which formed the remainder. Analysis of these Hb F and Hb A_2 fractions by fingerprinting gave findings indistinguishable from those of normal Hb F (cord blood) and normal Hb A_2.

The three non-investigated siblings of the two homozygotes died before the age of one year from causes probably unrelated to the genetic defect of their brothers. The six investigated children from the homozygotes could only be partially examined since no permission was given for venepuncture. Nevertheless the findings from finger-prick blood (red cell morphology, mean corpuscular volume (MCV) and Hb F-levels) leave no doubt that they are all actually heterozygotes for the thalassaemia gene in question.

Both parents and 13 other relatives of the two brothers in generations IV and V appear to be heterozygotes for a β-thalassaemia and present a haematological picture which is in all respects typical for thalassaemia minor. A further 24 relatives were, on haematological examination, entirely normal. The red cell morphology of the heterozygotes conformed to the usual one for β-thalassaemic heterozygotes, including marked basophil stippling, while the MCV had an average value of 73 μ^3 and the mean corpuscular haemoglobin concentration (MCHC) an average value of 31 per cent. The haemoglobin A_2 level (determined by starch-block electrophoresis at *p*H 8·6) was raised in all heterozygote individuals, together with a marked increase in foetal haemoglobin (as determined by the alkali denaturation technique).

The Hb A_2 concentrations varied between 5·3 and 7·6 per cent (with an average of 6·6 per cent) of total haemoglobin whereas the Hb F content varied between 5·1 and 14·4 per cent (average 8·3 per cent). These results

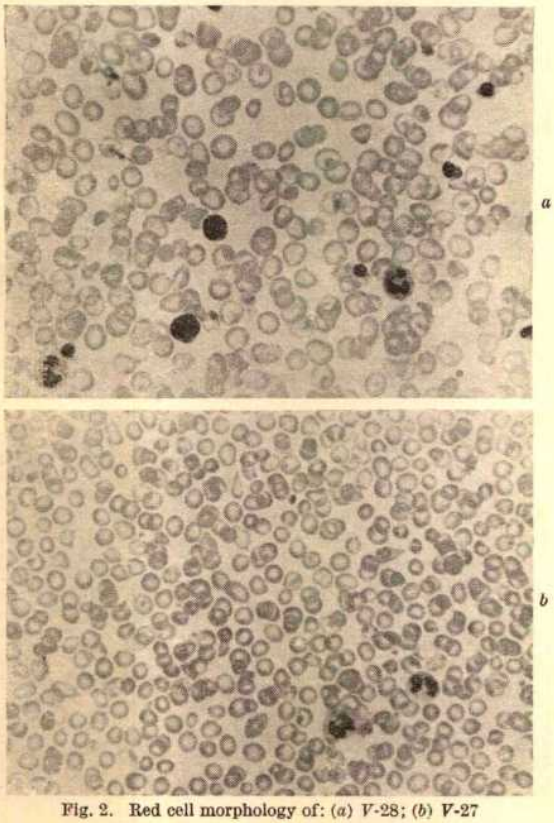


Fig. 2. Red cell morphology of: (*a*) *V*-28; (*b*) *V*-27

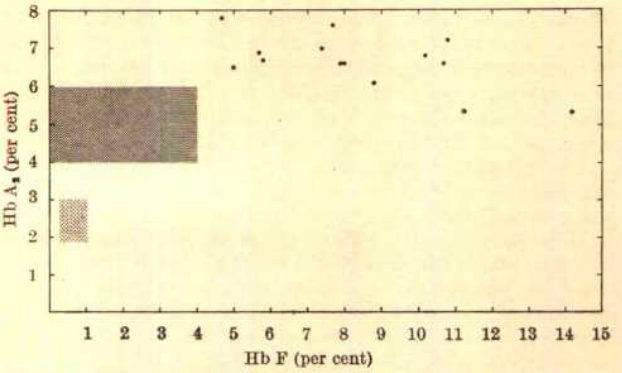


Fig. 3. Hb A_2- versus Hb F-levels in 14 heterozygotes and 24 normal individuals from the family of Fig. 1, compared with the range of usual findings in β-thalassaemia heterozygotes (shaded area). The findings on the 24 normals come too close together to be individually indicated and are therefore represented by the stippled area

were confirmed by parallel investigations in different laboratories both in Holland (Amsterdam and Leiden) and in the United States (Atlanta, Georgia). They are definitely very unusual when compared with the common findings in β-thalassaemia: Hb A_2 ranging between 3·5 and 7 per cent (with an average of 5 per cent) and Hb F ranging between 0·3 and 6·5 per cent[2,9–12]. The distribution of Hb F over the red cells of the heterozygotes, using the acid elution technique of Kleihauer-Betke[13], was found to be very uneven. Moreover, the 24 non-thalassaemic family members had Hb A_2-levels between 1·9 and 3·0 per cent, and Hb F-levels below 1 per cent (Fig. 3), therefore completely in agreement with findings from the literature on normals.

The genetic defect in the family described is in our view undoubtedly a form of β-thalassaemia. The heterozygotes conform in all respects to the accepted criteria for β-thalassaemia, that is, an increased level of Hb A_2, a typical morphological picture with a low MCV in the presence of a normal MCHC and an uneven distribution of Hb F over the red cells[12]. The two brothers (*V*-27 and *V*-28) are undoubtedly true homozygotes since their parents are second cousins and the mutation in question must be extremely rare. A severe impairment of β-chain synthesis (evidence for β-thalassaemia) exists since no Hb A is formed at all.

The difference with the usual heterozygous form of β-thalassaemia is evident since in our family the average Hb A_2-level is higher (6·6 per cent against 5 per cent) and the average Hb F-level (8·8 per cent) is above the highest reported figures which range for the usual form from normal to 6·5 per cent. In Fig. 3 the Hb F- versus Hb A_2-levels for 14 of the 15 adult heterozygotes are individually plotted. For comparison are also indicated the area in which all the findings on the 24 normal individuals of our family belong and the area in which approximately 95 per cent of reported findings on the common β-thalassaemia heterozygotes fall.

The absence of Hb A in our homozygotes appears to be rather unusual when compared with the more common thalassaemia homozygotes, but has recently been described with considerable frequency notably by Fessas from Greece[7,14,15]. In a personal communication, Weatherall (1964) has reported the finding of two sisters with fairly mild thalassaemia major with complete absence of Hb A. In the heterozygous state of these thalassaemias from Greece and the United States, however, the more usual form of β-thalassaemia with only a minor increase of Hb F appeared to be present.

Recently, many instances of apparently true β-thalassaemias with markedly increased levels of Hb F—which is unevenly distributed over the red cell population—have been reported[3,7,14,16]; in this so-called F-thalassaemia or β-chain thalassaemia type II (Fessas), however, the A_2-levels are without exception within the normal range. Homozygotes for this condition appear not to have been

described up to now. (*Note added in proof.* C. Brancati reported at the "Dixième congrès de la Société Européene d'Hématologie" in Strasbourg, August 1965 (résumé No. 270), a near-certain case of homozygosity of this F-thalassaemia. The haemoglobin consisted of Hb F only, with complete absence of both Hb A and Hb A_2.)

The hereditary persistence of foetal haemoglobin or the high F gene[2,12,15] is undoubtedly a genetically different entity because in the heterozygote carriers of this F gene a normal red cell morphology, a slightly decreased Hb A_2-level and an even distribution of Hb F over the red cells are being found[12]. The only reported case of homozygosity for the high F gene[17] is not anaemic. This 2-year-old girl also completely lacks Hb A, but in contradistinction has no Hb A_2 either.

On the basis of all these arguments the family described must carry a new type of β-thalassaemia mutation and provides further evidence for the genetic heterogeneity of thalassaemia. A further comparison and analysis are presented elsewhere[18].

The first discovery of the thalassaemic nature of the abnormalities in our family was made by Drs. H. K. Prins and M. Oort from the Central Laboratory of the Netherlands Red Cross Blood-transfusion Service in Amsterdam. We thank Dr. F. Westendorp Boerma for the immunological investigations, Dr. T. H. J. Huisman for chromatographic investigations and Dr. C. Baglioni for the fingerprint analysis and for his advice.

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TRANSFER OF CONDITIONED RESPONSES FROM TRAINED RATS TO UNTRAINED RATS BY MEANS OF A BRAIN EXTRACT

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THREE independent investigations reported during the past year have suggested that learned responses may be transferred from one vertebrate brain to another by means of a brain extract. The first of these, by Fjerdingstad, Nissen and Røigaard-Petersen[1], demonstrated that an intracisternal injection of an extract from the brains of rats trained to run to a signal light facilitated the learning of the same task by naïve rats. It was assumed, in this investigation, that the information-bearing molecule in the extract was RNA.

The second investigation, by Ungar and Oceguera-Navarro[2], utilized an extract from the brains of rats habituated to the sound of a hammer dropping on a metal plate. This extract, injected intraperitoneally into mice, facilitated the habituation of the mice to the same stimulus, as measured by startle responses. These workers suggested a peptide or protein molecule as the active component.

The most recent investigation, by Babich, Jacobson, Bubash, and Jacobson[3], again used an RNA extraction procedure, with an intraperitoneal injection in rats, and the task transferred was that of running to a food cup in a Skinner box in response to the click of a pellet dispenser. The results of this experiment, although again making use of a small number of animals, seemed to be most significant statistically, and the experiment had the further advantage of demonstrating transfer of the learned response without retraining or reinforcement, eliminating the possibility that the effect might involve merely a general sensitization or facilitated learning. The method of preparation and a biuret test performed in these experiments seemed to eliminate protein as a plausible molecule, and it was assumed that RNA was the information carrier. This assumption is in line with the earlier speculation by Hydén *et al.*[4-6] that RNA was a likely candidate for a memory encoding molecule. Alternative models have emphasized the possibility of chemical memory trace mechanisms which would lead to synaptic facilitation, rather than recording the experiential information directly in the form of a molecular code[7-9]. In such a model, RNA changes would merely form part of an induction cycle, leading, for example, to modified membrane proteins and alterations in the 'adhesive codes'[10] governing the synaptic connectivity of specific sets of neurones.

The work recorded here was designed, first, to replicate the results of the previous experiments with a larger number of animals, and, second, to test explicitly the conjecture that RNA might be responsible for information transmission by the brain extracts. The method of Babich *et al.* was used, the only major departure being in the use of a pooled extract from the brains of 20 trained rats in each experiment, rather than one-to-one matching of donors and recipients.

A total of 140 female albino rats was used (supplied by Holtzman, 190–210 g), including 60 donors and 80 receivers. These animals were divided equally between two experiments, the second being essentially a repeat of the first, with slight variations in technique and localization of brain regions employed in the extracts. In each experiment, 20 donors were trained to run to the food cup in the corner of a Skinner box (supplied by Scientific Prototype) in response to the click of the pellet dispenser. Reinforcement consisted of 45-mg Noyes pellets, given intermittently, on about 50 per cent of the clicks. Training was maintained for five days, with a maximum of 200 clicks or 1 h of training per day for the first four days, and 100 clicks on the fifth day. The animals were starved for two days before starting training, and the training methods were copied as nearly as possible from those of Babich *et al.*[3]. Ten untrained donors, in each experiment, were used for the preparation of a control extract. All 30 donors were killed at the same time, by decapitation, and the extract for each group of receivers obtained from the pooled brains of the donors.

Brain extracts were prepared by the RNA extraction method described by Babich *et al.*, except for the variations noted. In all, eight extracts were prepared, as follows.

Extract 1. The midsections (exclusive of frontal regions, cerebellum and brainstem) of the brains of ten untrained rats were utilized. These brain sections were ground with 80 ml. of fine sand in a large cold mortar, with 100 ml. phenol (90 per cent) and 100 ml. cold saline. This resulted in a large volume of liquid in which the brain tissue was very difficult to homogenize. The liquid remained quite thin and was decanted from the sand and centrifuged at 8,000*g* for 20 min at 0–4° C. The aqueous phase (about 40 ml.) was collected, and magnesium chloride was added to bring the concentration to 0·1 M. Two volumes (80 ml.) of cold ethanol were added and the mixture was allowed to stand in the cold for 30 min. The precipitate (presumed to be chiefly RNA) was collected by centrifugation at 35,000*g* for 30 min. The ethanol-water mixture was decanted and the residue dried *in vacuo*. The residue was taken up in 11·5 ml. saline. This solution was used for injection. The RNA concentration in this extract was estimated as 0·172 mg/ml. by determination on a Beckman *DB* spectrophotometer at 260 mμ (total RNA was 1·98 mg). This value, however, may be spuriously high due to a contribution from residual phenol in the extract, which peaks at 264 mμ.

Extract 2. Midsections of the brains of ten of the trained rats were employed. To avoid the difficulty of the first extract, only 10 ml. of phenol and 10 ml. of saline were added to the sand and brains. This resulted in a paste which homogenized easily. The remaining 80 ml. of the 1 : 1 phenol-saline mixture was then added. The remainder of the procedure was identical with that used for Extract 1. The RNA concentration was estimated to be 0·327 mg/ml. (or 3·76 mg total), again with the possibility of some phenol contamination.

Extract 3. The brain tissue used consisted of 10 midsections and the 20 residual portions (frontal and midbrain) from the trained rats. This had been frozen rapidly and stored for 48 h. The total weight was about 25 g. The extraction procedure was the same as for Extract 2, with 200 ml. of the phenol-water solution. The final residue was taken up in 22 ml. saline. The RNA concentration was estimated as 1·31 mg/ml. (or 28·8 mg total).

Extract 4. Half the solution obtained in Extract 3 was used (11 ml.). 1·1 mg of ribonuclease was added, and both Extracts 3 and 4 were incubated at 40° C for 1 h. The solutions were then stored at 0–4° C overnight before injection.

Extract 5. The entire cerebral sections of the brains of ten more untrained rats were employed. The extraction was performed as above, except that the brains were first homogenized, three or four at a time, with about 20 ml. of the saline in a Potter–Elvehjem homogenizer. The remaining 30 ml. saline and 50 ml. phenol were then added together with the sand, and the resulting mixture treated as before. Considerable care was taken in this and following extracts to get rid of all the liquid phase before drying the precipitate, to eliminate possible phenol contamination. In addition, a phenol blank run through the same procedure showed that all detectable traces of phenol had vanished after vacuum drying. The RNA concentration of the resulting extract was 0·52 mg/ml. (5·98 mg total yield).

Extract 6. The cerebellar portions (cerebellum and brainstem) of the brains of the second 20 trained rats were used. The extraction procedure was identical to that used for Extract 5, and the RNA concentration was 0·56 mg/ml. (6·94 mg total).

Extract 7. The cerebral portions of the brains of the second 20 trained rats were extracted by the same method as above, after having been frozen for 48 h. The RNA concentration was 0·42 mg/ml. (average of two determinations), with a total yield of 10·2 mg.

Extract 8. Half of Extract 7 was incubated with RNase by the same procedure as Extract 4. A measurement of the resulting solution at 260 Å indicated the equivalent of an RNA concentration of 0·57 mg/ml. (average of two determinations). The higher concentration obtained in the incubated extracts is an indication that the RNase was effective in breaking up the RNA into nucleotide components. A further test, to make sure the RNase was fully effective, was run by column fractionation on 'Sephadex *G*50' medium. A known RNA sample, used for comparison, gave one peak before incubation with RNase, and a single, distinctly separate peak after incubation. The same separation of peaks was found for Extracts 7 and 8, and it was estimated that residual RNA must have been reduced to something less than half of a per cent of the original content.

The injected animals were divided into eight groups of ten, each group (labelled *A*–*H*) receiving one of the eight extracts. Each rat was injected with 1 ml. of extract, intraperitoneally. Table 1 shows the treatment of the eight injected groups. The first experiment consisted of the training of the first 20 rats, the preparation of Extracts 1–4, and the injection and testing of Groups *A*, *B*, *C*, and *D*. The second experiment consisted of the training of the second group of donors, with the remaining extractions and tests of Groups *E*, *F*, *G*, and *H*. The rats were tested in four sets of 20, the first consisting of Groups *A* and *B*, the second of Groups *C* and *D*, the third of *E* and *F*, and the fourth of *G* and *H*. Each group of 20 was divided at random between the two types of extract employed, and tested by observers who were not informed of the identity of the rats until the tests were completed.

Each of the injected rats was given a total of 30 min in the Skinner box, over the two days prior to injection, and was starved throughout this habituation period. A total of ten clicks from the empty pellet dispenser was given to each rat during this period, and her responses were recorded. In testing the animals, the criteria for administering a stimulus corresponded to those employed by Babich *et al.* Clicks were given after the rat had been in the box for two minutes, and were spaced at least 1 min apart. The animal was required to be facing at least 90° away from the food cup in order to receive a click. Records of the time taken to reach the food cup were kept by means of a stop-watch. For the first experiment, a response was counted if the rat's nose passed within 2 cm of the food cup. For the second experiment, the response criteria were tightened, and responses were counted only if the rat was sniffing directly at the food cup, or had her nose over the rim. This led to a significant reduction in the number of responses counted in the second experiment, but it was hoped that spurious responses would be more effectively eliminated.

Each of the first five test sessions for each rat (after injection) consisted of five unreinforced clicks, response times being recorded as already described here. This was followed by a sixth session with a pellet in the cup at the outset, followed by five reinforced clicks. This was followed by a seventh, unreinforced, session of five clicks. In these last two sessions, the time to pick up the pellet was recorded, and responses were counted only if the rat's nose was over the edge of the food cup. For the first series of runs in Experiment 1, a second observer was employed who counted all approaches of a rat to the food cup, to obtain some measure of total activity. As there was no indication that this total activity measure was any different for controls and experimental groups, this measurement was discontinued in later runs. In the first experiment, each rat was given one pellet of lab chow between sessions 5 and 6; in the second experiment, they were given a pellet of lab chow after session 4.

Table 1. Groups of injected Rats (10 Rats in Each Group)

Group	Injections	RNA dosage (mg)
A	Extract 1 (control)	0·17
B	Extract 2 (trained cerebrum)	0·33
C	Extract 3 (trained whole brain)	1·31
D	Extract 4 (Extract 3 + RNase)	—
E	Extract 5 (control)	0·52
F	Extract 6 (trained cerebellum)	0·56
G	Extract 7 (trained cerebrum)	0·42
H	Extract 8 (Extract 7 + RNase)	—

Table 2. Response Rates of Each Group as a Percentage of Responses within 2, 3, and 5 Sec

Group	Unreinforced tests (1–5)			Reinforced tests (6–7)			Combined tests (1–7)		
	2″	3″	5″	2″	3″	5″	2″	3″	5″
A	1·6	2·4	5·2	3	10	21	2·0	4·6	9·7
B	2·4	3·6	7·6	20	23	29	7·4	9·1	13·7
C	2·8	5 2	7·6	12	16	29	5·4	8·3	13·7
D	1·6	2·8	6·8	13	17	28	4·9	6·9	12·9
E	0·0	0·4	1·2	5	6	13	1·4	2·0	4·6
F	2·0	2·0	3·6	5	7	19	2·8	3·4	8·0
G	0·0	0·0	0·4	7	10	12	2·0	2·8	3·7
H	1·2	1 6	3·2	4	9	21	2·0	3·7	8·3
Controls:	0·8	1·4	3·2	4·0	8·0	17 0	1·7	3·3	7·2
Exptl.:	1·7	2·5	4·9	10 2	13·7	23·0	4·1	6·0	10·1
RNase:	1·4	2·2	5·0	8·5	13·0	24·5	3·5	5·3	10 6

Table 3. Numbers of High and Low Rats in Control and Experimental Groups. High Rats have 3 or more Responses within 2 Sec in Exp. 1, or 2 or more Responses within 2 Sec in Exp. 2

Group	No of rats Low	High
A	10	0
B	6	4
C	7	3
D	6	4
E	10	0
F	6	4
G	9	1
H	9	1
All groups	63	17
Controls (*A*, *E*)	20	0
Trained extract	43	17
RNase (*D*, *H*)	15	5

The first test was administered about 4 h after injection, and the following three sessions spaced about 3 to 4 h apart. Session 5 was given the following day, and sessions 6 and 7 within 24 h afterwards.

The principal results of these experiments are presented in Tables 2 and 3. The distribution of fast responses (5 sec or less) seemed to be most indicative of the difference between experimental and control rats. Table 2 shows the percentage of responses within 2, 3, and 5 sec for each group, and for the combined controls, combined 'experimental' groups (*B*, *C*, *D*, *F*, *G*, *H*) and combined RNase groups. The response times for the pre-injection tests (10 clicks per rat) were recorded for the 40 rats used in Experiment 2. This yielded a total of 3 responses (0·75 per cent) within 2 sec, 3 within 3 sec and 11 within 5 sec. It appeared from these data that the injections had 'taken' on some of the rats, but not on others, conceivably due to immunological factors. A 'take' was characterized by a relatively large number of fast responses, typically within 2 sec or less, and was generally brought out quite reliably by the 'retraining' procedure of Sessions 6 and 7. Several of these rats, after eating their first pellet, scarcely missed a response during any of the subsequent ten clicks, wheeling rapidly (within 1 sec) from any starting position, and dashing directly to the food cup. By contrast, records of an exactly similar procedure for the second group of 20 donors, for 5 reinforced and 5 unreinforced clicks at the outset of their training, yielded a total of only 2 responses within 2 sec (1 per cent), and 7 responses within 3 sec. Although these figures agree closely with those of the injected control groups, they have not been treated as additional control data as the degree of starvation was not the same in both cases.

The time taken to recognize and eat their first sucrose pellet appears not to differ significantly for the control and experimental groups. The most sensitive discrimination of controls from experimental animals was found to result from a dichotomization of the injected rats into 'high' and 'low' categories. Since the response criteria were tighter on Experiment 2, slightly different cutting points had to be chosen in each case. For Experiment 1, a 'high' rat is one with a total score of three or more 2-sec responses in the seven sessions, while for Experiment 2 (Groups *E*–*H*) a 'high' rat is one with 2 or more 2-sec responses. These criteria give a total of 17 high rats out of the 80 injected animals, 11 in the first experiment and 6 in the second. The distribution of high and low rats for the different groups and important combinations of these is shown in Table 3. Combining the results of the two experiments, we find that among the 20 controls, there were no 'high' rats, all 17 'highs' being distributed among the 60 animals receiving some form of 'trained' extract. This difference is significant at a level of 0·4 per cent by a Fisher exact probability test[11]. The comparison of the controls with the trained extract rats from Groups *B* and *F*, which were tested at the same time (guaranteeing uniform treatment), gives a significance level of 0·16 per cent. (It should be noted that this is a very conservative test for these data, taking no account of the actual numbers of responses, and making no parametric assumptions.) If the 'retraining' data (sessions 6–7) are neglected, and a criterion of 2 or more 3-sec responses is taken to characterize a 'high' rat, then there are 9 'high' rats from the trained injections, and one from the controls ($P = 0{\cdot}08$). Although these results are clearly significant, they show a weaker effect than that observed by Babich *et al.* with the same training and extraction procedures. The possibility that this difference was due to the use of pooled brain extract instead of 1 : 1 injections has been checked in a subsequent experiment (to be reported in detail in a later publication) with negative results. Some more subtle differences either in training or in extraction procedures must, therefore, be responsible for the magnitude of their effect. The trained cerebellar extract seemed to be as potent as the trained cerebral extract, and the incubation with RNase did not eliminate the effect, even though RNA was shown to be eliminated from the extract. The difference between the combined RNase groups and the controls is significant at 2·4 per cent, and the difference between the cerebellar group and its corresponding control group (Groups *E* and *F*) is significant at 4·3 per cent. No other significant differences were found.

Since the biuret test employed by previous investigators[3] is insensitive to quantities under 1 mg, a more sensitive protein determination, using the Folin–Ciocalteau method[12], was carried out on Extract 7. This showed a concentration of 1·1 mg/ml. in this extract. This result is consistent with the work of Ungar and Oceguera-Navarro[2], who report that their effect is eliminated by chymotrypsin.

These results seem to confirm the possibility of 'memory' transfer by means of a brain extract, for a simple operant response to an auditory stimulus. It appears that the information-bearing molecule is not RNA, but might be a small soluble protein or polypeptide. Subsequent experiments by our group (to be reported later in detail) have shown that a bar-pressing response in a Skinner box can also be transferred, and that the molecular weight of the information-bearing molecule seems to be in the range of 1,000–5,000, as measured by 'Sephadex' fractionation. While consistent with the polypeptide hypothesis, this weight range would exclude any known form of RNA. The appearance of the effect from both cerebral and cerebellar extracts (if verified) argues against sharp localization, but this may be simply a reflexion of the complexity of the task employed, with different types of conditioning being recorded at each level.

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REPAIR OF X-RAY DAMAGE TO THE DEOXYRIBONUCLEIC ACID IN *Micrococcus radiodurans*

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THE dose of radiation required to inactivate a cell is determined by the extent of radiochemical damage and the capacity of the cell to restore this damage. While much evidence implicates DNA as the site of the primary radiochemical lesion, several possibilities have been considered to explain why cells such as *Micrococcus radiodurans* exhibit extreme resistance to X-rays[1], including critical structures which require exceptional doses of radiation to be damaged, the possession of intracellular protective agents[2], and the operation of very efficient repair processes[3]. *Micrococcus radiodurans* is also remarkably resistant to the lethal action of ultra-violet irradiation[4], but Setlow and Duggan[5] found that the DNA of this organism, as judged by thymine dimer production, is not particularly resistant to ultra-violet radiation damage and they suggest that the bacterium possesses a very efficient DNA repair system for ultra-violet damage. Furthermore, the ultra-violet action spectrum points to a possible role of deoxycytidine or protein damage in cell inactivation as well as in the delay in DNA synthesis observed at sub-lethal doses[6].

Possession of an intracellular protective agent has been proposed to account for the resistance of *M. radiodurans* to X-rays[2] and support for this hypothesis is lent by the finding that hydroxymercuribenzoate and iodoacetamide—both powerful sulphydryl poisons—enhance the inactivation by X-rays[7–9]. Radiosensitization by iodoacetamide, however, is not due to removal of intracellular —SH prior to irradiation, and the possibility has been considered[3] that these sensitizers act by enhancing the X-ray inactivation of the metabolic repair processes in radio-resistant organisms.

M. radiodurans shows 100 per cent survival following irradiation with 110 krads of X-rays under anoxia, a dose which has been shown to produce considerable physico-chemical damage to the DNA of *E. coli B/r* (ref. 10). Until recently it has not been possible to determine the nature and extent of the *in vivo* X-ray damage to the chromosome of *M. radiodurans* since no suitable method for isolation of the DNA has been available. The culture fluids of *Streptomyces albus G* have been shown to contain a number of bacterial cell-wall degrading enzymes[11,12], and one of these, the '*R*1 fraction'[13], was found to be lytic towards *M. radiodurans*[14]. This observation has led to the development of a suitable procedure for isolation of DNA from this bacterium. This communication describes some of the changes which occur in the DNA of *M radiodurans* following X-irradiation.

Radiosensitivity of M. radiodurans DNA in vivo. Exponentially growing cells of *M. radiodurans* were obtained by diluting a 14-h-old culture in TGY medium (0·5 per cent tryptone, 0·1 per cent glucose, 0·3 per cent yeast extract) three-fold with fresh medium and incubating in shake culture at 30° C until the optical density at 650 mμ reached a value of 0·8. The culture was chilled, the cells collected in a Sharples centrifuge, washed once in 0·04 M veronal buffer *p*H 8·6 and resuspended in the same buffer at a concentration of approximately 5×10^{10} cells/ml. Stirred samples were irradiated at 4° C under nitrogen with 220 kV X-rays at a dose rate of $1{\cdot}1 \times 10^4$ rads/min. A survival curve for cells irradiated under these conditions is presented in Fig. 1 and shows that no cell inactivation occurs at doses below 0·5 Mrad.

To extract the DNA, 20-ml. samples of the suspension in veronal buffer were incubated with 5 mg *Streptomyces* '*R*1 fraction'[13] for 15 min at 30° C. This suspension was then cooled in ice and 10-ml. samples irradiated at 4° C. Immediately after irradiation, 4-ml. samples of this suspension were added to a solution containing 50 ml. TGY medium, 15 ml. 0·2 M citrate-phosphate buffer *p*H 5·6 and 13 ml. 20 per cent sodium dodecyl sulphate. After incubation for 15 min at 30° C to initiate lysis the samples were left to stand at room temperature until dissolution of the cells was complete (about 1–2 h). The clear, highly viscous solution was made 1 M with respect to sodium chloride and the sample shaken gently at room temperature for about 12 h. After centrifugation at 10,000*g* for 10 min to remove precipitated protein and polysaccharide, an equal volume of 2-ethoxyethanol was added slowly to the supernatant to precipitate the DNA. The fibrous material was collected, rinsed briefly in absolute ethanol and redissolved in 30 ml. of M/100 sodium chloride. 3 ml. RNase solution (4 mg/100 ml.) were added and the samples incubated at 25° C for 4 h. After addition of 1·5 ml. of 20 per cent sodium dodecyl sulphate and 8 ml. of 5 M sodium chloride, the samples was shaken gently for 15 min and any insoluble material was removed by centrifugation at 15,000*g* for 10 min. The DNA was precipitated by addition of an equal volume of 2-ethoxyethanol and the fibres washed twice in absolute ethanol, once in acetone and air dried. The yield of DNA was 50–80 per cent. The treatment with *Streptomyces* '*R*1 fraction' alone caused no cell inactivation during a period of 2 h and the X-ray survival curve for enzyme treated suspensions is identical to that shown in Fig. 1.

The molecular weight of the DNA was estimated by measurement of its intrinsic viscosity (η) in a Couette viscometer and its sedimentation coefficient $S_{20,w}$ in the Spinco model *E* ultracentrifuge with ultra-violet optics. From these measurements it is possible to determine the nature of the changes in macromolecular structure following X-irradiation[15]. Fig. 2 shows the reciprocal values of the intrinsic viscosities of DNA samples extracted

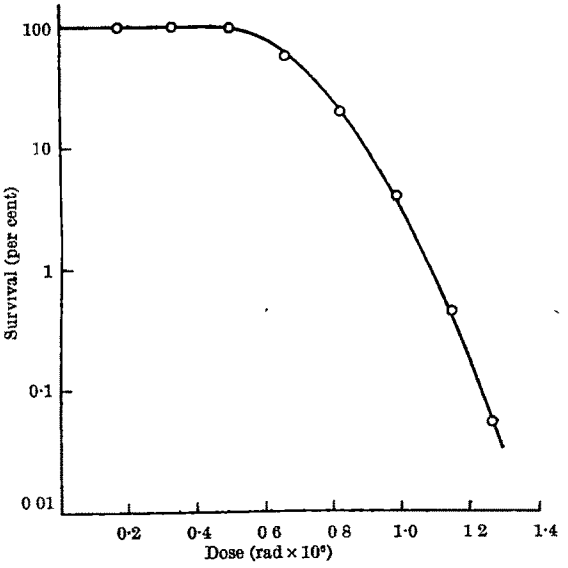


Fig 1. Survival curve of *M. radiodurans* irradiated under anoxia

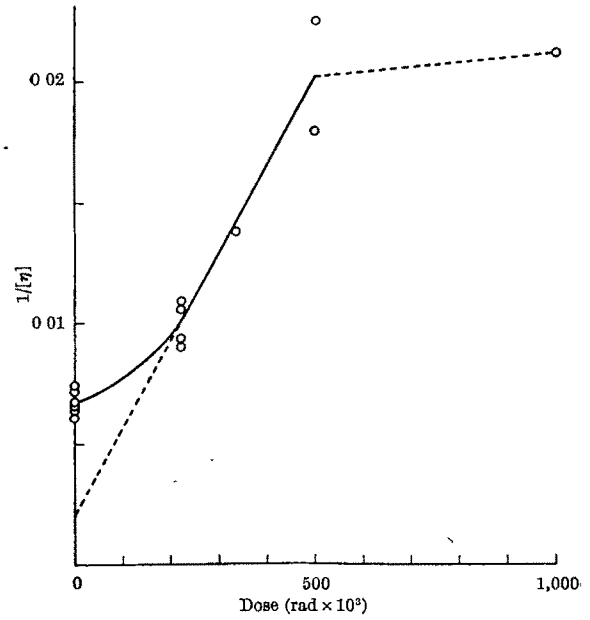


Fig. 2. Effect of X-rays *in vivo* on the reciprocal intrinsic viscosity of *M. radiodurans* DNA

from cells immediately after irradiation with doses of 220, 330, 495 and 990 krads. The results show that there is a considerable decrease in viscosity of the irradiated samples and that the extent of the decrease is dose dependent.

When DNA is irradiated with sparsely ionizing radiations *in vitro*, the change in its intrinsic viscosity is a direct measure of the number of scissions in the twin helix (double breaks) caused by a given radiation dose[15]. The physico-chemical changes observed in the DNA extracted from *M. radiodurans* indicate that over a limited dose range a similar relation obtains as in Table 1.

Table 1. Viscosity of DNA isolated from X-irradiated Cells during Post-irradiation Incubation at High Cell Concentrations (about 5 × 10⁸/ml.)

Dose (krads)	Incubation time	$S_{20,w}$	$[\eta]$ (dl./g)	% Yield DNA	Molecular weight[26] calc. from $[\eta]$	Molecular weight[26] calc. from $S_{20,w}$
0	0	27·2	151	77	13·7 × 10⁶	13·1 × 10⁶
220	0	24·2	92	55	8·8 × 10⁶	9·5 × 10⁶
0	4 h at 30° C	—	150	73	—	—
220	4 h at 30° C	—	93	60	—	—

The plot of the reciprocals of the intrinsic viscosities of *M. radiodurans* DNA with increasing cellular X-ray dose is shown in Fig. 2 and it can be seen that linear extrapolation gives a much higher zero dose intrinsic viscosity than the actual experimental controls. The behaviour above 500 kr. may indicate the presence of cross-linking[15].

DNA of high molecular weight is very susceptible to hydrodynamic shear[16]. A given shear rate will reduce all molecules above a certain size to a lower size distribution, but longer shearing at the same rate will have no further effect. In a similar fashion different shear rates will cause plateaux at different size distributions[17]. If the DNA extraction procedures utilized for *M. radiodurans* impose approximately constant shear rates this would explain why the function in Fig. 2 does not extrapolate to the control viscosity because on this basis the rate of shear will reduce control and irradiated DNA alike to a limiting size, unless the irradiation alone reduces the molecular weight below this limit. Thus the control viscosities so far as irradiation is concerned would not be the 150 dl./g measured experimentally, but could be nearer the 500 dl./g indicated by the extrapolation in Fig. 2.

The linear portion of the graph in Fig. 2 suggests that *M. radiodurans* DNA is about 25 per cent more resistant to X-ray-induced double breaks than the DNA of *E. coli B/r* (ref. 10) when irradiated *in vivo*. At a sensitivity of 140 eV per number-average double break the *M. radiodurans* DNA is about six times less resistant than salmon and herring sperm DNA irradiated *in vitro*[15]. The apparent increased radiosensitivity of DNA *in vivo* may be due to a large contribution by indirect action[18], or to 'energy transfer' from the proteins associated with the DNA[19]. However, it can also be explained as another result of hydrodynamic shear since irradiation of DNA also causes single breaks, that is, scission of only one chain of the twin helix[15]. These single breaks constitute points of weakness in the DNA molecule and the intact strand may rupture at these points under shear rates which would not be expected to produce double breaks in an undamaged molecule, with the net result of more double breaks than were actually caused by irradiation. If the X-ray sensitivities of herring and salmon sperm DNAs irradiated *in vitro* represent the true situation occurring in *M. radiodurans*, then this would require that at least five single breaks are produced for every double break in the bacterial DNA on X-irradiation. The extrapolation in Fig. 2 is very uncertain because both the types of shearing damage discussed above can occur at the same time and there is also no reason to expect the intrinsic viscosity to be linearly related to the molecular weight above molecular weights of 20×10^6. All that can be said is that the true control molecular weight is very large, perhaps encompassing all the DNA in the cell. There can be no doubt, however, that the DNA of *M. radiodurans* has sustained considerable damage at doses where the cells exhibit 100 per cent survival. If the DNA of *M. radiodurans* is contained in a single chromosome as described for *E. coli*[20], then it must possess the capacity for the repair of double breaks in the twin helix.

Post-irradiation incubation. Cells were grown and irradiated as described and then diluted into fresh medium and incubated at 30° C with shaking. Incubation was examined at two concentrations, namely, *c.* 4×10^9 cells/ml. and 2×10^8 cells/ml. At the former concentration no cell growth would be expected while at the latter, unirradiated cells will undergo exponential growth for at least 3 h. During incubation, samples of the cultures were taken for determination of optical density at 650 mμ, DNA content by the method of Burton[21], and acid insoluble protein by the procedure of Lowry *et al.*[22]. When cells which had been irradiated to a dose of 220 krads were incubated at the higher cell concentration in fresh medium no change in either DNA or protein content was observed during a 4-h incubation period at 30° C. Neither was there any detectable alteration in the viscosity of the DNA extracted from cultures during incubation (Table 1) suggesting that under non-growth conditions no degradation, synthesis, or repair of DNA occurs.

Cultures of unirradiated cells incubated at the lower initial cell concentration show an exponential increase in turbidity, DNA and protein content with a doubling time of 120 min. Cells irradiated to doses of 220, 330 and 495 krads, however, show no net increase in DNA during the early period of incubation (Fig. 3*a*). After this lag, the length of which is dose dependent, DNA synthesis commences at a rate at least equal to that of the control culture. The cells irradiated to 495 krads show a small but significant decrease in DNA content during the period before DNA synthesis starts, indicating that some degradation of the DNA occurs during this time. Both the turbidity and protein content (Fig. 3*b*) of the irradiated cultures increase during the initial period of incubation, but at a rate which is both slower than the control and also dependent on the dose of radiation received. Pronounced degradation of DNA during post-irradiation incubation has been reported in *E. coli* and *E. coli B/r*, but only after doses of X-rays which are both lethal and an order of magnitude less than those described in the present communication. At doses where survival was

50 per cent or more, neither DNA degradation nor delay in synthesis was observed[23,24].

Clearly, at doses of X-rays which cause no cell inactivation, profound changes occur in the metabolic activity of *M. radiodurans*, the most remarkable being the delay in DNA synthesis, a phenomenon also observed following ultra-violet irradiation[5]. Since no increase in cell number occurs until after DNA synthesis resumes, we may conclude that during this delay part or all of the lethal damage in the DNA is repaired. While evidence of synchronized growth is seen in the irradiated cultures, signs of partial synchrony at least can be seen in the unirradiated cultures as would be expected for cell cultures held at the high concentration and low temperature for the 30–60 min required for irradiation.

Repair of the X-ray lesion in DNA. It was found that during post-irradiation growth the cells repaired the partial degradation of the cell wall resulting from treatment with the *Streptomyces R*1 fraction so that cells no longer lysed on treatment with dodecyl sulphate. The DNA isolation procedure was therefore modified. After harvesting from the post-irradiation growth media, the cells were resuspended in 0·04 M veronal buffer *p*H 8·6 at a concentration of *c.* 10^{10} ml. and treated with '*R*1 fraction'. The remaining steps in the isolation procedure were then carried out as described previously.

Marked changes in both the viscosity and extractability of the DNA occur during post-irradiation incubation under growth conditions. After approximately 2 h incubation, the DNA extracted from cells which had received 220 krads of X-rays gave the same viscosity as that from unirradiated cells (Table 2). DNA extracted from cells incubated for 1 h, however, showed a lower viscosity than the zero time irradiated samples, indicating that some degradation of the DNA occurs during the 'lag period'. During the 'lag period' considerable difficulty was encountered in preparing samples of fibrous DNA by the standard technique from cells which had been irradiated to doses of 330 and 495 krads. After DNA synthesis had commenced, samples of fibrous DNA could again be prepared, and it is apparent from Table 2 that even after doses of 330 and 495 krads the viscosity of the DNA had returned to that of the control value. The inability to prepare samples for viscosity measurement from heavily irradiated cells during the early part of the repair process is due, in part, to the non-precipitability of the DNA in 2-ethoxy ethanol. This loss in DNA precipitability does not seem to be due to extensive denaturation because the buoyant density of DNA extracted from an irradiated cell lysate by caesium chloride equilibrium density centrifugation is the same whether the cells were taken immediately after irradiation, in the middle of the post-irradiation lag phase or after the onset of DNA synthesis. The data would therefore indicate a further reduction in the molecular size of the DNA. It is also possible that during this time part of the DNA is rendered non-extractable because of chemical combination with protein such that the buoyant density is not altered in caesium chloride.

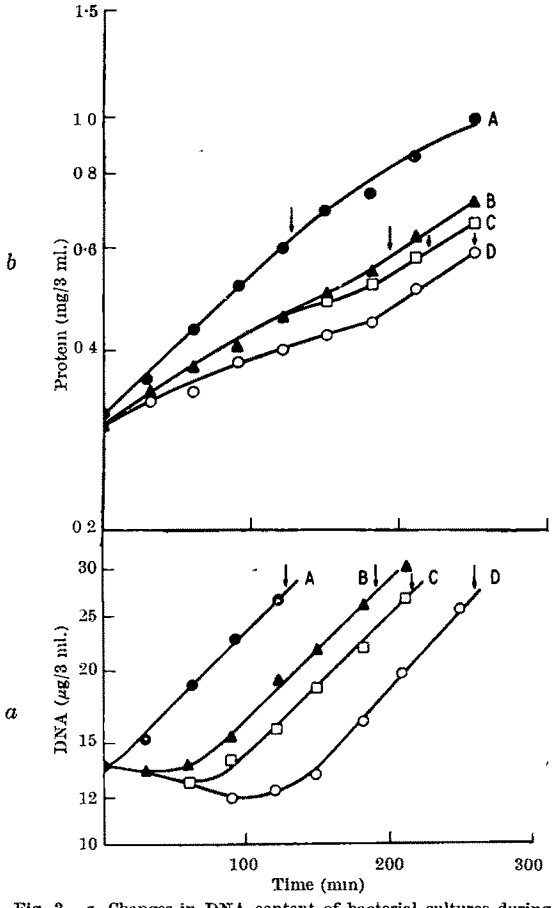


Fig. 3. *a*, Changes in DNA content of bacterial cultures during post-irradiation incubation under growth conditions, *b*, protein content of cultures during post-irradiation incubation under growth conditions. *A*, Control (●), *B*, 220 kr (▲); *C*, 330 kr (□), *D*, 495 kr. (○)

Table 2. CHANGES IN VISCOSITY (AS PERCENTAGE OF CONTROL) OF DNA ISOLATED FROM X-IRRADIATED CELLS DURING POST-IRRADIATION INCUBATION AT LOW CELL CONCENTRATIONS (ABOUT 2 × 10^8 CELLS/ML. INITIAL CONCENTRATION)

Dose (krad)	(η) % control at time (h) 0	1	1·5	2·0	2·5	3·5	4·0	4·5
220 (i)	69·0	60·5	—	99·0	—	—	—	—
220 (ii)	65·5	48·5	—	91·0	—	100	—	—
330	49·5	—	*	—	69·0	—	96·0	—
495	33·0	—	—	—	*	—	—	95·0

— No sample taken * No fibrous DNA could be prepared at these times (see text)

It is quite clear from these results that *M. radiodurans* possesses a most efficient DNA repair system which is even capable of restituting 'double breaks' in the twin helix. The decrease in molecular size seen during the early part of the repair process indicates that damaged regions of the DNA are being removed. Confirmation of such an excision process has been found following X-irradiation of cells of *M. radiodurans* labelled in their DNA with tritiated thymidine. Measurements of tritium activity of cold perchloric acid (PCA) extracts of cells and also of the media suggest that tritium label passes first into the PCA soluble fraction and then into the medium (Fig. 4). The excision process begins immediately on incubation and the quantity released reaches its maximum at the time when DNA synthesis commences. Thus during the 'lag period' when no DNA synthesis is observed, excision of damaged regions of the DNA is occurring.

Four possible schemes for the repair of X-ray damaged DNA in *M. radiodurans* may be considered, namely: (1) Excision, followed by restitution, followed by synthesis; (2) excision, followed by restitution and synthesis together; (3) excision and restitution together, followed by synthesis; (4) all three together.

In all four cases the term 'excision' may include removal of areas adjacent to the damaged sites, as has been envisaged by Boyce and Howard-Flanders[25] following ultra-violet damage.

The release of labelled material reaches its maximum before the onset of DNA synthesis; therefore, these two processes would seem to be divorced from one another. This argues against scheme 4. While labelled material is being released from the cells the amount of DNA estimated by the Burton method also drops, and while it is not possible at present to compare accurately the relative losses it is evident that more than 75 per cent of the excision loss is not restituted prior to the onset of synthesis proper. This argues against schemes 1 and 3. If restitution is not substantially complete before the onset of synthesis, the DNA might be expected to show symptoms of its unrestituted state. This may explain

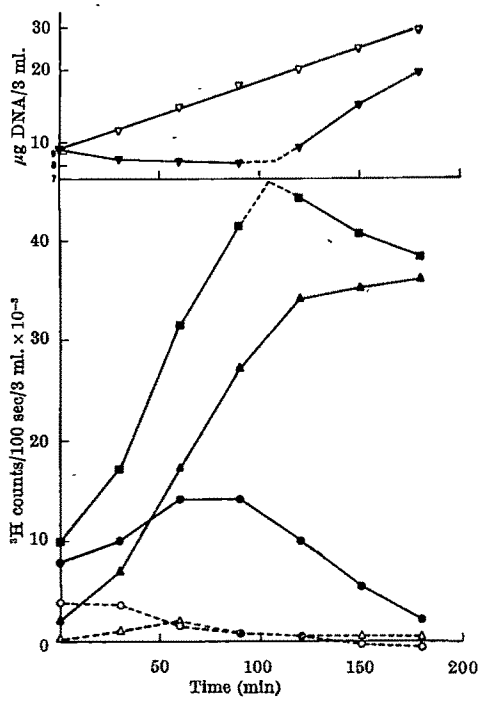


Fig. 4. The release of tritium activity from the DNA of cells given 330 kr. of X-rays, on post-irradiation incubation under growth conditions in TGY medium supplemented with cold thymidine. *a*, Into PCA soluble fraction (○●); *b*, into medium (△▲); *c*, total counts (■). Also shown is the total PCA insoluble DNA content measured by the Burton method. Open symbols are control and closed symbols are irradiated samples. Total activity released 13·6 per cent (of DNA); maximum loss in activity from DNA 14 per cent; maximum loss in DNA by Burton method 12 per cent

why the difficulty in extracting the DNA from lysates of irradiated cells increases during the period of excision, reaches a maximum prior to the beginning of DNA synthesis and then decreases as DNA synthesis proceeds. Once the irradiated cell suspension has doubled its initial DNA content the DNA is indistinguishable, by the physico-chemical methods employed, from control DNA. Most of the evidence would therefore point to scheme 2, and if this is the case then a DNA polymerase may possess the ability to effect both restitution and synthesis.

More difficulty is experienced in explaining the repair of the physico-chemical damage suffered by the DNA on biological grounds. From the interpretation given earlier for the change in molecular size of the DNA on irradiation (Fig. 2) it follows that if the bacterial DNA is all in one piece, and shearing is excluded, the effect of 330 kr. of X-rays will be to cause several hundred scissions of the twin helix (double breaks)—quite apart from single breaks and other damage—and in addition, more than 10 per cent of the DNA components will have been excised from the DNA before the onset of restitution and synthesis. Yet, by the time that the irradiated suspension has doubled its initial DNA content, all these scissions of the twin helix will have been repaired. Either the nucleoprotein structural proteins and/or RNA must play a very important part in maintaining the DNA template or else *M. radiodurans* can tolerate errors in its genetic code and still appear unchanged so far as currently available biological parameters are concerned.

While the restoration of X-ray induced DNA damage in cells of *M. radiodurans* is clearly of importance in terms of the survival of the cells, it cannot be stated with certainty at the present time that cell death occurs because some of the DNA damage is not repaired. Moreover, the restoration of radiation damaged DNA may represent but a part of the capacity of this extraordinary micro-organism for the restoration of cellular damage.

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EVALUATION BY RADIOIMMUNOASSAY OF FACTORS CONTROLLING THE SECRETION OF PARATHYROID HORMONE

Intravenous Infusions of Calcium and Ethylenediamine Tetraacetic Acid in the Cow and Goat

PREPARATION of purified bovine parathyroid hormone (PTH) has permitted more definitive characterization of its chemical and immunological properties, as well as its physiological behaviour[1]. In 1963, Berson, Yalow, Aurbach, and Potts[2] reported the development of a specific and sensitive radioimmunoassay for the bovine hormone. In principle, this technique was similar to the methods developed for the measurement of insulin and growth hormone[3], and depended on the inhibition by unlabelled hormone of the binding of hormone labelled with iodine-131 to specific antibody. With recent improvements in the sensitivity of the assay, particularly the use of non-equilibrium conditions[4], it has been possible to measure reproducibly the concentration of PTH in normal bovine plasma and perform a number of physiological studies concerning the regulation of hormone secretion[5].

Bovine PTH was purified by gel filtration[6] and ion exchange chromatography[1]. This highly-purified product was labelled with iodine-131 (IsoServe Corporation, Cambridge, Massachusetts) by the method of Hunter and Greenwood[7] and was used as the test antigen. Antibody produced by repeated injections of a crude hormone preparation into guinea-pigs was used at a final dilution of 1/300,000 in the assay[2].

Parathyroid hormone in bovine or caprine plasma was measured by a modification of the method of Berson *et al.*[2]. In the non-equilibrium assay, unlabelled hormone or unknown plasma was pre-incubated at 4° C with antibody for 4–5 days; labelled hormone was then added to the mixture for 24–48 h. This technique offered several advantages: (1) the assay was more sensitive, since there was a greater decrease in bound to free ratio for a given amount of unlabelled hormone; (2) the shortened incubation period markedly decreased radioactivity 'damage'[3] and allowed the use of labelled hormone of high specific activity (700–1,000 μc./μg); (3) the binding of unlabelled hormone was facilitated by keeping the concentration of antibody greater than the final dilution (1/300,000) during the pre-incubation phase. With these specific improvements, concentrations of hormone in normal unextracted bovine plasma could easily be measured at a 1/5–1/10 dilution, and absolute amounts of hormone as small as 10 μμg could be detected.

Samples of jugular venous blood were obtained from normal cows under control conditions and during infusions designed to alter plasma calcium concentration. Disodium EDTA or calcium chloride was administered by intrajugular catheter to conscious animals, while periodic samples for calcium determination and immunoassay were obtained from the other jugular vein. Plasma calcium was measured by a modification of the EDTA method[8], the standard deviation being ±0·1 mg/100 ml. Concentrations of calcium in plasma were consistently 0·1–0·2 mg/100 ml. greater than similar measurements in serum, but the results of immunoassay were not significantly different. Bovine plasma was routinely assayed in duplicate at a dilution of 1/5, the standard deviation being ±0·065 mμg for concentrations less than 1·0 mμg/ml. and ±0·171 mμg for concentrations of 1·0–6·0 mμg/ml.

Similar infusions were administered to goats anaesthetized with methoxyfluorane through an endotracheal tube. Solutions of calcium gluconate or disodium EDTA were infused via a femoral vein, and jugular venous samples were obtained periodically for measurement of calcium and PTH concentration.

Initial investigations of the cross-reactivity of purified bovine PTH and the endogenous hormone in bovine plasma indicated a reaction of complete identity (Fig. 1). Likewise, there was excellent cross-reactivity between endogenous caprine hormone and purified bovine PTH. While this reactivity was not complete, it was sufficient to permit measurement of PTH concentration in normal caprine plasma at a 1/5–1/1 dilution.

The concentration of PTH in the plasma of 24 normal cows varied from 400 to 1,800 μμg/ml., with a mean of 1,070 (Fig. 2). There was a reciprocal relationship between plasma calcium and the concentration of PTH in the normal animal; this relationship was statistically significant with a correlation coefficient of −0·6.

Infusions of calcium chloride, with corresponding elevation of the plasma calcium, consistently decreased the measureable concentration of hormone in peripheral bovine plasma. This is shown in Fig. 3, where gradual elevation of plasma calcium was associated with a marked reduction in the concentration of PTH. Two hours after beginning the infusion, hormone was not detectable, and even 5 h after the infusion, PTH concentration had not returned to normal. In three different experiments, elevation of blood calcium to 14 mg/100 ml. was accompanied by a complete disappearance of measurable hormone (less than 100 μμg/ml.).

With prolonged infusions of disodium EDTA in two animals (Fig. 4), a decrease in plasma calcium was associated with: (1) a marked and sustained increase in the concentration of PTH; (2) elevation of PTH concentration to three to four times the control value during maximal stimulation; (3) persistence of increased concentrations of hormone despite a prolonged hypocalcaemic stimulus (20 h in one animal and 36 h in the other).

The reciprocal relationship between calcium and PTH concentration was shown more vividly when alternate infusions of calcium, EDTA, and calcium were administered (Fig. 5). Despite the constant rate of fall in plasma calcium with EDTA from 14·5 to 6·1 mg/100 ml., there was no real rise in PTH concentration until the plasma calcium was less than 10 mg/100 ml. In addition, stimulation of the gland with EDTA following suppression with calcium (Fig. 5) produced a smaller increase in PTH

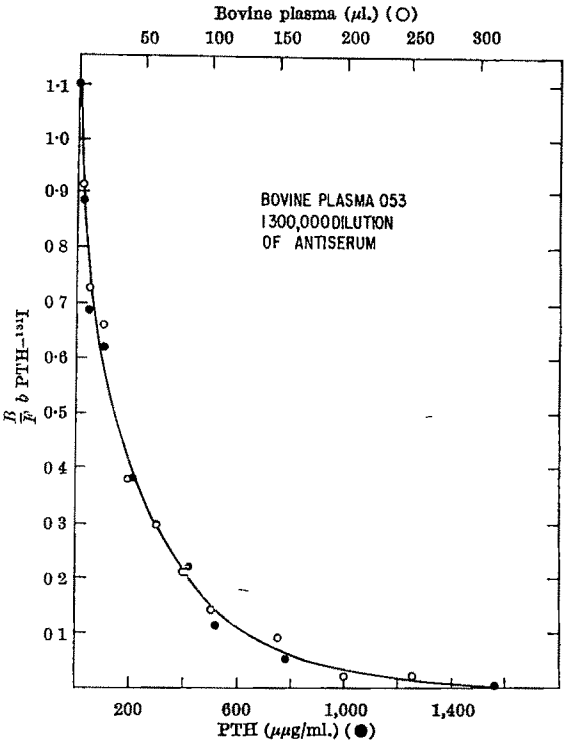


Fig. 1. Standard curves obtained with a purified preparation of bovine PTH (closed symbols, μμg/ml.) and with aliquots of bovine plasma (open symbols, μl. of plasma added to total incubation mixture of 0·5 ml.). The curves through the two sets of points are identical, suggesting a reaction of complete identity between the purified preparation and endogenous hormone in bovine plasma

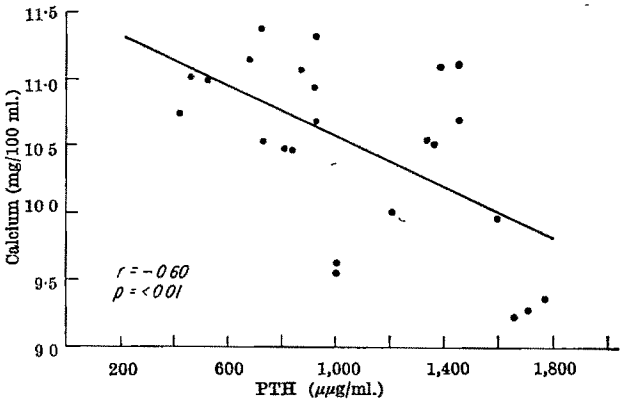


Fig. 2. Distribution of PTH concentration in 24 normal animals. Each assay point represents duplicate determinations in two separate assays (the mean of four values). There is a statistically significant inverse correlation (*P* value less than 0·01) between calcium and PTH concentration in normal animals

concentration than did stimulation without prior administration of calcium (Fig. 4).

The role of calcium ion in the regulation of PTH secretion was also studied during normal lactation and during the syndrome of parturient paresis (or 'milk fever'). Mild physiological hypocalcaemia accompanying the onset of normal lactation (about 8 mg/100 ml.) was frequently

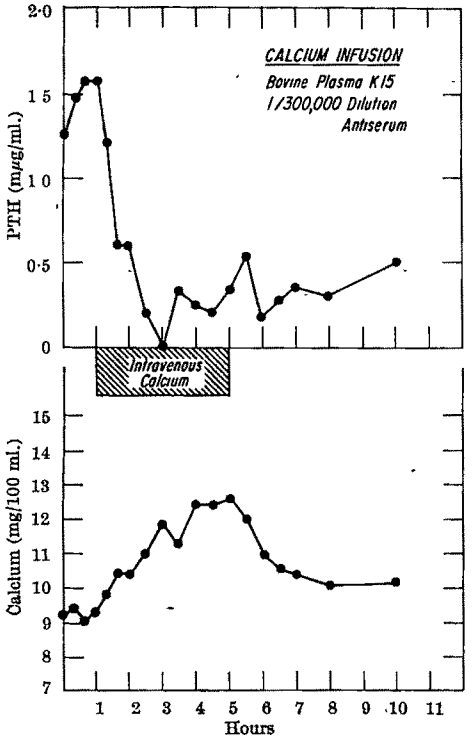


Fig. 3. The effect of a 4-h infusion of calcium chloride (99·6 m.equiv./h) on plasma PTH and calcium concentration in the cow

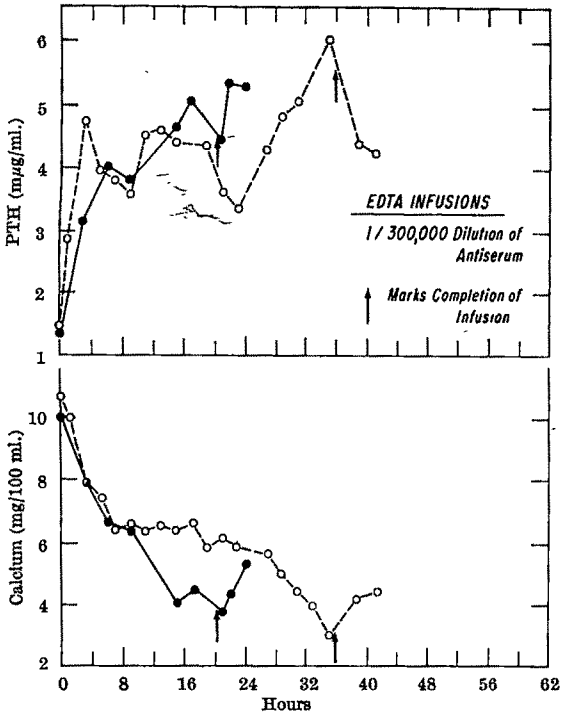


Fig. 4. Infusions of disodium EDTA in two normal cows for 20·5 h (closed symbols, 40 m.equiv./h) and 36 h (open symbols, 57·5 m.equiv./h), respectively; the effects on plasma calcium and PTH concentration are shown. Arrows mark the end of each infusion. Of note is the continued stimulation of PTH production despite the prolonged hypocalcaemic stimulus

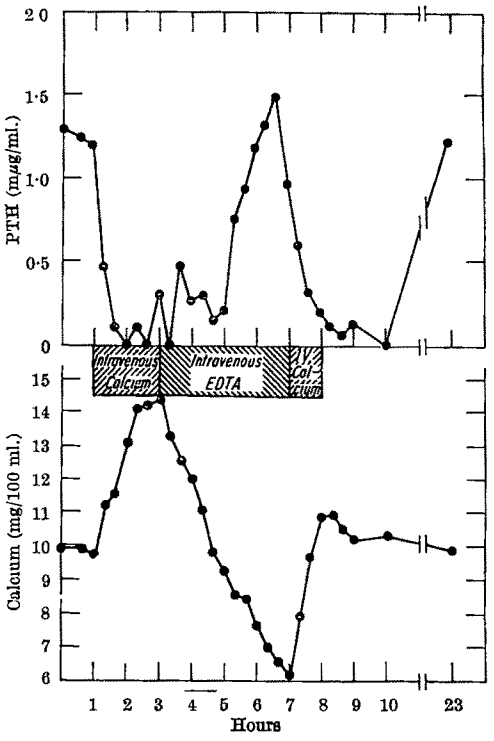


Fig 5. Concentrations of PTH and calcium in bovine plasma in response to alternate infusions of calcium chloride (196·4 m equiv./h), disodium EDTA (115 m.equiv /h), and calcium chloride (196·4 m.equiv./h)

associated with an increase in PTH concentration to two to three times normal. In addition, animals with parturient paresis, a syndrome characterized by marked hypocalcaemia and flaccid paralysis during the postpartum period, showed elevated concentrations of PTH (similar to the concentrations produced by maximal stimulation of the gland with EDTA).

Parathyroid hormone in normal caprine plasma was measured in conjunction with parallel experiments involving perfusion of the isolated superior parathyroid gland of the goat[9]. Variation of the plasma calcium concentration by alternate infusions of EDTA and calcium produced changes in PTH concentration (Fig. 6) which fully corroborated the findings with bovine plasma and permitted direct correlation with the results of isolated parathyroid gland perfusion.

It is reasonable to assume that there is a close correlation between the estimates of PTH concentration obtained by immunological methods and the actual content of biologically active hormone in plasma. The evidence in favour of this approach, as well as the sources of potential error, has been extensively discussed[3,10]. The results reported in this and the accompanying article[9] at least satisfy empirical criteria; the changes in hormone concentration follow the pattern predicted by earlier investigations[11–14] of hormone secretion during physiological variations in plasma calcium. In addition, evidence is provided from isolated gland perfusions in a sheep and goats that the changes in PTH concentration in peripheral plasma are due to actual alterations in the secretion rate of hormone and not merely to changes in its distribution, excretion, or rate of destruction[9].

It is generally believed that the concentration of ionized calcium in the blood directly controls the secretion rate of PTH. Production of parathyroid hyperplasia by diets low in calcium[11], perfusion investigations by Patt and Luckhardt[12] and Copp and Davidson[13], and the *in vitro* investigations of parathyroid cells by Raisz[14] all provide evidence to support this hypothesis. With the use of a sensitive assay to detect PTH in plasma, it has been possible to confirm directly the control of hormone secre-

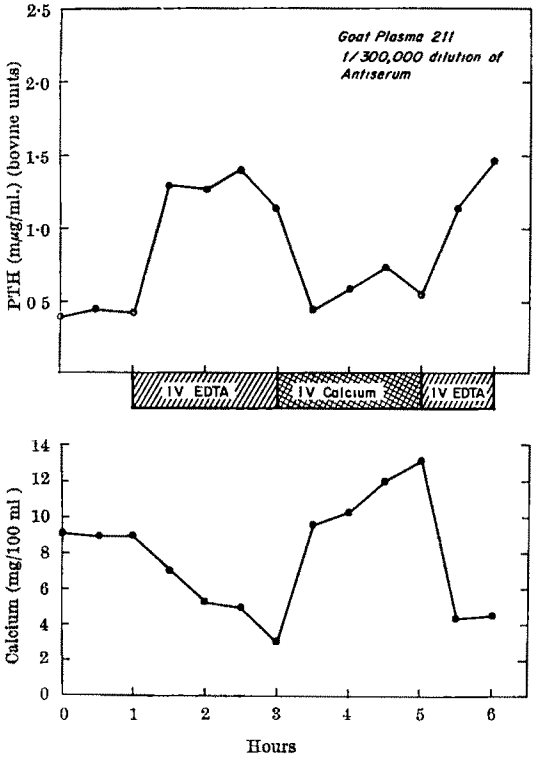


Fig. 6. Concentrations of PTH and calcium in caprine plasma in response to alternate infusions of disodium EDTA (6·44 m.equiv /h), calcium gluconate (9·5 m.equiv /h), and EDTA (6·44 m.equiv./h)

tion by blood calcium. In addition, the presence of an inverse correlation between calcium and PTH in the normal animal suggests that the hormone is being continuously secreted, and that it is under the fine and precise control of blood calcium level[15,16].

With maximal suppression or stimulation of the gland, peripheral hormone concentrations may vary from undetectable values (less than 0·1 mμg/ml.) to 6·0 mμg/ml. The normal range in the animals so far examined has been between 0·4 and 1·8 mμg/ml. Severe hypocalcaemia associated with parturient paresis produced the same increase in PTH concentration noted after maximal stimulation with EDTA, suggesting that an absolute defect in hormone secretion is not the cause of this disorder. An increase in PTH concentration during hypocalcaemia in the cow was thus directly demonstrated without the necessity of administering EDTA acutely to lower plasma calcium.

The maintenance of blood calcium in its narrow homoeostatic range is a highly complex process. It seems clear that changes in PTH secretion play a major part in this homoeostasis, but the interrelationships of PTH, thyrocalcitonin[17], and/or possible hypocalcaemic factors from the parathyroid gland[15] remain to be elucidated. The work recorded here indicates that changes in PTH concentration may appear as rapidly as 20 min after starting an infusion of calcium or EDTA. The diminished response to EDTA stimulation after previous infusion of calcium may reflect a delay in the restoration of hormonal synthesis following suppression of the gland. Preliminary results by the immunoassay method also suggest that magnesium acts like calcium in suppressing PTH secretion[9,18], and that plasma phosphate only indirectly affects PTH secretion by influencing the concentration of ionized calcium in the blood[18].

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Perfusion of the Isolated Parathyroid Gland of the Goat and Sheep

The investigations of Sherwood *et al.*[1] provide direct evidence from intravenous infusions of calcium and EDTA in the cow and goat that the concentration of parathyroid hormone (PTH) in peripheral plasma varies inversely with blood calcium. The development of techniques for perfusing the isolated superior parathyroid gland of the goat or sheep[2,3] and the strong degree of cross-reactivity between bovine and caprine hormone[1] permitted the direct measurement by radio-immunoassay of the concentration of PTH in the venous effluent of the perfused gland[4]. Variations in the amount of hormone leaving the gland undoubtedly reflect alterations in its rate of secretion; direct assessment of the factors which influence hormone secretion is thus made possible.

(*A*) *Acute Experiments.* Three 12-month-old Nubian Cross female goats, weighing 25–39 kg, were used for the perfusion investigations. The operative procedure was carried out under methoxyfluorane/oxygen closed circuit anaesthesia. Via a midline incision, both thyroid lobes containing the inferior parathyroid glands were removed. The arterial supply and venous drainage of the left superior parathyroid were identified; they were usually in common with those of the left apical lobe of the thymus. The external maxillary artery and vein and the left femoral artery were catheterized with silicone elastic tubing (external diam. 2·1 mm, internal diam. 1·0 mm). Except for those vessels related to the parathyroid and

thymic lobe, all branches of the left carotid artery and jugular vein were ligated between the levels of catheterization and the ligated left superior thyroid artery and vein[2]. Thus, the blood supply to, and drainage from, the left superior parathyroid gland could be isolated from the general circulation by clamping the carotid artery and jugular vein caudal to the points of emergence of the parathyroid vessels. The left apical lobe of the thymus, which contained the left superior parathyroid gland, was separated from the remainder of the thymus. The right superior parathyroid was not disturbed.

After the goat was treated with heparin (250 units/kg initially, then 125 units/kg every 2 h), the left parathyroid gland was perfused with blood of controlled calcium concentration, as described by Care *et al.*[3]. Parathyroid venous blood was obtained via the catheter in the external maxillary vein, after the left jugular vein had been clamped caudal to its junction with the parathyroid vein. The plasma calcium concentration in the perfusate was varied over a wide range (0–13·8 mg/100 ml.) by the addition of either disodium EDTA or calcium gluconate, in 0·9 per cent saline. In two experiments the gland was perfused with blood containing a high concentration of magnesium (2·8–3·7 m.equiv./l.).

The flow-rate of perfusate in the afferent and efferent vessels was measured; they should be equal when satisfactory surgical isolation of the parathyroid *in situ* has been achieved. The concentrations of calcium (by a modification of the method of Hill[5]) and parathyroid hormone (by a modification[1] of the method of Berson *et al.*[4]) in parathyroid venous plasma were determined at suitable intervals. Plasma magnesium was measured by flame photometry[6]. At the end of each gland perfusion, indian ink was added to the perfusate and the perfused gland excised for histological examination to ensure that the parathyroid gland had, in fact, been perfused.

(*B*) *Conscious Animal.* A gland perfusion was performed in one 18-month-old Herdwick sheep (28 kg). The operative procedure, similar to that already described here, was performed under aseptic conditions on the day preceding the parathyroid perfusions. The carotid artery caudal to the parathyroid vessels, with its branches ligated, was placed in a skin tube for ease of temporary occlusion during the gland perfusion. The jugular vein could be occluded with a 2/0 mersilene snare placed caudal to its junction with the parathyroid vein when collection of parathyroid venous blood was required.

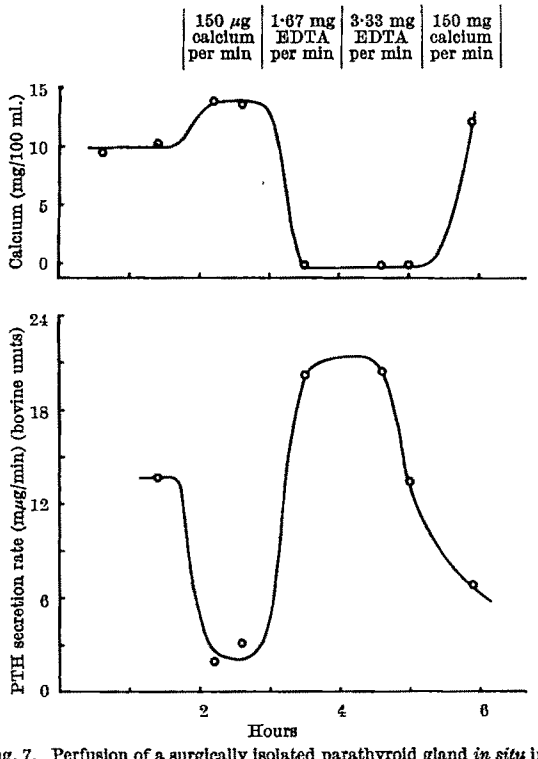


Fig. 7. Perfusion of a surgically isolated parathyroid gland *in situ* in an anaesthetized goat The thyroid and inferior parathyroid glands were removed. Secretion rate of PTH varied inversely with changes in plasma calcium concentration. Blood flow through the gland was maintained at 2·7 ml./min

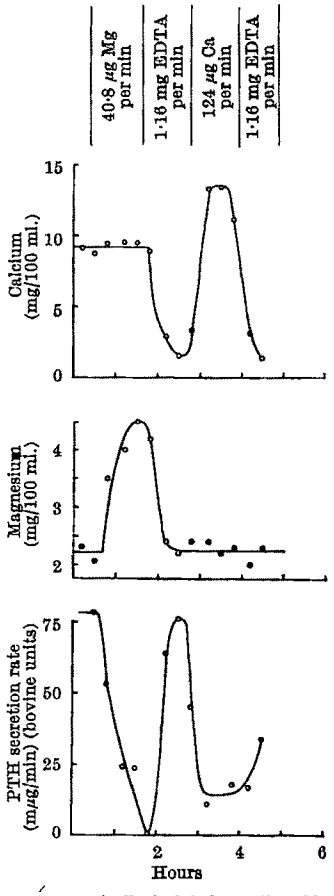


Fig. 8. Perfusion of a surgically isolated parathyroid gland *in situ* in an anaesthetized goat. The thyroid and inferior parathyroid glands were removed. Blood flow through the gland was maintained at 2·7 ml./min. The effects of hypermagnesaemic, hypocalcaemic or hypercalcaemic perfusion on PTH secretion are depicted

The isolated parathyroid gland was perfused for 1·8 h with hypercalcaemic blood, and several parathyroid venous blood samples were collected, some of which were assayed for PTH.

The immunological similarity of bovine and caprine hormone has already been described[1]. Similar cross-reactivity over the most sensitive portion of the standard curve was also observed between bovine and ovine PTH.

The influence of plasma calcium on the secretion of PTH by the perfused gland is shown in Fig. 7. Perfusion of the gland with hypercalcaemic blood (14 mg/100 ml.) caused a marked suppression of PTH secretion to 20 per cent of the initial value. Subsequent perfusion with EDTA resulted in a stimulation of hormone secretion which could easily be suppressed with a second perfusion of calcium. The secretory rate changed rapidly in response to calcium fluctuations, alterations in hormone concentration being evident within 20 min after the calcium concentration of the perfusate was altered[1]. Changes may be even more rapid than this, but in these initial experiments no samples earlier than 20 min were obtained. The secretory rate during the control period (before calcium or magnesium perfusion) appeared to be high, relative to that seen after hypocalcaemic stimulation. This may have been due to the administration of anaesthesia or to the fact that the inferior parathyroids were surgically removed. Still another possibility, suggested by the results obtained with

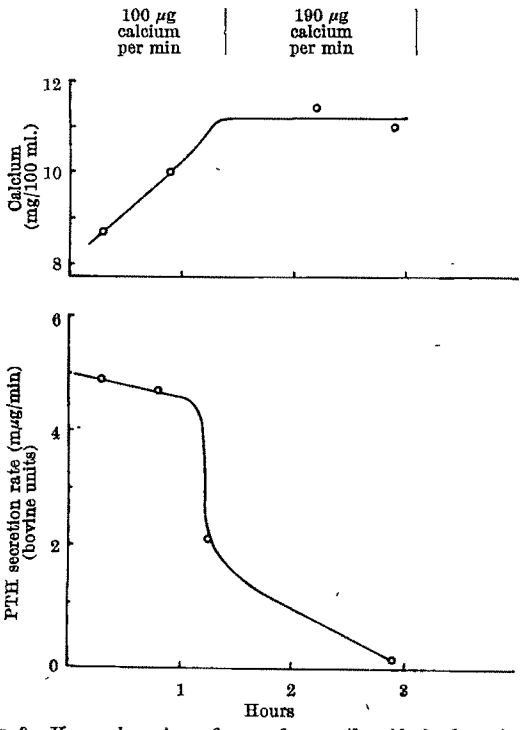


Fig. 9. Hypercalcaemic perfusion of a parathyroid gland *in situ* in a conscious sheep. This gland had been surgically isolated on the previous day, when the thyroid and inferior parathyroid glands were both removed. Blood flow was maintained at 2·2 ml./mn. The effect of hypercalcaemic perfusion on PTH secretion is shown

intravenous infusions in the cow[1], is that the initial perfusion with calcium or magnesium may have partially suppressed the response to subsequent EDTA stimulation.

Similar changes in PTH secretion rate were observed in a second anaesthetized goat (Fig. 8). In addition, perfusion of the gland in this animal with hypermagnesaemic blood caused a decrease in hormone secretion similar to that noted with hypercalcaemia. In a third experiment of the same type, an 80 per cent increase in plasma magnesium concentration resulted in a 30 per cent decrease in PTH secretion within 6 min.

In a conscious sheep, surgically prepared 24 h earlier, perfusion of a parathyroid gland with hypercalcaemic blood caused a similar decrease in the secretion rate of PTH (Fig. 9).

Direct evidence has already been presented[1] that the concentration of PTH in peripheral plasma responds in a reciprocal manner to alterations in plasma calcium concentration. Direct assessment of hormone secretion rate by determination of PTH concentration in the venous effluent of the isolated perfused parathyroid gland has fully confirmed these results. In addition, the perfusion studies indicate: (1) that the initial response of the gland to changes in calcium is rapid (less than 20 min); (2) that the receptor site for calcium regulation is in the parathyroid glands, as previously suggested[7-9]; (3) that the changes in plasma PTH concentration represent actual changes in secretion rate of hormone. Whether there are also variations in the distribution, excretion, or rate of destruction of the hormone in response to changes in concentration of calcium and other ions was not separately tested in these experiments.

There is an interesting similarity in the response to hypercalcaemic or hypermagnesaemic perfusion. Whether magnesium plays a significant part physiologically in regulating PTH secretion, as suggested by MacIntyre *et al.*[10], awaits additional investigation (the use of physiological changes in magnesium concentration by dietary or other means and evaluation of the effects of hypomagnesaemia).

The precise interrelationships of parathyroid and thyroid polypeptides in maintaining calcium homeostasis are now being investigated in a number of laboratories. Direct measurement of these hormones by radioimmunoassay, utilizing both isolated gland perfusates and peripheral plasma, should provide valuable information concerning their physiological function. Precise evaluation of the factors controlling hormone concentration, the rapidity and extent of changes in secretion rate, and alterations in the distribution, excretion or destruction of these proteins will allow more definitive assessment of the finely adjusted hormonal control of blood calcium concentration.

The major part of this work was completed while one of us (A. D. C.) was a visiting scientist in the U.S. National Health Institute, Bethesda, Maryland.

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RELATIONSHIP BETWEEN ENVIRONMENTAL TEMPERATURE AND THE DEATH RATE OF WOMEN FROM NEOPLASMS OF THE BREAST

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IT has been shown for the countries of Norway, Sweden, Scotland, and England and Wales that the death rate from neoplasms of the breast in women is closely correlated with the mean annual temperature[1]. The investigation of this relationship has been extended to all countries for which comparable data were available, and the results are now reported.

The evidence on death rates was taken from a report by Segi and Kurihara[2]. Of the twenty-four countries with which these authors dealt, four were excluded for the following reasons: Canada, United States, and Australia, because the countries are so large that the idea of a mean annual temperature becomes absurd, and Israel because the bulk of the population has only been there for the

past 40–50 years. For the remaining twenty countries the death rates are expressed per 100,000 living and adjusted for age. For Belgium the rates are given only for the years 1954–61, for Portugal 1956–61, and for Chile 1950–57 and 1960–61. For the other countries the rates are for the years 1950–61. The mean values for these periods have been used.

Mean annual temperatures were calculated by a method of weighting. The populations of the largest towns, districts, counties and other divisions of the various countries are given in *Whitaker's Almanack* (1964) (ref. 3) and the *Statesman's Yearbook* (1964–65) (ref. 4). For most of these subdivisions one or more places are quoted by the Meteorological Office, and the mean annual temperature was obtained from published tables[5]. However, for many countries this left a large proportion of the population unaccounted for, and they were assumed to live at the mean temperature for the whole country. Then, by using the population living at each mean annual temperature as a weight, a weighted mean annual temperature for the country as a whole was obtained.

The mean annual temperatures were then plotted against the death rates from neoplasms of the breast, and it was at once obvious that the straight line relationship previously found for Norway, Sweden, Scotland, and England and Wales did not hold for the other countries, although a second degree curve gave a reasonable fit.

At this stage of the investigation it was thought that if the mean temperature influenced the death rate from breast neoplasms, then the range of temperature might also be a controlling factor. This seemed not unreasonable since Greenwood has shown[6] that of nineteen hens kept in a standardized environment (temperature 65° F, relative humidity 60 per cent, artificial light only) eighteen developed adenocarcinoma of the ovaries or oviducts, as compared with a complete absence of such tumours in a control series of fourteen hens kept under normal conditions for the same period.

The Meteorological Office includes in its published tables: (1) the monthly averages of the daily maximum and minimum temperatures; (2) the averages of the extreme values of the daily maximum and minimum for each month; (3) the absolute extremes which have occurred for each month during the entire period of recording. Of these three records (1) was chosen for the present purposes as it was considered to give a better reflexion of the temperature range than the others, because (2) is the average of the abnormal values and (3) is even more abnormal than (2).

The highest and lowest monthly averages were, therefore, taken as representing the range of temperature, and a 'range' for each country calculated by the method of weighted means as described for mean annual temperature. Various combinations of mean annual temperature and 'range' were plotted against the death rates, and it was found that the best fit was obtained by using mean annual temperature + 'range'.

Further evidence of the usefulness of this combination of mean annual temperature + 'range' in biological systems was received from Dr. P. S. Dixon of the University of Liverpool, where a similar result had been found experimentally for the effect of environment on the distribution of marine algae[7].

The technique of multiple regression was then applied to the data to obtain the best combination of mean annual temperature and 'range' for the observed death rates from breast neoplasms. It must, however, be kept in mind that the estimations of both death rates and temperature are known to be inaccurate and that it is not possible to estimate the degree of these inaccuracies. It follows that standard errors for the data derived from the foregoing calculation would have to be regarded with considerable suspicion, and it was finally decided that their use would tend to cause an impression of accuracy which could not be justified from the data.

It was found that:

$$y = 69{\cdot}5614 - 0{\cdot}5249R - 0{\cdot}6200M$$

where y is the expected death rate from neoplasms of the breast, R is the 'range' and M the mean annual temperature, from which it will be seen, bearing in mind the inaccuracies in the data, that 'range' and mean annual temperature produce approximately equal effects.

This provided an additional reason for using the combination of mean annual temperature + 'range', and it was decided to use this representation of the data for the continuation of the work, and to obtain data for all countries which presented them in such a way that they could be reduced to the Segi–Kurihara standard population[2].

Data for twelve more countries which fulfilled this condition were obtained from *Annual Epidemiological and Vital Statistics 1950 to 1961* (ref. 8). In addition it was found that the distribution of the female population of Iceland for the 1960 census did not differ significantly from the Segi–Kurihara standard, so that the crude death rate for that country could be used[9]. Finally the death rate for breast neoplasms for Singapore, adjusted to the Segi–Kurihara standard, is given by Muir[10]. Thirty-three countries were now available, and the data for them are shown in Table 1, with the 'best straight line' calculated from the data, and the resulting graph in Fig. 1. Calculation of the correlation between death rate and 'range' + mean annual temperature gave $r = -0{\cdot}7208$, $n = 31$, and P much less than 0·001.

Table 1. Death Rates from Breast Neoplasms (Age-adjusted), Mean Annual Temperature (MAT), 'Range' and Mean Annual Temperature + 'Range' for 33 Countries

	Country	Death rate	MAT	'Range'	MAT + 'range'
(1)	Ireland	25·7	49·0	30	79·5
(2)	Switzerland	21·5	44·0	46	90·0
(3)	Sweden	18 2	38·0	54	92·0
(4)	Norway	17·1	35·5	58	93·5
(5)	Finland	12·1	35·0	61	96·5
(6)	Denmark	24·0	45·5	40	85·5
(7)	Austria	14 9	47·0	51	98·0
(8)	Portugal	11·0	60·5	36	96·5
(9)	Italy	13·6	59·0	47	106·0
(10)	Japan	3·8	56·0	53	109 0
(11)	Belgium	19 8	49·5	40	89·5
(12)	France	14·4	53·0	44	97·0
(13)	The Netherlands	23·4	49·0	38	87·0
(14)	Germany	15·5	46·5	44	90·5
(15)	England and Wales	23·2	49·5	32	81·5
(16)	Northern Ireland	18·9	48·0	29	77·0
(17)	Scotland	22·8	46·5	29	75·5
(18)	Chile	7·1	56·5	31	87·5
(19)	New Zealand (Whites)	21·6	55·0	31	86·0
(20)	Singapore	6·2	80·5	16	96·5
(21)	Yugoslavia	6·9	55·0	54	109 0
(22)	Hungary	12 4	50·5	57	107·5
(23)	Spain	6·5	60·0	47	107·0
(24)	Czechoslovakia	13 1	48·0	51	99·0
(25)	Greece	4·5	63·5	46	109·5
(26)	Poland	6·8	47 0	51	98 0
(27)	Romania	7 5	46·5	59	105·5
(28)	Bulgaria	7 2	53·5	60	113 5
(29)	Iceland	26·0	41 0	29	70·0
(30)	South Africa (Whites)	21·7	63·0	42	105 0
(31)	Mexico	3·8	75·0	32	107·0
(32)	Venezuela	8·0	72·5	21	93·5
(33)	Columbia	5·6	70·0	18	88·0

The nature of the relationship between the environmental temperature and the death rate was found to be that the rate fell with increase of both mean annual temperature and 'range'. This, however, was complicated by an apparent repulsion between mean annual temperature and 'range'. Consideration of mean annual temperature and 'range' for all those places dealt with by the Meteorological Office, that is most of the inhabited world, showed that there was a very marked negative correlation

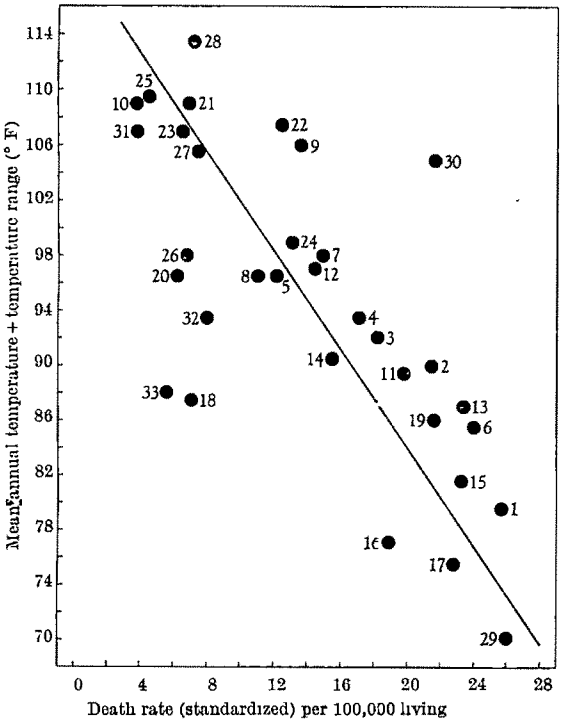


Fig. 1. See Table 1 for identification of numbers

between mean annual temperature and 'range', P again being much less than 0·001.

Mr. R. H. Clements of the Meteorological Office[11] has pointed out that this correlation is to be expected on an ideal world, because as one approaches the poles from the equator the mean temperature decreases but the conditions become more favourable for low temperatures in the winter half-year and for high ones in the summer half, hence the range increases. On the actual world the situation is, of course, more complicated by the atmospheric circulation, variations in cloudiness, and especially by the nature of the surface, land and sea. This explains some of the more remarkable results of Fig. 1, for example Iceland with mean annual temperature + 'range' of 70·0 and death rate of 26 as compared with Norway's mean annual temperature + 'range' of 93·5 and death rate of 17, the distance of the two countries from the equator being approximately the same.

It is now clear that the relationship previously found between mean annual temperature and the death rate from breast neoplasms for Norway, Sweden, Scotland, and England and Wales was a first approximation to a more complex relationship, and that the combination of mean annual temperature and 'range' provides a much wider relationship between environmental temperature and the death rate from these neoplasms. The same procedure carried out for other neoplasms produced no similar pattern, or in fact any pattern other than a random distribution. There are, of course, bad points on the graph, but in view of the uncertainties in the data this is to be expected. It may be of some importance that the countries which diverge most from the straight line are those which have been colonized from European countries, namely South Africa, Venezuela, Columbia and Chile.

Two factors which may influence the incidence of breast neoplasms have previously attracted attention, lactation and social conditions. The influence of lactation is based on experimental work in mice, but investigations of such a possibility in humans have failed because of the difficulties encountered in obtaining lactational histories in normal women[12]. While it is just conceivable that variations in breast-feeding in different countries might account for the observed differences in the death rate from breast neoplasms, the evidence which could prove or disprove this does not exist. Stocks[13] found an association between a social class index and the mortality index for breast cancer in women in thirty large towns in England and Wales for the years 1921–30, the correlation coefficient being $-0{\cdot}357 \pm 0{\cdot}159$. This is not significant at the 1 per cent level, and Stocks stated that there was apparently some factor other than economic and social differences operating to affect this cancer. As economic differences have been greatly reduced since 1921–30 it seems very improbable that such a factor could explain the variations in death rates shown in the present article.

The nature of this relationship remains completely obscure, but in view of the very high correlation there can be little doubt of its reality. Certain possibilities suggest themselves, but as they are at present without supporting evidence it is not proposed to discuss them at this stage.

I thank Sir Austin Bradford Hill, Mr. R. H. Clements, Prof. Hans Gruneberg, Dr. G. F. Marrian, Prof. C. A. B. Smith and Dr. C. C. Spicer for their advice and Dr. P. S. Dixon for permission to use unpublished material.

Correction added in proof. The death rate for Ireland (1) has been shown in Fig. 1 and Table 1 as 25·7. This is an error and should read 18·9 in both places.

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CORTICAL LESIONS AND INTERHEMISPHERIC COMMUNICATION IN MONKEYS (*Macaca mulatta*)

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THE technique of restricting the visual connexions of each eye to the ipsilateral hemisphere of the brain by midline section of the crossed optic nerve fibres has permitted investigation of the function of the forebrain commissures in interhemispheric transfer of visual discrimination habits[1–3]. It has been reported[4] that if optic chiasma-sectioned monkeys are taught pattern discriminations with one eye open, the problems are correctly performed with only the other eye open, without further training. If, after thirty trials using this second eye, the forebrain commissures are cut (that is, the anterior commissure and the corpus callosum, plus un-

avoidably the psalterium situated beneath the callosum), it is found that the monkeys perform correctly with the first eye open, but imperfectly with the second eye. This suggests that communication between the hemispheres is incomplete or absent during the initial training. However, the amount of training necessary to bring the monkeys back to the correct performance with only the second eye open is significantly less than that given with the first eye open. It seems that communication occurs during the testing of the naïve side, before cutting the commissures, and the animal requires only a few more trials to complete the learning on that side. Further problems were then taught with only one eye open after the commissures had been cut, and these were not correctly performed with the second eye alone open until the monkey had received an amount of training comparable with that originally required[4]. Because of these and similar results in other species it is generally considered that the forebrain commissures mediate the communication of visual pattern training with one hemisphere to the other.

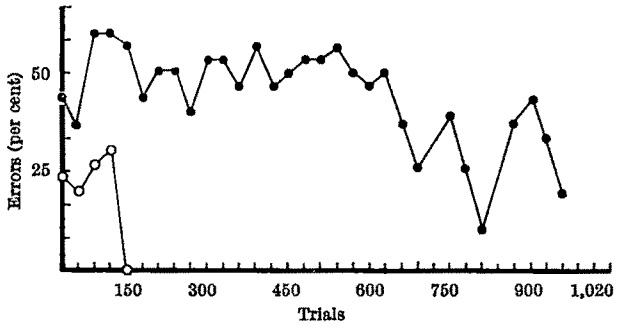


Fig. 1. Percentage of errors per 30 trial training session. Lesion, left inferotemporal; problem, 6- versus 5-point star. ●, Initial training on damaged side; ○, subsequent testing on intact side

Since the forebrain commissures concerned arise and terminate within the cerebral cortex, it was thought that suitable cortical lesions might disrupt interhemispheric communication. This has now been tested with three types of lesion. Various aspects of this work have been reported elsewhere[5–7]. The two optic chiasma-sectioned monkeys in Group I each sustained a large lesion of the frontal parietal and temporal cortex on one side, sparing the visual and motor areas. Before making the cortical lesion, these monkeys had been taught to discriminate + from ○ using the eye on the side later to be damaged, and a more difficult problem, ▲ from ■, using the other eye. After the cortical removals the first problem was solved correctly on the trained, damaged side only, even though the neocortical commissures had not been sectioned. The second discrimination was made correctly without further training with either eye open, although this second task was more difficult than the first, as judged by the number of trials needed in training. Thus the lesion interfered with communication from the damaged side to the undamaged side but not the reverse, producing an unexpected asymmetry in interhemispheric communication. This result would not have been possible if the communication between the two hemispheres had been completed during the initial training. A similar deficit in performance on 'difficult' discriminations, when using the eye projecting to the untrained side following cortical lesions on the trained side, has been found by Myers[8] in cats.

Post-operatively, these two monkeys were taught to discriminate Y from T using the eye on the damaged side, and ⊤ from ⊣ with the eye on the intact side. The first discrimination was performed correctly without further training using the eye on the untrained side, but the second discrimination was not. The lesion thus disrupted communication from the intact hemisphere to the damaged one. This asymmetry in the communication of post-operatively acquired learning is in the opposite direction to that found for pre-operatively acquired learning. These results were consistent in the two monkeys and present a challenge for interpretation. It was decided to repeat the experiments with more complete lesions confined to specific areas of cortex.

The three optic chiasma-sectioned monkeys of Group II sustained unilateral lesions in the inferotemporal area, which is concerned in the discrimination of visual patterns. Lesions in this area are known to produce specific visual deficits[9]. The pre-operative training was the same as for the previous group. Post-operative testing showed that communication was able to take place between the hemispheres in both directions. The asymmetry found in Group I was absent. The monkeys were then given 1,000 trials with the eye on the damaged side attempting to discriminate ⊛ from ⊛, but did not reach the criterion of three errors in each of two successive sessions of thirty trials, although the proportion of errors was slowly falling (Fig. 1). When the monkeys were tested on the same problem using the opposite eye, they reached criterion in about 150 trials. The score for other chiasma-sectioned monkeys with intact cortex trained monocularly on this problem is about 600 trials. Thus the inferotemporal lesion made learning on the damaged side slow and incomplete, yet the number of trials required on the intact side was only one quarter of that which would be expected if the latter had received no training. There is evidence[10–12] that in the situation described the remaining intact inferotemporal area is necessary for the slow learning of visual discriminations through the eye projecting to the damaged side. The results recorded here suggest, therefore, that communication from the damaged to the intact side during learning is enhanced by the participation of the intact inferotemporal area in the learning of visual discriminations.

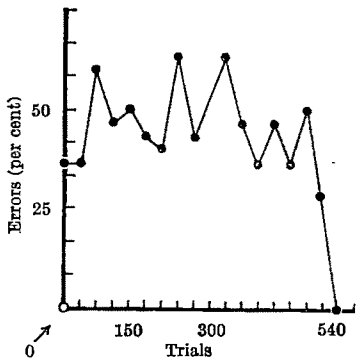


Fig. 2. Percentage of errors per 30 trial training session. Lesion, left inferotemporal; problem, ⊤ versus ⊣. ●, Initial training on intact side; ○, subsequent testing on damaged side

When the monkeys had reached criterion in discriminating the five- and six-point stars using the eye projecting to the intact hemisphere, they were re-tested with the other eye. They at once solved the problem correctly, in spite of the previous slow progress in 1,000 trials on this side. This result could mean that the solution to a problem is more easily communicated to the opposite hemisphere than is information about a discrimination problem that has not yet been solved completely. The result could, however, also be interpreted as an asymmetry such that communication from the intact hemisphere to the damaged hemisphere is more effective than in the opposite direction. To test that the communication from the intact side to the damaged side seen in the foregoing did not depend on the previous training given to the damaged side, a new problem, ⊤ versus ⊣, was trained

on the intact side. The animal performed correctly when the damaged side was tested (Fig. 2), showing perfect communication in this direction. A further control is now being undertaken using monkeys with inferotemporal lesions. This consists of varying the amount of training given to the damaged side on the six- versus five-point star problem to see if the amount of training necessary to complete the learning on the second, or intact, side varies correspondingly.

The experiments were repeated on the two chiasma-sectioned monkeys of Group III with unilateral lesions in the frontal granular cortex. The lesions were centred on the sulcus principalis and included the frontal eye fields (Fig. 3). Frontal lesions cause a variety of deficits and sometimes interfere with certain types of visual discriminations[14]. The pre-operative training was the same as in Group I and Group II. Post-operative testing revealed effective communication between the hemispheres in both directions, as in Group II. The post-operative training was the same as for Group II and again the six- and five-point star problem was unsolved after 1,000 trials with the eye on the damaged side open. When the other eye was tested, 600–800 trials were required to learn the problem, so there was no saving as in Group II. Moreover, subsequent re-testing with the eye projecting to the damaged hemisphere revealed a continued performance at chance level. The frontal lesion seemed, therefore, to have reduced communication between the two hemispheres in both directions (Fig. 4). This result was further tested by training a new problem, discriminating ∓ from ∔ using the eye on the normal side. This problem was solved correctly on the normal side, but subsequent testing through the eye on the damaged side again showed a performance at the level of chance. These results were consistent in the two monkeys used.

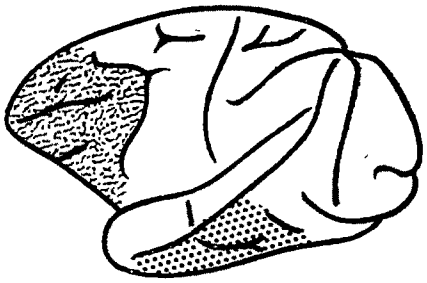


Fig. 3. Diagram to show extent of cortical lesions sustained by animals in Group II and Group III. Regular stippling (below), inferior temporal lesion (Group II); irregular stippling (above), frontal lesion (Group III)

These experiments on the effect of cortical lesions on interhemispheric communication have produced a number of unexpected results, and have suggested several further experiments. In the first place, properly designed experiments are needed to make certain that interhemispheric communication normally takes place during the testing of the naïve hemisphere rather than during the initial training. Secondly, the asymmetry found in the first group of monkeys in the communication of pre-operatively learned problems needs to be confirmed in a larger group, for it was not seen after frontal, or inferotemporal lesions, and is at present difficult to explain. It implies that the lesion disrupts communication from the damaged to the intact side without affecting retention on the trained, damaged side.

The most surprising finding was that interhemispheric communication can be enhanced as well as obstructed by appropriate cortical lesions. The explanation suggested for the asymmetry in the second group of monkeys was that communication from the damaged to the intact side during learning is enhanced by the necessity for the intact inferotemporal area to participate in the learning of visual discriminations seen through the eye on the damaged side. This explanation might also apply to the similar asymmetry in the communication of post-operative learning in the first group of monkeys, except that the lesion in these monkeys involved only a small part of the posterior inferotemporal area. The prestriate cortex was, however, involved in the lesion and this would have reduced the visual input to the inferotemporal area. The frontal lobe was also extensively damaged in these first two monkeys; but the results are not similar to those for the third group of monkeys with pure frontal lesions.

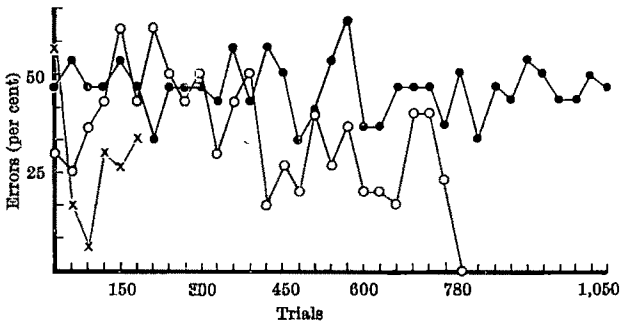


Fig. 4. Percentage of errors per 30 trial training session. Lesion, left frontal; problem, 6- versus 5-point star. ●, Initial training on damaged side; ○, subsequent testing on intact side; ×, subsequent re-testing on damaged side

The inability of the third group of monkeys which had frontal lesions to learn the visual discrimination given on the damaged side was unexpected, though similar cases of increased difficulty in learning have appeared in the literature[10,13]. The frontal lesions prevented the correct performance of a visual discrimination using the eye projecting to the damaged side even after it had been trained to criterion on the intact side. Whether this is due to some quite different disability in the testing situation such as increased distractability, visual neglect or lack of drive inhibition as described by Brutkowski *et al.*[14] is at present unknown. Further results suggest that the animals with frontal lesions respond more quickly to the discrimination stimuli when using the eye projecting to the damaged side than when using that on the normal side. Possibly the response time is insufficient to allow adequate examination of the stimuli. Comparison with the deficit in the second group of monkeys and the explanation offered for it suggests that the deficit produced in the monkeys with frontal lesions cannot be compensated by the activity of a similar intact structure on the other side, so that interhemispheric communication is not promoted. These results suggest that the examination of the effect of cortical lesions on interhemispheric communication is a useful method of exploring aspects of the functioning of cerebral cortical areas, and a fertile source of further problems.

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LETTERS TO THE EDITOR

ASTRONOMY

Jupiter's Great Red Spot

The great red spot of Jupiter has puzzled astronomers since its discovery in 1857 (or since 1664 if it is identical with the feature seen by Robert Hooke). It is generally conceded to-day that Jupiter is approximately 80 per cent hydrogen by weight[1-3], and the best modern evidence does not conflict with the assumption that hydrogen predominates in the atmosphere of Jupiter to the same extent as in the planet's bulk[4]. It has proved difficult to conceive of an 'object' able to 'float' in a surfaceless ocean[5] of supercritical hydrogen gas. Therefore the suggestion of Hide[6] that the red spot might not be a flotation phenomenon at all but rather a 'Taylor column' was an intriguing alternative. Unfortunately, it has not been possible as yet to prove hydrodynamically that a Taylor column can be realized in Jupiter's atmosphere; for one does not know enough about the hydrodynamic conditions of the atmosphere (we shall return to this point later). Moreover, certain observed phenomena appear to indicate that the rather restrictive requirements for the existence of a Taylor column are not met by the red spot. Without here going into these arguments, we merely wish to propose a novel and alternative explanation based on a theoretical prediction of J. D. van der Waals[7] which has been verified in some detail by Krichevsky and Zichs[8], Kennedy and Takenouchi[9] and others.

Multi-component fluids with one polar component can show phase separation over a broad range of temperatures. As a rule the temperature range of immiscibility of fluid phases begins somewhere between the highest and lowest critical temperatures of the pure components and extends indefinitely upward, in so far as is known. In two-component two-phase systems the phases generally differ in density, but continuous changes in pressure and temperature can reverse the sign of the difference in density of the two phases. Moreover, there is a finite (univariate) range of conditions for which the two distinct phases have equal densities[9]. The two-component system is univariate in the sense that one of the two degrees of freedom allowed by the phase rule is lost owing to the stipulated equality of densities. However, three-component systems (for example, hydrogen, helium and ammonia) could show decomposition into two phases having equal densities over a finite area of the p,T plane.

Since the atmosphere of Jupiter is predominantly composed of the non-polar gas hydrogen, though the polar gas ammonia is known to be present, we feel that possibly the red spot is merely a region where phase separation occurs under the condition that the two distinct phases have the same density, but different compositions. Hence the 'red spot phase' would be buoyantly neutral with respect to its surroundings—a thermodynamic Cartesian diver.

We anticipate objections to this view, which we shall not attempt to meet here. Experiments we hope to see forthcoming in the near future may very well prove that a multi-component gas matching Jovian surface conditions does not show the behaviour of the systems already investigated[8,9]. In that case the objections would stand with no rebuttal possible. On the other hand, if phase separation does occur in such gases, it is clear that the hydrodynamic theory of the Jovian atmosphere may be significantly—if not profoundly—affected. We shall then present a more detailed set of reasons for our belief that the red spot may be, after all, merely a normal manifestation of the thermodynamics of multi-component fluids.

We would like to underscore the fact that our remark concerning the possible effects of the separation of fluid phases on the hydrodynamics of the Jovian atmosphere would deserve attention even if Jupiter had no red spot. As a concrete illustration of how the phase separation phenomena in supercritical gas mixtures might alter hydrodynamic flows one can imagine a cylinder in the laboratory filled with pure water vapour maintained at 260° C and the pressure raised from 1,500 to 2,500 bars and thereafter reduced to 1,500 bars. Naturally, owing to the small displacements, the hydrodynamic mass velocities are small and at most of the order of the piston velocities, except near the region where the moving piston meets the cylinder walls so that the usual local lubrication flows would arise. However, if the cylinder is filled with a mixture of carbon dioxide (say, 30 per cent by mole) and water and the same process repeated, a glance at Fig. 8 in ref. 9 shows that the same isothermal cycle in pressure would cause dramatic effects. For at 1,500 bars the bottom of the cylinder would contain a phase with 17·5 per cent mole per cent of carbon dioxide, while a lighter phase with 50 per cent carbon dioxide would fill the top. On compression to 2,200 bars the carbon dioxide content of the bottom phase would rise to about 21 per cent and that of the phase on top would be depleted to approximately 47 per cent. Further compression would again lower the mole per cent of carbon dioxide in the phase which originally was at the bottom; but then the bottom phase becomes less dense, and in general, if the cycle is not too rapid, the two phases must reverse positions in the cylinder every time the pressure crosses 2,200 bars, approximately. Consequently, in this very simple system the hydrodynamic regime is altered drastically by the phenomenon of phase separation.

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ASTROPHYSICS

X-ray Emission from Very Dense Stars

In recent years few localized astronomical X-ray sources have been discovered by rocket- and balloon-borne instruments. To explain the X-ray emission by celestial bodies several production mechanisms have been invoked such as the inverse Compton effect, bremsstrahlung in a 'hot' plasma cloud, synchrotron radiation and thermal emission.

These attempts have been reviewed by Giacconi and Gursky[1], by Manley[2] and by Burbidge *et al.*[3].

The purpose of this communication is to consider yet another mechanism, which accounts in a satisfactory manner for the emission of X-rays from punctual sources. It fails, however, to account for the emission from extended sources.

Consider a very dense neutron star such as was described by Hoyle, Narlikar and Wheeler[4]. They showed that for a star possessing rotation, the usual treatment of the maximum mass does not apply. One can obtain a disk-like structure, of radius a, thickness b, rotating around an axis perpendicular to the disk in its centre, where the mass M may be made as large as one may desire.

They pointed out that since a neutron star is thought to be formed catastrophically, it will arrive at its stable configuration through large dynamical oscillations, thus dissipating a total energy of 10^{54} ergs for a star of mass $M = 5\,M_{\odot}$.

Such a star, the density of which is of the order of 10^{15} g cm^{-3} and the dimensions of which are of the order of a few kilometres, will develop a polar magnetic field H of the order of 10^{10} gauss.

Dynamical oscillations of the main body of a neutron star imply oscillations of the associated magnetic field. As the density ρ falls steeply at the surface, the hydromagnetic wave speed $v = H/\sqrt{4\pi\rho}$ becomes of the order of the velocity of light and the oscillations become electromagnetic rather than hydromagnetic. Outside the immediate surface of the star, electromagnetic waves of wave-length λ are generated, with $\lambda/2\pi$ of the order of the linear dimensions of the star.

The polar magnetic field H can be expressed in the form: $H = H_0 + \delta H \cos \omega t$, where $H = 10^{10}$ gauss is the stationary field and $\delta H \cos \omega t$ is the variable part of the field due to electromagnetic oscillations, with $\omega = v/a$.

If one relates the magnetic field of the star to its angular momentum, it would seem reasonable to admit that during the dynamic oscillations of such a disk-shaped star the relative variation of angular momentum, and thus the relative variation of the magnetic field, is of the order of $\delta H/H \cong 10^{-4}$.

Although neutrons are dominant in the star, electrons and protons are also present. The electromagnetic waves of frequency ω will exert radiation pressure, particularly near the rim of the star, and will accelerate electrons to an average energy of 10^9 eV. These electrons will be trapped in the magnetic field of the star, and will spiral tightly around the magnetic lines of force emitting synchrotron radiation.

The tightly spiralling electrons will create a toroidal magnetic field H_t of the same order of magnitude as the polar field H.

Let n be the average density of electrons creating the toroidal field, then:

$$H_t = H = \frac{4\pi I}{l} = \frac{4\pi e c n \pi a b}{2\pi a}$$

where e is the charge of the electron and c the velocity of light (the electrons being relativistic). If a is taken to be 3×10^6 cm, and $b = a/10$, one has $n = 1{\cdot}1 \times 10^{13}$ electrons cm^{-3}.

The radiation pressure at the rim of the disk is:

$$\frac{(\delta H)^2}{12\pi} \cong 2{\cdot}6 \times 10^{10} \text{ ergs cm}^{-3}$$

Each electron thus gains an average energy of:

$$E = \frac{2{\cdot}6 \times 10^{10}}{1{\cdot}1 \times 10^{13}} \cong 2{\cdot}4 \times 10^{-2} \text{ erg}$$

say 10^9 eV.

Electrons with an average energy of 10^9 eV spiralling in a magnetic field of $H = 10^{10}$ gauss emit synchrotron radiation with a spectrum in which the peak flux has a characteristic frequency:

$$\nu_c = 1{\cdot}26 \times 10^6 H \left(\frac{E}{mc^2}\right)^2 \cong 5 \times 10^{22} \text{ c/s} \cong 2 \times 10^8 \text{ eV}$$

that is γ-rays of 200 MeV.

The total power radiated by an electron is:

$$p = 10^{-3} H^2 \left(\frac{E}{mc^2}\right)^2 \text{ eV.sec}^{-1} =$$

$$1{\cdot}6 \times 10^{-15} H^2 \left(\frac{E}{mc^2}\right)^2 \text{ergs.sec}^{-1} = 0{\cdot}6 \times 10^{12} \text{ ergs.sec}^{-1}$$

The star will radiate a total power of:

$$P = 2\pi a b^2 n p \cong 10^{43} \text{ ergs.sec}^{-1}$$

The dynamical energy of oscillations of 10^{54} ergs will be dissipated in 10^{11} sec ($\cong$ 3,000 years). However, if one considers the gravitational redshift, which is quite high, that is:

$$\frac{\Delta\nu}{\nu} = -\frac{GM}{c^2 a} = -0{\cdot}25$$

the whole spectrum should be shifted towards the lower frequencies and the time of dissipation of the dynamical energy will be somewhat longer.

The frequency analysis of the synchrotron radiation could be obtained if the energy spectrum of electrons were known. To simplify matters it will be assumed hereafter that the spectrum of synchrotron radiation is of the form A/ν (ref. 5). The total power emitted is then:

$$P = \int_{\nu_1}^{\nu_2} A \frac{d\nu}{\nu} = A \log \frac{\nu_2}{\nu_1}$$

The lower limit of integration, ν_1, occurs quite naturally at the frequency at which the dielectric constant:

$$1 - \frac{n e^2}{\pi m \nu^2}$$

of the electron cloud becomes zero. Thus:

$$\nu_1^2 = \frac{ne^2}{\pi m} = 9 \times 10^{20} \text{ and } \nu_1 = 3 \times 10^{10} \text{ c/s}$$

For the higher limit of integration, ν_2, one may take $2\,\nu_c$—ν_c being the characteristic frequency. This is justified by the fact that for frequencies higher than ν_c the energy in the synchrotron pulse falls off exponentially It can be remarked here that the value of A is very insensitive to the limits of integration. One finally has:

$$A = P/\log \nu_c/\nu_1 \cong 3{\cdot}5 \times 10^{41} \text{ ergs.sec}^{-1}$$

The number of X or γ photons emitted per sec by the star in the bandwidth $\Delta\nu$ is then:

$$\Delta N = \frac{A\Delta\nu}{h\nu^2}$$

photons sec^{-1} (h is Planck's constant). Consider a star the distance of which from the Earth is R, then the number of photons, X or γ, received at the surface of the Earth (neglecting absorption) per unit area during one second in a band-width $\Delta\nu$ is:

$$\Delta n = \frac{A\Delta\nu}{4\pi R^2 h\nu^2}$$

As an example, consider a star for which $R = 5 \times 10^{22}$ cm and compute Δn for X-rays of 5 KeV, that is $\nu = 1{\cdot}2 \times 10^{18}$ sec^{-1}. Assuming $\frac{\Delta\nu}{\nu} = 0{\cdot}25$, one has $\Delta n_x \cong 3{\cdot}5 10^2 X \text{ cm}^{-2}$ sec^{-1}. The number of γ-photons of 200 MeV received under the same conditions will be $\Delta n_\gamma \cong 10^{-2}\,\gamma$ cm^{-2} sec^{-1}. These numbers are somewhat higher than those obtained experimentally[6,7]. If, however, one considers the gravitational redshift the discrepancy between calculated and observed numbers decreases. The corresponding number of optical photons, calculated under the same general conditions, is:

$$\Delta n_{ph} \cong 10^6 \text{ ph.cm}^{-2} \text{ sec}^{-1}$$

Whereas the absorption by interstellar matter is quite small for X-rays and γ-rays, it is by no means negligible

for optical photons. In fact, if account is taken of absorption by interstellar matter, the star is scarcely detectable in the optical band.

It is interesting to compute the unattenuated power which one could expect to receive on the surface of the Earth in the radiofrequency band, above the cut-off frequency ν_1. For $\nu = 10^{10}$ sec^{-1}, one has $\Delta p = 10^{-18}$ W m^{-2} (c/s)$^{-1}$. This is a very high figure indeed. However, these waves would be strongly attenuated in regions of the electron cloud, where the densities are higher than the average calculated. In spite of this fact it would be interesting to observe X-ray emitters in the centimetre band of radio frequencies.

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SPACE SCIENCE

An Effect of Nuclear-reactor Operation in Space

There is considerable development effort towards producing nuclear reactors for SNAP (systems for nuclear auxiliary power) applications. Obviously the performance of these reactors in the space environment is a matter of some concern.

Both the effect of the reactor system on the space environment and the effect of the space environment on the reactor system should be carefully considered. Recently it was pointed out that a nuclear reactor could have a definite effect on its environment while orbiting in the geomagnetosphere[1]; now it appears that the environment can have a very definite (and unexpected) effect on the reactor system.

In particular, the photonic flux from a nuclear reactor will produce Compton electrons in nearby material. Under planetary conditions, these electrons would dissipate their energy through atomic interactions, and then return to the immediate vicinity of their origin because of electrostatic attraction. In space, however, energy dissipation is small enough to be neglected, and magnetic fields will at best cause only a small percentage of the ejected electrons to follow paths returning them to the reactor system, so electrostatic forces must play an especially large part in establishing a steady state condition. This suggests that the reactor system might achieve a noticeable positive potential while in operation. The magnitude of this effect can be determined from a few rough approximations.

Let us assume that a 1 MW (thermal) nuclear reactor is in polar orbit at an altitude of 1,000 km above the surface of the Earth. Let us further assume that the heat rejection radiator is placed about the reactor as a spherical surface. Since the radiator area would be about 120 m^2 (ref. 2), this yields a thin spherical shell (perhaps 2 cm thick) of 3·1 m radius. At the centre of the shell would be located the reactor and reflector. The reactor should be no more than 30 cm in radius (assumed spherical here), and the reflector would perhaps be 4 cm of beryllium.

It is reasonable also to assume that the average power density of the reactor is less than twice that of the power density at the reactor's edge. This provides a power density near the reactor's edge of $4{\cdot}4 \times 10^{-6}$ MW/cm^3, which in turn yields a fission density of $1{\cdot}36 \times 10^{11}$ fissions/cm^3-sec. Each fission can be considered to yield 7 photons at 1 MeV per photon[3].

Now 1 MeV photons have an e-folding path length in plutonium fuel of 0·7 cm, so the photon production region can be taken as $7{\cdot}9 \times 10^3$ cm^3. This yields a thin shell source of $7{\cdot}5 \times 10^{15}$ photons/sec (of 1 MeV each). However, geometric considerations should lower that figure to about $3{\cdot}5 \times 10^{15}$ photons/sec.

The 4 cm of beryllium should attenuate the photons to approximately 2×10^{15} photons/sec. So, 2×10^{15} photons/sec (of 1 MeV each) should strike the inner wall of the radiator. If we assume the radiator to be 2 cm (of aluminium) thick, the photons/sec through the outer surface should approximate $1{\cdot}4 \times 10^{15}$ photons/sec. When only those electrons within an electrons e-folding distance of the radiator's outer surface are considered, 3×10^{13} electrons/sec are Compton scattered.

If we consider further the angular spread of Compton scatter, about 5×10^{12} electrons/sec of 0·8 MeV maximum energy can be considered as ejected from the reactor system. If no other factors were considered, the reactor system would rapidly approach a positive potential of 0·8 MV. However, there are other factors which should be included.

Over the reactor's orbit, there are flux levels of trapped electrons and positive ions as low as 10^3 particles/cm^2 sec and lower[4]. The positive ions would tend to be repelled from a positively charged reactor system, while the electrons would just as effectively be attracted to the system. The large surface area of the reactor system would tend to acquire about 1×10^9 electrons/sec. This, of course, assumes the very doubtful complete repulsion of all positive ions.

We now have a Compton electron current away from the reactor system, and a trapped radiation electron current into the system. When the energy distribution of the Compton electrons is considered in light of the electrostatic build-up, a steady state potential of about 0·79 MV is found to exist on the reactor system.

The importance of this potential can be better evaluated by noting that the reactor system described would have a capacitance of $3{\cdot}4 \times 10^{-10}$ farads. So the electrostatic energy stored would be 100 joules.

The discharge of 100 joules might puncture a very thin surface; but it would appear that this effect would provide merely a nuisance for the case investigated. The positive charge would tend to make objects in contact with the reactor system surface be repelled from that surface, and from each other.

However, it would seem advisable to determine the magnitude of this effect to be expected for the particular space mission planned for each nuclear reactor.

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PHYSICS

Attenuation of Shock Waves by Cooling

The attenuation of shock waves was the subject of a recent investigation[1], where energy loss through heat transfer was suggested as the mechanism.

Experiments conducted by me[2] have illustrated that heat loss from shock waves can be an effective method of attenuation. Here, liquid nitrogen was injected into shock waves.

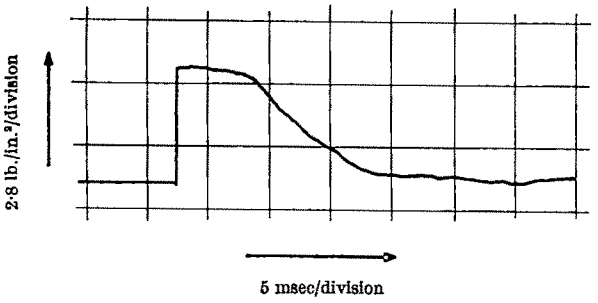


Fig. 1. Pressure-time record of undisturbed shock wave

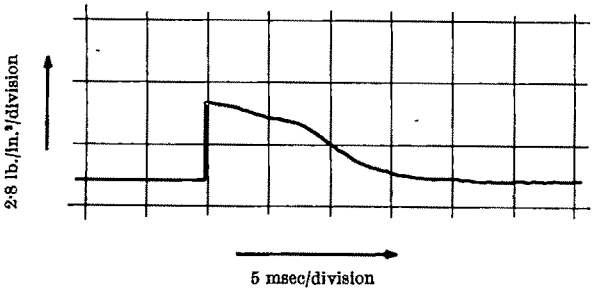


Fig. 2. Pressure-time record of similar shock wave following injection of liquid nitrogen

Fig. 1 illustrates a pressure-time record of a shock wave in a 2 × 1·5 in. shock tube. Fig. 2 illustrates the pressure-time characteristics of a similar shock wave, following the injection into the wave of 3·8 c.c. of liquid nitrogen.

The total impulse in the shock wave was reduced from 46 lb/in². msec to 31 lb/in². msec.

Although the quantities of liquid nitrogen required limit the usefulness of this technique on any large scale, its use on a small scale would appear to present many intriguing possibilities.

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Lifetimes of Saturable Absorbers

The use of saturable absorbers such as aluminium phthalocyanine for the passive Q-switching of ruby lasers[1] raises the question of the lifetime of the active molecule. We have measured the lifetime of that state of aluminium phthalocyanine which corresponds to absorption at or near 6943 Å. Similar data for magnesium phthalocyanine have been obtained, for comparison with literature values.

In their theoretical treatment of passive Q-switching, Szabo and Stein[2] point out that this may need modification for the case of short lifetimes, and the reservations of Armstrong[3] concerning the effect of short spontaneous lifetime on the application of Franz and Nodvik's[4] saturable amplifier theory to saturable attenuators also showed the need for lifetime measurements to be made *in situ* or in high-flux environments similar to the laser resonator. Using the low-flux phase-shift fluorometer method, Dmitrievskii *et al.*[5] have found that the excited-state lifetimes of magnesium and zinc phthalocyanines in various solvents lie in the ranges 6·5–8·6 nsec and 3·0–4·6 nsec respectively. Gires and Combaud[6] have established that the excited-state lifetime of chloro-aluminium phthalocyanine in chloronaphthalene is less than 10 nsec.

We have photographed directly the time variation of the fluorescence of a solution of chloro-aluminium phthalocyanine in chloronaphthalene when excited by a giant pulse from a Q-switched ruby laser. Comparison with a simultaneous photograph of the exciting light yielded the lifetime.

The output from a 2 in. × 0·25 in. ruby laser, with external reflectors and a Pockels cell Q-switch, was focused into a 6 × 10^{-4} M solution of chloro-aluminium phthalocyanine to give a line source of fluorescence about 0·3 mm wide. This was focused on the photocathode of an S.T.L. image converter camera, operating in the streak mode, the line of fluorescence thus constituting the effective slit of the streak recording system. A small fraction of the laser beam was deflected on to an opal glass screen giving a spot of 0·2 mm diameter which was also in the field of view of the camera. Records were made on Kodak 'Royal-X Pan' film which was carefully calibrated for density versus relative incident light intensity. As there is no reciprocity failure for exposure times shorter than 1 msec (ref. 7) this film calibration was performed with a xenon flash lamp and standard step wedge.

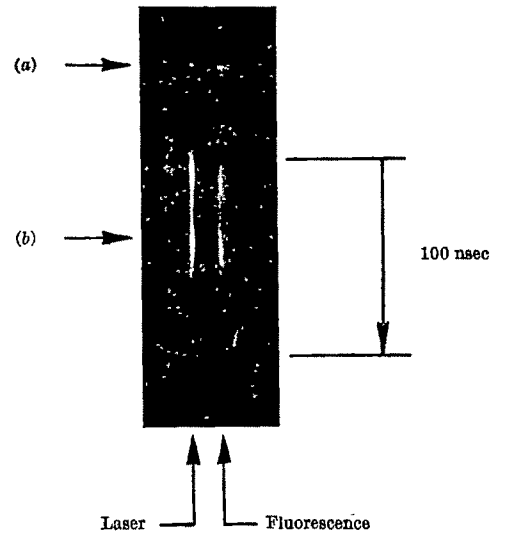


Fig. 1. (*a*) Stationary image; (*b*) streak record, time increasing downwards

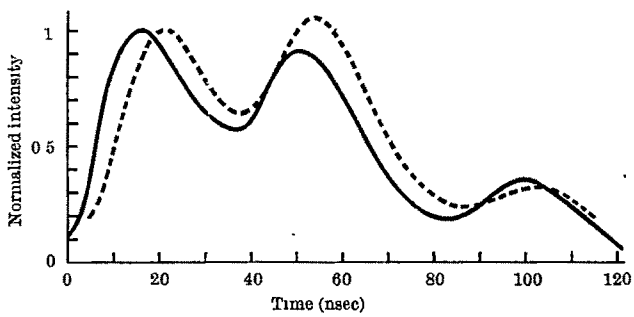


Fig. 2. —, Laser intensity; - - -, fluorescent intensity (normalized to unity near 20 msec)

The experimental records are shown in Fig. 1. The stationary patterns *a* show the effective slit during the course of the experiment. In the streak mode *b* the time-variation of the laser and fluorescent intensities are shown and the delay in fluorescent output due to the lifetime of the absorber may be seen by eye. Scattering at 6943 Å was found independently to be less than 4 per cent. The streak records were scanned along the time axis with a microdensitometer, and the wave-forms (Fig. 2) obtained after applying the film calibration. These show the gross effect of a time delay of about 5 nsec, but a more careful analysis was thought warranted.

Assuming that the excitation process is a simple two-level transition with a single decay process, and that

the number of molecules, n, in the upper state is small compared with the number in the ground state, then:

$$\dot{n} = PI - n/\tau$$

where I is the normalized incident intensity, PI is the rate of induced absorption, and τ is the lifetime of the excited state. The fluorescent intensity I_F is proportional to n and hence, by integrating over times t_1 to t_2, we obtain:

$$\frac{I_F(t_2) - I_F(t_1)}{\int_{t_1}^{t_2} I\,dt} = Q - \frac{1}{\tau}\frac{\int_{t_1}^{t_2} I_F\,dt}{\int_{t_1}^{t_2} I\,dt}$$

where Q is a constant.

The integrals of this equation were evaluated for intervals $(t_2 - t_1) = 10$ nsec over the wave-forms of Fig. 2 and the left-hand side plotted against the multiplier of $1/\tau$. A least squares fit of a straight line to these points gave a value of 5·3 nsec for τ (estimated standard deviation, 1 nsec). A second set of results gave 4·4 nsec.

To examine the validity of the method, an observation of the lifetime of magnesium phthalocyanine in chloronaphthalene was made and gave 9 nsec, which is in substantial agreement with the results of Dmitrievskii *et al.*[5], who used different solvents.

A lifetime of 5 nsec is not large compared with the usual photon lifetime in a laser resonator and this could limit the application of the simple theory of the passive Q-switch laser. A quantitative analysis of the possible effects of this parameter is at present being carried our in these laboratories.

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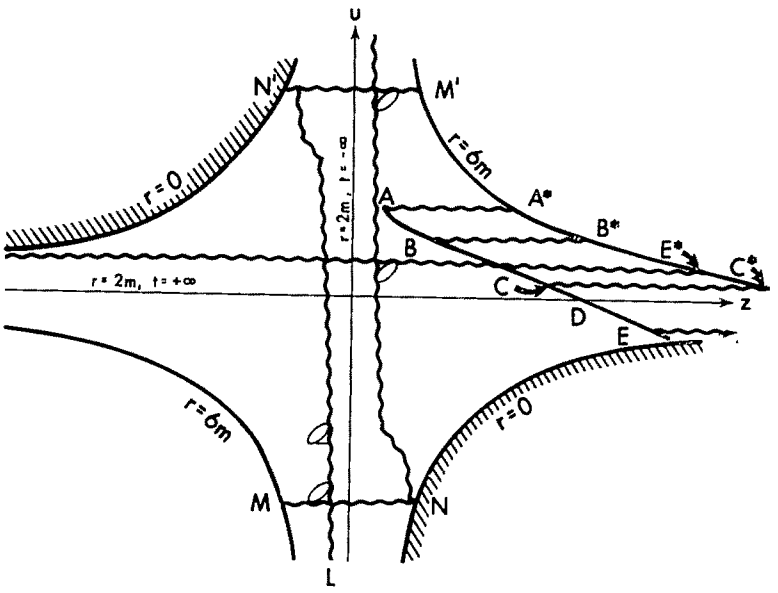


Fig. 1 The uz plane, showing null (wavy) lines and future null cones in the domain $(r > 2m)$ where they can be defined. Accepting a Euclidean topology for space-time, it is natural to interpret the figure as a map of the union of the 2-spaces $\theta = c_1$, $\varphi = c_2$, and $\theta = \pi - c_1$, $\varphi = \pi + c_2$. Thus, M, M' are events occurring at diametrically opposite points of a 3-space $t = \text{const.}$, and N, N' are to be identified. The light wave $LN'M'$ sent radially inwards by a stationary observer at $r \gg 2m$ reaches the diametrically opposite observer after a co-ordinate time-lapse $\Delta t \approx 2r$. Radial light-signals emitted by a freely falling clock at successive moments A, B, C, D, E are received by an external stationary observer at A^*, B^*, C^*, never, and E^*. Light signals emitted along section DE are received in inverted order, and may be said to be red-shifted into negative frequencies

Radial Motion in the Schwarzschild Field and the Issue of Gravitational Collapse

According to present relativistic ideas, a test particle released from rest at a distance r_{max} from a central point mass m appears (from the point of view of an external observer) to slow down as it falls toward the Schwarzschild radius $2m$, reaching it only asymptotically. This is in contrast with the Newtonian picture of a rectilinear oscillation with half-period $\pi(r^3_{max}/2m)^{1/2}$.

In fact, the proper time required to reach $r = 2m$ is finite, and the particle's career can be explored beyond this. But it is thought that, having once disappeared behind the observer's event horizon, the particle remains permanently sealed off from optical observation. It is concluded that the Schwarzschild sphere acts as a 'unidirectional membrane'[1] for particles and light-pulses dropped in from outside. Since both the field equations and the static boundary conditions are reversible, this conclusion (while not logically impossible) is difficult to understand.

Exactly the same considerations apply to the homologous gravitational collapse of a pressure-free sphere of mass m, since particles on the surface are effectively test particles in the exterior Schwarzschild manifold $V(m)$. One encounters the same disparity between the Newtonian picture of a reversible pulsation and the strange but generally accepted[2] contemporary description[3] of asymptotic shrinkage to $r = 2m$ and isolation from the external observer.

The current relativistic conception rests on the implicit assumption (for which no justification is apparent)* that events occurring 'later' in the history of a test particle in $V(m)$ also occur later in the history of an external observer. I shall present arguments to support the contention that this assumption and the conclusions derived from it are fallacious.

The line-element:

$$ds^2 = 2du\,dz + (z^2/2mr)\,du^2 + r^2\,(d\theta^2 + \sin^2\theta\,d\varphi^2)$$
$$r = 2m + uz/4m$$

is regular for $r > 0$ and convertible to the standard Schwarzschild form by the substitution:

$$t = r - 2m\ln(u/2z)$$

in the domain $(uz > 0)$ where the co-ordinate t is defined. It therefore represents an analytic extension of the Schwarzschild manifold, and it may be shown to be geodesically complete.

For a test particle started from rest at $r = r_{max} = 2m\csc^2\alpha$, the (analytically extended) parametric equations of the path are:

$$r(\Theta) = 2m\sin^2\Theta\csc^2\alpha$$
$$u(\Theta) = 4m\csc\alpha\sin(\Theta + \alpha)\exp G$$
$$z(\Theta) = 2m\csc\alpha\sin(\Theta - \alpha)\exp(-G)$$

where:

$$4\sin^2\alpha\,G = 1 + \csc\alpha\sin(2\Theta - \alpha) - 2\cot\alpha\,(1 + 2\sin^2\alpha)\Theta$$

* An appeal to extraneous considerations of causality does not justify recoiling from a consistent follow-through of the motion according to the principles of general relativity. Relativity is not automatically consistent with intuitive preconceptions of causality. The new description presented here indeed appears to violate causality, though only within a radius comparable with the Schwarzschild radius.

When $r_{max} = 2m$ this time-like geodesic is a closed loop (Fig. 2). This means that the accustomed global assignment of a time-arrow breaks down inside the Schwarzschild sphere. That is, each individual observer traverses his world-line in a definite sense, so that the relation 'earlier-later' retains a meaning for him (except at isolated points); however, it is impossible, in the domain $r < 2m$, to distinguish 'past' and 'future' halves of the null cone in a globally consistent way[4].

In general, the motion is oscillatory, the co-ordinate time between successive maxima being:

$$\Delta t = 2\pi m \csc^3\alpha \cos\alpha (1 + 2\sin^2\alpha)$$

For $r_{max} \gg 2m$ this formally agrees with the Newtonian result.

A remarkable feature of the motion is the existence of events such as X (Fig. 2) at which the world-line intersects itself. For large r_{max}, the radial co-ordinate at this event has the value:

$$r(X) \approx 2m + 4m \exp(-\pi/2\alpha^3) \quad (\alpha \to +0)$$

The well-known paradoxes[4] associated with self-collision are invalidated if one assumes that exposure to conditions at the singularity $r = 0$ erases every type of organized structure, in particular (human or mechanical) 'memory'.

A detailed description of the homologous collapse of a ball of dust would be complicated. However, the preceding equations apply to the geodesics followed by particles at the surface, and therefore give a correct account of the superficial aspects of the motion. The history of the surface is thus depicted by an external observer as a V-shaped curve of the form $BXF \ldots$ (Fig. 2). This means the ball is observed to be pulsating elastically, with maximal radius r_{max} and a minimal radius between 2 m and 2·5568 m.

For a sphere of 10^8 solar masses collapsing from the Chandrasekhar radius of instability[5], the period of pulsation is about 20 years. In practice, the pulsations will be slowed by anisotropies and viscous effects, and quickened by loss of amplitude due to radiation, neutrino emission and ejection of matter streams with escape velocities (mainly at the minimal radius). It seems that this picture offers hope of understanding the extended lifetimes, light variation and coherence of flashes which have been such puzzling features of the quasars.

The final stage in the evolution of such an object supervenes when the amplitude of the pulsation has died down to the Schwarzschild radius. The interior is in a state, characterized by multiple self-collisions, which can properly be termed primordial chaos. It is interesting to speculate that in this condition the body is thermally dead. All irreversible processes must have ceased, since there is no preferred time-direction in which they could operate.

Fuller accounts of this work will appear shortly in *The Physical Review* and *Il Nuovo Cimento*.

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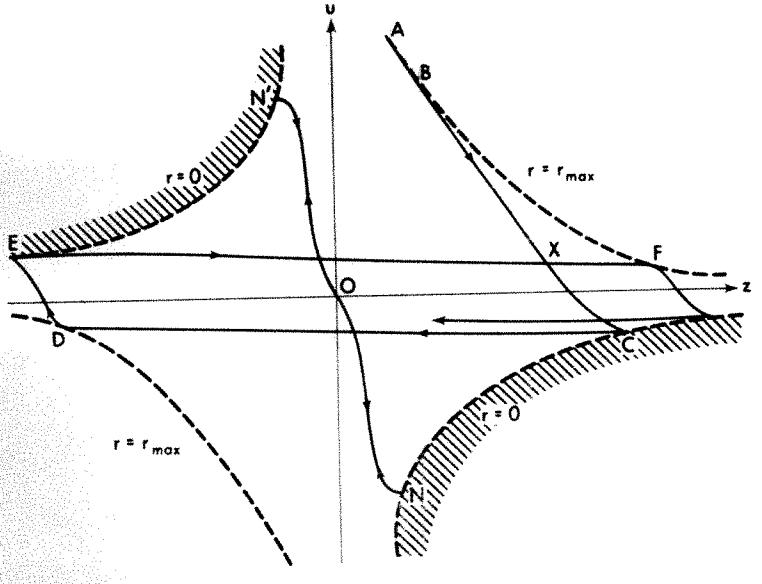


Fig. 2. Impressions (not to scale) of the time-like geodesic paths of a test particle with $r_{max} = 2m$ (the loop $ON'ON \ldots$) and a particle with $r_{max} > 2m$ (the curve $BCDE \ldots$). The curve $ABCDE \ldots$ may also be regarded qualitatively as the history of a massive thin spherical shell which has been held at rest (section AB), then released and allowed to fall under its own weight, as seen in the exterior Schwarzschild space. In this case, diametrically opposite points of the figure should be identified, and the first self-collision is not at X, but a point X^* in the first or third quadrant obtained by intersecting the curve with its reflexion through the origin

GEOLOGY

Terrestrial Origin of the Igast Objects

THIS communication presents evidence for the terrestrial origin of the objects which allegedly fell from the sky at Igast, Estonia, in 1855 (ref. 1).

The Igast objects have been of interest in relation to the tektite problem, because if it could be proved that they were of extraterrestrial origin, they would be the first such material with the chemical composition of a tektite. It was suggested by us[2] that, since much of the Igast material referred to in published papers could be shown to be spurious, the genuine Igast specimens should be re-examined. Through the courtesy of Dr. M. Hey and Mr. M. Frost of the British Museum (Natural History) we were able to make a re-examination of specimen No. 36271 (British Museum (Natural History) number).

The specimen, which is part of the British Museum (Natural History) collection of pseudo-meteorites and is so labelled, weighs about 1·5 g. It is brownish lavender in most places, but is reddish-brown in a zone a few millimetres deep along one side and around some of the vesicles. The material is chiefly vesicular glass, but under the microscope can be seen to grade into a sintered mass of quartz grains. Several individual quartz grains and clusters of quartz grains are present.

The specimen examined is definitely one of the original ones collected by Grewingk and Schmidt (1864) as shown by its resemblance to their description and by the Museum label, which shows that it was acquired in 1864 directly from Grewingk. (In the 1880's the Russian dealer, J. Siemaschko, distributed specimens labelled Igast which were not acknowledged by Grewingk and were entirely different in their characteristics.) However, the specimen was completely fused in the Earth's atmosphere rather than being superficially melted, as are

meteorites. Evidence for this conclusion consists of the presence of abundant iron oxide throughout the specimen and the absence of a fusion crust. There is a smooth quenched skin which might be mistaken for a fusion crust, but is clearly not one because it grades into, and is identical with, the smooth surface lining vesicles.

This Igast specimen closely resembles the pseudoscoria formed by burning coal and lignite beds, such as described by Lydon[3] and others. It particularly resembles material discovered by Elbert A. King in Eocene sediments of the coastal plain near Huntsville, Texas, and shown by field relations to have been formed by burning lignite seams. Such material is much more common than formerly realized, since numerous specimens have recently been recovered from Iowa and surrounding states in a search for possible extraterrestrial materials resembling the Igast objects (Table 1).

Table 1

	Igast[1]	Iowa *W*-9	Iowa *W*-21
SiO_2	80·9	63·4	67·3
Al_2O_3	9·9	16·5	19·0
Fe_2O_3	2·4	1·8	1·0
FeO		5·6	3·2
MgO	1·6	3·2	2·6
CaO	0·75	2·8	0·08
Na_2O	0·76	1·1	0·74
K_2O	3·1	2·5	4·7
H_2O-	n.d.	0·35	0·12
H_2O+	n.d.	1·4	0·61
TiO_2	n.d.	0·78	0·57
P_2O_5	n.d.	0·24	0·15
MnO	0·20	0·21	0·04
CO_2	n.d.	0·07	< 0·05
Ignition loss	0·32	—	—

Analyses of Iowa pseudoscorias by Rapid Rock Analysis Section of the U.S. Geological Survey.

We thus conclude that the Igast objects are of terrestrial origin and are probably pseudoscorias formed by burning coalbeds.

We thank Dr. M. Hey and Mr. M. Frost of the British Museum (Natural History) for their co-operation, Mr. E. A. King of the Manned Spacecraft Center (National Aeronautics and Space Administration) for bringing the Texas pseudoscorias to our attention; Mr. P. Nielsen of Missouri Valley, Iowa, and Mrs. D. Cooper of Blencoe, Iowa, for specimens; and the U.S. Department of Agriculture for its collaboration in the search.

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Metamorphic Differentiation in Crenulated Schists

It has recently been suggested by Rast[1] that the common development of modal variation in structures of the strain-slip[2] or crenulation cleavage[3] variety in quartz–mica schists is essentially a matter of mica recrystallization in "the points of maximum flexure" of the earlier developed schistose fabric. I suggest, however, that on the contrary in most cases the mobile constituent is silica, and that it is the recrystallization of quartz that is the basis of this type of metamorphic differentiation.

Such a view has been expressed already by Voll[4], who attributes a high surface energy to the junction of quartz and mica compared with quartz/quartz junctions or quartz and other minerals. In line with these views of Voll and the petrographic evidence, it seems reasonable to suppose that where a schistose quartz–mica rock is folded and develops zones of steeply dipping and highly strained schistosity in one set of limbs of asymmetric folds, the quartz diffuses from these zones and migrates to more gently dipping and less strained limbs and crystallizes there.

The typical crenulated schist contains asymmetric folds resembling kink-bands[5] and shows mica concentration not where there is the greatest flexure of first generation mica, as claimed by Rast, that is, along fold axial planes, but in the alternate limbs of the folds. Comparison of such rock with that in which the crenulation structures are not developed shows that the mica proportion in broad and only moderately rotated limbs is very close to the proportion in rocks without the crenulations, but that the narrow and strongly rotated limbs show pronounced deficiency of quartz. In thinly layered quartz–mica schists, quartz-rich layers may be followed into the steep limbs of such folds to thin greatly as the mica-rich layers on either side almost merge, but to thicken as the next broad limb is reached.

It is characteristic of mica-rich zones such as those of the folds of Fig. 1 to have as good a schistosity as limbs rich in quartz, and there is no difficulty in explaining the precise continuity of mica fabric by proposing silica migration and quartz recrystallization as, throughout the folds, the mica fabric is an arrangement directly inherited from an earlier phase of deformation and crystallization. In a hypothesis of mica recrystallization, however, there is more difficulty, as the common situation of Fig. 1 would then suggest the need for a very strict control of the attitude of the new micas, the crystallization of which supposedly took place only under the control of residual stresses[6]. This is not to say that the diffusion of micaceous

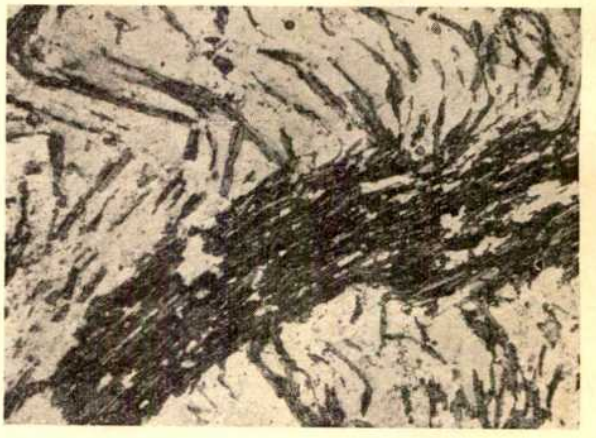


Fig. 1

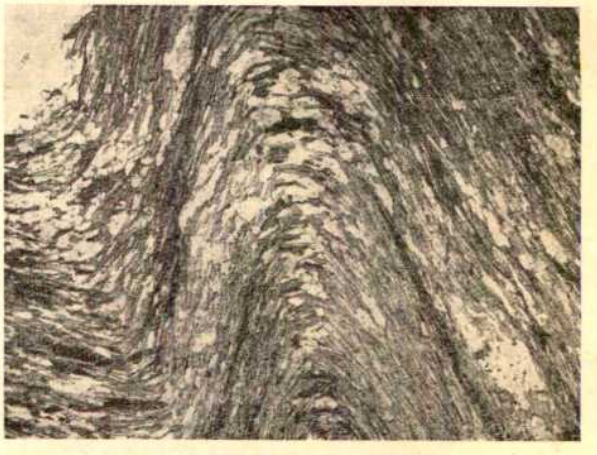


Fig. 2

components does not take place, but that these common cases of differentiation do not seem to show it. The hypothesis of quartz migration allows in addition a ready explanation of the development of differences of quartz fabric in the two limb types of asymmetric folds where grains in the two limbs often are of a different shape[7,8]. Thus, difference in mineral shape accompanies mobility of constituents.

Fig. 2 shows a rock in which crenulations are almost symmetrical. Here there is no formation of fold limbs alternately rich in quartz and in mica, but instead of quartz-rich zones along the axial plane traces. Here it seems that, since all limbs lay equally obliquely across the principal stress and were equally strained, silica-seeking areas of minimum strain concentrated in the fold hinges.

Thus the petrographic evidence is in favour of silica migration and quartz recrystallization rather than mica recrystallization as this allows crenulation differentiation (a conclusion suggested by Dewey[8] for kink bands). The ready diffusion of silica in a schistose quartz–mica fabric during deformation thus seems to be a most important element in the metamorphic differentiation of such rocks, and post-kinematic mica recrystallization is relegated to a role in developing other mica arrangements not possessing the continuous and well-marked schistose arrangement common to those illustrated here.

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CHEMISTRY

Fluorescence Quantum Yield of 1-Dimethylaminonaphthalene-5-sulphonate

Fluorescence quantum yields may be determined with spectrofluorometers simply by comparing the corrected emission spectrum of an unknown substance with that of a compound of known fluorescence yield[1], provided that the relative numbers of photons reaching the solutions are known. Recently, 1-dimethylaminonaphthalene-5-sulphonate (DNS) in water at 25° was used by Churchich[2,3] as a fluorescence standard which was assumed to have a quantum yield of 0·53 as reported by Weber and Teale[4]. The assumption that this quantum yield figure was correct was also made by Slayter and Hall[5] for the purpose of calculating the efficiency of energy transfer in protein-dye conjugates. The purpose of the present communication is to direct attention to the fact that while DNS may be a suitable fluorescence standard, the quantum yield figure of 0·53 is incorrect. In fact, Bowen and Seaman[6] have already published data indicating that the quantum yield of DNS in water at 20° C is only about 0·29. These authors found that the quantum yields were higher in solvents of lower dielectric constant and took pains to exclude oxygen quenching by purging the solutions with a stream of carbon dioxide. Measurements of DNS quantum yield have been performed recently in this laboratory.

An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) was calibrated by modifications of standard techniques (see ref. 7); and the fluorescence quantum yields were measured by comparison with quinine—it is well established that the latter has a quantum efficiency of 0·55 at 25° C in 1 N sulphuric acid[1,8]. The apparatus and its associated instrumentation have been described previously[9,10].

Table 1. Quantum Yields of 1-Dimethylaminonaphthalene-5-sulphonate at 23°

Solvent	Q
Water (purged with N_2)	0 37 ± 0·01
0 1 M $NaHCO_3$	0·36 ± 0·01
0·5 M $NaHCO_3$	0 33 ± 0 01
1 0 M $NaHCO_3$	0·31 ± 0·01
95 per cent ethanol (purged with N_2)	0 72 ± 0 02

The results obtained are shown in Table 1. DNS was found to have a quantum yield of 0·37 in water, but the fluorescence was quenched in bicarbonate solutions. The value $Q = 0{\cdot}31$ in 1 M sodium bicarbonate is reasonably close to that reported by Bowen and Seaman[6], so it can be assumed that their low figure was due to quenching as a result of the use of carbon dioxide to purge their solutions of oxygen. These authors also showed that the quantum yield of DNS in ethanol at 20° C, also purged with carbon dioxide, rose to about 0·75. This figure compares well with our measurements in ethanol purged with nitrogen. In ethanol, then, there is no appreciable quenching by carbon dioxide. Since the solubility at 25° C of carbon dioxide in ethanol is about four times that in water[11], the quenching observed in aqueous solutions is probably due to the HCO_3^- species.

DNS in 0·1 M sodium bicarbonate has now been used in this laboratory as a fluorescent standard ($Q = 0{\cdot}36$), and the results are the same as those obtained when quinine is used as the standard. DNS is easily crystallized, reasonably stable, and there is little overlap of the absorption and emission bands. Experience with our instrumentation leads us to agree with Parker and Rees[1] that determinations of quantum yield utilizing a comparison with a standard are probably more accurate than 'absolute' methods which attempt to measure Q directly.

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Cadmium-109 in New Zealand Rainwater

Cadmium-109 has been detected in rainwater collected at the Institute of Nuclear Sciences site, Lower Hutt, New Zealand. This isotope is presumed to have been produced during the high altitude nuclear detonation, 'Starfish Prime', carried out over Johnston Island on July 9, 1962. The cadmium-109 was introduced at an altitude of 400 km to investigate distribution and fallout for debris injected at the outer edge of the ionosphere[1].

Cadmium-109, which has a half-life of 470 days, decays by electron capture to silver-109*m* ($t_{\frac{1}{2}} = 40$ sec). In the experiments to be reported the specific activity of the cadmium-109 in the samples was determined by measurements on the 22–25-keV *K* X-rays which follow the electron capture process and which also arise from internal conversion of the 88-keV radiation in the decay of silver-109*m*.

Measurements have been made on monthly rainwater samples collected between April and October 1964. Initially samples were collected in a stainless steel vessel; this was later supplemented by a polythene-lined tray. Both vessels yielded similar activities in the October collection, but the September results are significantly

different. The reason for this discrepancy is not yet understood. At the beginning of each month the bottoms of the collectors are covered with distilled water and appropriate carrier solutions added for the radioisotopes now being examined (caesium-137, strontium-90, barium-140, cerium-144, cerium-141, cadmium-109). For cadmium-109, 10 mg cadmium as sulphate is used. At the end of the sampling period, the rainwater containing the carriers and their associated activities is transferred to the laboratory where the cations are absorbed on 'Dowex 50 × 8' cation exchange resin at 80° C. To remove cadmium and the alkali metals, the resin column is eluted with 0·7 M hydrochloric acid and cadmium is associated with sodium in the early part of this elution. The cadmium fraction is evaporated to dryness and the residue redissolved in 0·25 M hydrochloric acid. Cadmium is then precipitated as sulphide by passing hydrogen disulphide through the solution. The precipitate is coagulated by heating on a water bath, and filtered on to a tared 1-cm diameter No. 42 Whatman filter paper disk, dried with acetone, and re-weighed. The filter paper disk and cadmium sulphide deposit is sealed by covering with transparent plastic tape. Blanks have been prepared from the carrier solution.

Since November 1964 no cadmium has been recovered because an unusually large amount of calcium, which was present in the rainwater, caused the cadmium to be removed from the 'Dowex 50' resin column during the cation absorption process.

The counting technique has been improved during the course of the work to be reported; the following procedure is now being used. To minimize errors which could arise from background variations a sample changer is used; this alternately moves a sample and a blank into the counting position. The pulses from the detector are routed to different segments of a pulse height analyser, the appropriate segment being determined by the position of the sample changer; sample changes are made every 40 min. Counting is carried out using a 0·080 in. thick × 0·5 in. diam. NaI(Tl) detector and a 14 stage photomultiplier. An X-ray spectrum from a 148-l. rainwater sample collected during October 1964 is shown in Fig. 1. This spectrum was obtained from a 2,040-min run with the counting time equally divided between sample and blank.

In an attempt to prove that the photopeak shown in Fig. 1 does indeed arise from cadmium-109 the October rainfall sample was ignited to remove the filter paper and plastic tape covers. The residue was digested with a few ml. of aqua regia on a water-bath and finally taken to dryness on a hot plate. The residue was dissolved in 0·25 M hydrochloric acid and cadmium precipitated as sulphide. The precipitate was filtered on to a tared 1-cm diameter No. 42 Whatman filter paper, dried with acetone and re-weighed. The cadmium sulphide deposit and filter were sealed with plastic tape. The specific activity of the treated sample was essentially the same as the original.

Identification based on the decay of the samples is being carried out. Measurements were made on the April sample during July and August 1964 and again in February 1965. During the intervening period the activity had decayed by a factor of 0·807 ± 0·153 which is in good agreement with the factor 0·766 obtained assuming a 470-day half-life.

Table 1

Collection date	Collector	Volume of rain water (l.)	Cadmium carrier recovery (per cent)	Fraction of total deposition	Activity (counts/min/l.)
April 1964	Stainless steel	41·2	81	1	0·185 ± 0·010
May 1964	Stainless steel	69 4	87	1	−0·002 ± 0·015
June 1964	Stainless steel	81 3	100	0 66	0 014 ± 0·015
July 1964	Stainless steel	75·2	100	0·44	0 031 ± 0·015
August 1964	Stainless steel	115·8	41·2	1	0 075 ± 0·015
September 1964	Poly-thene	157·0	84	1	0 097 ± 0·006
	Stainless steel	147·8	100	1	0·047 ± 0·006
October 1964	Poly-thene	42 9	21·8	1	0·204 ± 0·030
	Stainless steel	40·4	100	1	0·220 ± 0·014

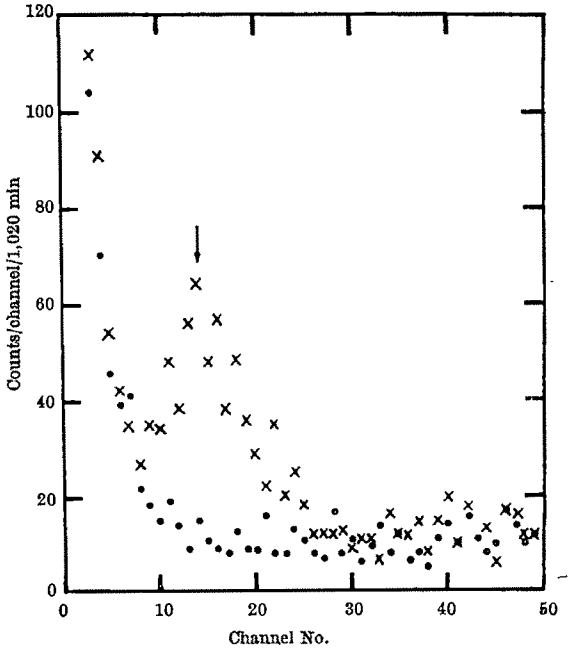


Fig. 1. Pulse height spectra obtained from September 1964 rainwater sample (×) and blank (●). The arrow indicates the expected position of the X-ray peak from cadmium-109

The results obtained so far are listed in Table 1. The results have been corrected for decay of the samples between collection time and counting. In evaluating the correction factor we have arbitrarily assumed that all the sample was collected on the first day of the month. The efficiency of the γ-ray spectrometer was determined using a cadmium-109 source kindly provided by the Health and Safety Laboratory, U.S. Atomic Energy Commission, New York.

The activity concentrations of the measured samples are of the order predicted by Salter[1].

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Crystallization Kinetics of 1 : 3 : 5 Tri-α-naphthyl Benzene

Some years ago an examination[1] of the physical properties of tri-α-naphthyl benzene revealed that this compound exhibits unusual behaviour, spontaneously forming a hydrocarbon glass on cooling. It was also noted that the pure material (which is non-polar) could also be crystallized with difficulty. Recently, more extensive investigations of the physical properties of this material have led to significant results, particularly in relation to the nature of the transport phenomena experienced in viscous flow and crystallization.

A detailed investigation has been made of the kinetics of crystallization, from temperatures a few degrees below the melting point (T_m = 472° K) to within 25° of the glass transition temperature (T_g = 342° K). Over this interval a gradual transition in morphology, ranging from single crystals to spherulites, is observed as the temperature is lowered. The crystal growth rate varies over six logarithmic decades in this temperature span and passes through

a maximum at $T_{max} = 448°$ K. Viscosity measurements[2] (covering fifteen logarithmic decades in viscosity) made through the same temperature range have enabled us to test the validity of some aspects of accepted theories of crystallization[3,4] according to which the crystal growth rate, G, is given by:

$$G = G_0 \exp - \Delta F^*/kT \exp - \Delta G^*/kT \qquad (1)$$

Here G_0 is a parameter considered constant, ΔF^* represents the activation energy characteristic of transport across the liquid-crystal interface (usually taken as the activation energy for viscous flow in the liquid). ΔG^* is a measure of the energy required to construct a stable nucleus of the appropriate geometry.

We find that a model invoking coherent surface nucleation[4] is adequate for our analysis. In this model, B is a constant and the undercooling, $\Delta T = T_m - T$. The transport term, rather than having the form for an activation process, is better represented by a Vogel relationship[5]. This relationship is derived from, and is compatible with, free volume concepts. Under these changes, equation (1) becomes:

$$G = G_0 \exp\left(-\frac{C}{T - T_\infty}\right) \exp\left(\frac{-BT_m}{T\Delta T}\right) \qquad (2)$$

By a Williams–Landel–Ferry analysis[6] of the crystal growth at large supercoolings between T_g and T_{max}, values of the constants C and T_∞ are obtained. Then rearranging equation (2) and plotting $\log G + \frac{C}{2{\cdot}303(T - T_\infty)}$ versus $1/T\Delta T$ gives an approximate value for B. With this derived value of B, the temperature dependence of the transport process involved in crystallization can be written as $G' \equiv G \exp\left(\frac{BT_m}{T\Delta T}\right)$. With these G' values, a more accurate estimate of C and T_∞ is made. In turn, a better value of B is obtained. Utilizing the deduced constants from this truncated iterative process, we find that we can fit all the crystal growth data by equation (2).

Fig. 1 shows reduced plots for G' and viscosity data over the same temperature interval. The temperature dependence for viscous flow and transport-dominated crystal growth G' differ substantially. The present study clearly demonstrates that it is not correct to use the temperature dependence of viscous flow to describe the crystal growth of 1 : 3 : 5 tri-α-naphthyl benzene.

Over the temperature range where a free volume expression fits the results, the calculated parameters[7] for viscous flow are $b = 5{\cdot}0$, $v_0 = 0{\cdot}807$ c.c./g and $T_\infty = 200°$ K; for crystal growth $b = 0{\cdot}84$, $v_0 = 0{\cdot}848$ c.c./g and $T_\infty = 300°$ K. Here b is the Doolittle parameter[8] and v_0 is the occupied volume[6,10]. According to Cohen and Turnbull[10], b is a measure of the minimum hole size necessary for mass transport. The fact that b for viscous flow is the larger indicates that more co-operative interactions are necessary for flow than for crystal growth. If T_∞ is the temperature where the effective free volume for a process disappears (under equilibrium liquid volume which is unattainable in reality), then it may be concluded that between 200° and 300° K viscous flow is possible, whereas crystal growth is not. In contrast, at higher temperatures (Fig. 1), where b is the dominating influence in determining the severity of the temperature dependence, then mass transport in viscous flow changes more rapidly than it does in crystal growth. It appears reasonable that v_0 for crystallization should be the larger because the crystal substrate is space-consuming and thereby lowers the local probability for density rarefactions near the crystal interface.

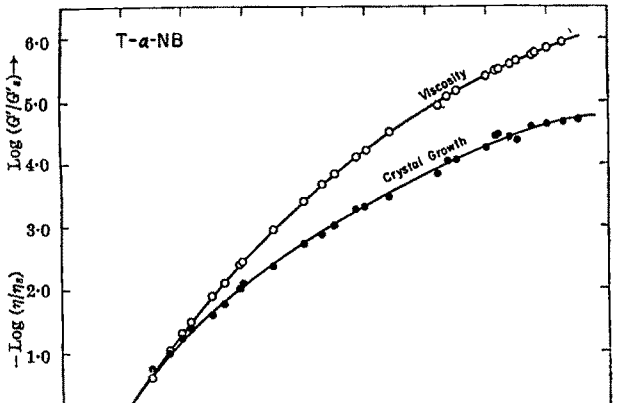


Fig. 1. Temperature dependence of reduced viscosity and transport dominated crystal growth rate of 1 : 3 : 5 tri-α-naphthyl benzene (reference temperature $T_s = 100°$ C). Subscript s indicates value of G' and η at T_s

Although these results apply specifically to a single compound, they may have implications relating to the behaviour of other crystalline materials.

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Covalent Radii of Noble Gas Atoms and Position of Hydrogen in the Periodic System of Elements

In connexion with the discussion initiated in the article by Dash[1] on the position of hydrogen in the periodic system of elements, I wish to say that one cannot disagree with the critical notice of Hawes[2], who has stressed that it is not correct to express the 'atomic size' of members of the noble gas family in terms of their van der Waals radii (the only radii which are conveniently measurable) while for other elements (in the comparisons presented by Dash) covalent or metallic radii are used. Although, as Dash[3] has stated in his reply, the noble gas elements do not form homopolar covalent aggregates, the discovery during 1962–64 of a large number of noble gas compounds makes possible nowadays the estimation of covalent radii for these elements on the basis of their heteroatomic compounds. Such an attempt has been made by myself and colleagues[4].

First, the electronegativity (X) of noble gas elements has been computed using the Mulliken method and expressed in Pauling's scale according to the equation: $X = (I + A)/2 \times 2{\cdot}78$, where I is ionization potential, A, electron affinity. The following values have been obtained: $X_{He} = 4{\cdot}33$, $X_{Ne} = 3{\cdot}73$, $X_{Ar} = 2{\cdot}65$, $X_{Kr} = 2{\cdot}31$, $X_{Xe} = 1{\cdot}94$, $X^{Rn} = 1{\cdot}67$ (for comparison: $X_F = 3{\cdot}78$, $X_{Cl} = 3{\cdot}03$, $X_{Br} = 2{\cdot}77$, $X_I = 2{\cdot}47$, $X_O = 2{\cdot}71$).

Afterwards, using our own modification of Batsanov's[5] method, the averaged values of electronegativity ($\overline{\Delta X}$) and the averaged values of ionicity degrees ($\overline{i}$) have been computed for Xe—F and Xe—O bonds in the known

compounds XeF_n, and XeO_n, where $n = 1, 2, \ldots, 6$ (the degree of ionicity has been calculated by the Hanney and Smith equation: $\bar{i} = 0{\cdot}16\overline{\Delta X} + 0{\cdot}035\ (\overline{\Delta X})^2$; for the increasing n values the starting value of xenon electronegativity increases gradually, for example, from 1·94 in XeF to 2·16 in XeF_6, while $\overline{\Delta X}$ and $\bar{i}$ values decrease respectively). Then, using for the Xe—F bond-length the known Schomaker and Stevenson formula in the form:

$$r_{XeF(in\ XeF_n)} = r_{Xe(in\ XeF_{n-1})} + r_F - 0{\cdot}09\ |X_F - X_{Xe(in\ XeF_n)}|$$

and for the Xe—O bond-length an analogous formula, and also the experimental values of these bond-lengths in the species: XeF_2, XeF_4, XeO_3, XeO_6^{4-}, and the known covalent radius values of fluorine (r_F) and oxygen (r_O), the following values for the average covalent radius of xenon atom have been obtained:

$$\text{at single bond } r_{(Xe-)} = 1{\cdot}41\ \text{Å}$$

$$\text{at double bond } r_{(Xe=)} = 1{\cdot}18\ \text{Å}$$

The covalent radius of xenon (1·41 Å), computed in this way, is only about 6 per cent larger than that of the preceding element—iodine (1·334 Å). It seemed more reasonable to evaluate the covalent radii of the other noble gas elements analogically, that is, increasing the values for the preceding halogens about 6 per cent, than to assume, like Waters and Gray[6], that the formation of an octet causes no expansion of the electronic shell, or to add, like Nesbet[7], a constant correction 0·10 Å to the covalent radius of halogen directly preceding the given noble gas element. The values of covalent radii, estimated in this way, are:

Ne	Ar	Kr	Xe	Rn
(0·76 Å)	(1·05 Å)	1·21 Å	1·41 Å	1·55 Å

Although these values are much less than those of van der Waals radii used in Dash's communication[1], in accordance with his expectation[3], taking into account such corrections does not change the general nature of periodic relationships shown by the atomic radii of elements plotted as a family of curves dependent on the principal quantum number. In particular, the slope of line halogen–noble gas element on Dash's graphs remains the same as that of line hydrogen–helium, and remains the reverse of the slopes for lines drawn for all the other pairs of *s* orbital elements (lithium–beryllium, sodium–magnesium, etc.). This supports the uniqueness of the first quantum shell emphasized by Dash. As is easily proved, besides the covalent radii and boiling points the melting points of elements, plotted as a family of curves dependent on the principal quantum number, also show the same regularities.

The other suggestions of Hawes[2], that one can find the specific properties of hydrogen that would make possible the comparison of this element with any desired element or family of elements in the Periodic Table, and particularly the suggestion of considering the hydrogen as a very light member of Group IV*b* (carbon family), seem only partly true.

The uniqueness of the 1*s* (ref. 1) position of hydrogen, justified by energy conditions (ionization potential and constant energy difference (CED) (ref. 8), cannot be called into question. Some similarity of hydrogen to carbon, but not to other elements of the carbon family, is expressed not only in the near electronegativity values ($X_H = 2{\cdot}1$; $X_C = 2{\cdot}5$ in Pauling scale), what has been stressed by Hawes[2], but mainly in the fact that only these two elements, unique in the Periodic Table, use in their compounds all their disposable orbitals in the appropriate for their principal quantum level. In the case of these two elements, their orbitals are exactly half filled. These conditions are not obeyed for heavier members of Group IV*b*.

Moreover, the electronegativity of carbon is very near to the arithmetical mean of electronegativity ($\bar{X}$) of extremely electropositive caesium ($X_{CS} = 0{\cdot}7$) and extremely electronegative fluorine ($X_F = 4{\cdot}0$). As shown by Haiduc[9] this is the condition of homoatomic as well as heteroatomic catenation. The former is shown only, beside carbon, by sulphur ($X_S = 2{\cdot}5$), selenium ($X_{SE} = 2{\cdot}4$), and iodine ($X_I = 2{\cdot}4$) in polyiodides; the latter is appropriate for about 65 pairs of elements of different electronegativities, but which obey the Haiduc condition $\bar{X} = 2{\cdot}5 \pm 0{\cdot}35$. Such catenation leads to the formation of chain and cyclic structures by alternation of elements. This capacity of carbon to form homogeneous chains, taken with the specific properties of hydrogen, means that carbon and hydrogen bind readily together, and form unusually numerous, nearly pure covalent, mother hydrocarbon systems, thermodynamically stable and moderately reactive. They provide the millions of organic compounds characteristic for the alive nature. The richness of forms which are known, uniform and simple (in sense of types of chemical bonds), has contributed to and exaggerated the role of electronic octet and binding pair (cf. ref. 10).

This is the limit of the structural similarity of carbon and hydrogen atoms, however; their physical and chemical properties differ from those expected for the true chemical analogue or homologues.

On the other hand, the structural similarity of hydrogens ($1s^1$) and halogens (ns^2np^5), and therefore also the similarity of their properties, as stressed by Dash[1,3], arising from the fact that these elements require attraction of one electron to get the especially stable even-electron noble gas configuration, seems to be proved.

Moreover, this can also be said of the uniqueness of the first quantum shell (H–He) as compared with whole periodic system, as well as of some uniqueness of the second shell (Li–Ne) as compared with the next ones. This is evident in the properties of second period elements sometimes very different from those of their heavier analogues. This is a consequence of the energetic relations, which manifest themselves, among other things, in the values of constant energy difference, CED, also computed by Dash (cf. Table 2, ref. 8).

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Labelled Products from the Internal Radiolysis of Liquid Cyclohexane saturated with Tritium Gas

Although the fate of the secondary electron in the radiolysis of water has been discussed for many years, most workers have assumed that it returns to the parent ion in condensed saturated hydrocarbon systems and have interpreted radiolysis data in terms of excited molecules and free radicals. However, the occurrence of ion-molecule reactions in such systems has been discussed by Libby[1] and Williams[2], both of whom have put forward indirect evidence for such reactions[3–6]. The action of several solutes has been interpreted in terms of secondary electron scavenging[7–9], and Freeman[10] and Allen[11] have shown by conductivity measurements on irradiated *cyclo*hexane and *n*-hexane respectively that low yields of free electrons and hence positive ions occur. However,

direct evidence for ion-molecule reactions in such systems is still lacking.

*Cyclo*hexane is the most studied liquid saturated hydrocarbon system and yields the smallest number of radiolysis products. Ho and Freeman[12] in a careful investigation of pure *cyclo*hexane have recently found primary products which had hitherto evaded detection.

We have investigated by gas chromatography the products formed by self-irradiation of *cyclo*hexane/tritium mixtures in the liquid phase and present the results as being pertinent to both the foregoing topics.

0·3 ml. samples of *cyclo*hexane (< 0·001 mole per cent impurity) were saturated with tritium gas at 44 mm and kept in a glass capillary at room temperature for up to two days. Fractions 20 cm from the top of the capillary were transferred to a side limb under vacuum and 2 μl. samples injected into a Pye-Argon gas–liquid chromatograph with proportional counter and ionization chamber attached. Preliminary experiments showed that no gas phase products formed above the surface of the liquid diffused rapidly enough to interfere with the results.

No compounds other than the parent *cyclo*hexane were detected with the ^{90}Sr β-ionization mass detector of the Pye chromatograph, but numerous labelled products were identified with the radiation detectors. Silicone oil, di(2-cyanoethyl)ether, and benzyldiphenyl were used as stationary phases. Chromatographic analysis was carried out at 25° C, limiting detection of labelled products to molecules with less than ten carbon atoms. Not all the products have been identified, but a list of labelled compounds with relative peak areas normalized with respect to *cyclo*hexane is shown in Table 1.

Table 1

2-methylbutane	0 01
n-pentane	0 17
2-methylpentane	0 08
n-hexane	0 20
methyl*cyclo*pentane	0·02
(C_6)	0 07
*cyclo*hexane	1·00
methyl*cyclo*hexane	0·10
*cyclo*hexene	0·04
ethyl*cyclo*hexane	0·22
(C_8 or C_9)	0·15
(C_8 or C_9)	0 32
(C_8 or C_9)	0·32

Although accurate G values have not been determined, the total yields are well above 0·02 indicating that tritium recoil labelling can account for only a small fraction of the products, and therefore most of the labelling is caused by internal tritium-β radiolysis of the *cyclo*hexane.

The implications of these results are threefold:

(1) Free-radical mechanisms are not dominant in the labelling reactions in this system. In radiolytic investigations of *cyclo*hexane it has been found that *cyclo*hexyl radicals are by far the most abundant free radicals as shown by the fact that di*cyclo*hexyl and *cyclo*hexene are the major products[12,13]. Consequently, if the tritium labelling of all products in the system discussed here occurred by free-radical reactions the yield of labelled by-products relative to that of labelled parent should be at least one order of magnitude lower. We conclude that in the production of labelled by-products, free radical reactions are not dominant. Ion-molecule reactions seem the most likely source of such by-products.

(2) There are many more primary products than have hitherto been detected in radiolytic investigations conducted without the use of tritium. Although reaction with tritium would alter the reaction pathway of the various intermediates, the formation of the many labelled products indicates that there are many more primary products.

(3) Fragmentation of *cyclo*hexane occurs in the liquid. The formation of carbon skeletons other than C_6 demonstrates that to a small extent fragmentation involving the breaking of two or more carbon–carbon bonds occurs in the liquid (as well as in the gas phase as shown by the mass spectral pattern) in spite of the cage effect of neighbouring molecules.

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BIOPHYSICS

3,2,1 : *A,B,C* Sub-unit Hypothesis for the α-Chains of Tropocollagen

The clear-cut demonstration by Piez[1] that the 3 α-chains of cod-fish skin tropocollagen are unique, and the likelihood that a similar condition is true for all species of tropocollagen, have led me to consider various simple arrays of sub-units within the chains which could explain their known compositional features. I have arrived at a model in which an α-chain consists of 6 sub-units, each of approximately 17,000 molecular weight, and falling into 3 different compositional types that I call A, B and C, respectively. A tropocollagen unit, then, in this model would consist of a total of 18 sub-units: $6A$, $6B$, $6C$. Of all the possible combinations of 6 sub-units of 3 types in an α-chain, one which fits the known analyses of the amino-acid compositions of the several types of α-chains in the various tropocollagens is the following:

$$\alpha_1 = 3A+2B+C;\ \alpha_2 = 3C+2A+B;\ \alpha_3 = 3B+2C+A$$

For purposes of reference we designate this particular model as the 3,2,1 : *A,B,C* model, indicating that each different α-chain in the tropocollagen unit consists of a different array of the sub-units, but similar in that it has 3 of one kind of sub-unit, 2 of a second, and 1 of the third.

The validity of this model has been tested as follows: First, it must rationalize the data for the composition of the 3 separate chains of tropocollagen of cod-fish skin as determined by Piez. This is the most exacting test of the model because similarly complete data are not available for any other species of tropocollagen. Secondly, the model must be thoroughly consistent with the compositional data known for all the other tropocollagens examined which have been separated into α-chains. The reason why the data on these latter collagens are incomplete is that they have been separated into α_2 and 'α_1' chains, but the latter have not yet been experimentally resolved into α_1 and α_3 components as in the case of cod-fish.

In making such tests of the model, I have in particular determined how it accommodates the analysis with regard to 10 amino-acids, selected because each is present in a relatively low level in the tropocollagen molecule or is distributed unequally among the different α-chains. This constitutes the most stringent test of the model, since obviously less unique models could also explain the composition with regard to amino-acids which appear to be distributed in equal and large numbers among the different α-chains.

Before presenting the fit of the known amino-acid compositions with the proposed model, let us consider some of the ideas leading to its development. In this laboratory I have been impressed with the recurrence of the number 6, or multiples of it, as various chemical and physical aspects of the tropocollagen structure have been

Table 1. Contents of Six Amino-acids* of Various Tropocollagens as Multiples of Six

Tropocollagen	Tyrosine	Hydroxy-lysine	Histidine	Phenyl-alanine	Methionine
Rat skin†	7·2 (6)**	18·2 (18)	12·7 (12)	36·7 (36)	23·7 (24)
Rat tail tendon†	12·1 (12)	21·4 (18, 24)	11·3 (12)	36·6 (36)	23·7 (24)
Human skin‡	9·7 (12)	18·0 (18)	17·2 (18)	36·9 (36)	16·6 (18)
Cod-fish skin§	10·9 (12)	20·0 (18)	25·0 (24)	36·0 (36)	61·7 (60)
Carp swim bladder†	8·8 (12)	22·1 (24)	10·2 (12)	41·2 (42)	50·8 (48)
Dogfish skin¶	7·4 (6)	19·4 (18)	38·3 (36)	38·1 (36)	56·1 (54)

* Expressed as residues per tropocollagen molecule (300,000).
† Calculated from Piez *et al.* (ref. 4).
‡ Calculated from Bornstein and Piez (ref. 8).
§ Calculated from Lewis and Piez (ref. 9).
¶ Calculated from Piez (ref. 1).
** Numbers in parentheses represent an integral multiple of 6.

Table 2. Experimental* and Calculated† Cod-fish α-Chain Analyses of the Basis of the Unified (3,2,1 · *A,B,C*) Sub-unit Model

Amino-acid		α_1	α_2	α_3	Sum	*A*	*B*	*C*	*n*
Tyrosine	Experimental	1·1	7·0	2·8	10·9				
	Calculated	2	6	4	12	0	0	2	2
Hydroxylysine	Experimental	5·5	9·1	5·3	19·9				
	Calculated	5	8	5	18	1	0	2	3
Histidine	Experimental	5·1	13·0	7·2	25·3				
	Calculated	4	12	8	24	0	0	4	4
Phenylalanine	Experimental	15·0	8·7	13·0	37·0				
	Calculated	15	9	12	36	3	3	0	6
Methionine	Experimental	16·0	19·0	17·0	52·0				
	Calculated	17	20	17	54	3	2	4	9
Valine	Experimental	16·0	23·0	23·0	62·0				
	Calculated	16	21	23	60	1	4	5	10
Leucine	Experimental	19·0	27·0	16·0	62·0				
	Calculated	18	26	16	60	4	0	6	10
Threonine	Experimental	23·0	29·0	25·0	77·0				
	Calculated	22	30	26	78	3	3	7	13
Lysine	Experimental	32·0	22·0	32·0	86·0				
	Calculated	32	21	31	84	5	8	1	14
Glutamic acid	Experimental	84·0	69·0	88·0	241				
	Calculated	84	68	88	240	12	20	8	40

* Calculated to a 100,000 molecular weight α-chain from experimental data of Piez (ref. 1).
† Calculated from the model $\alpha_1 = 3A+2B+C$; $\alpha_2 = 3C+2A+B$; $\alpha_3 = 3B+2C+A$ with integer amino-acid assignments to each of the sub-units.

analysed. Thus, there are 6 'ester-like' bonds per α-chain[2], and 12 hexose units per tropocollagen molecule[3]. Even more significantly, if one examines the amino-acid compositions of various species of tropocollagen as determined by Piez *et al.*[4], one is struck by the fact that amino-acids, critical according to the criteria already described here, occur in multiples of 6. Table 1 illustrates this circumstance for 5 amino-acids as they occur among 6 different tropocollagens. Thus, apart from the implications of the ester-bond and hexose analyses, there are reasons which compel one to consider a sub-unit hypothesis for the α-chains. (*Note added in proof.* Recently, Hörmann *et al.*[10] have shown the presence of an average of 6 N^{α}-acetyl residues per α-chain in tropocollagen and have interpreted this finding as consistent with an average of 6 sub-units per α-chain.) If one looks closely at the amino-acid compositional data, one sees that there is more regularity than contained in the simplest model that states that there are 3 different α-chains, 2 of which (α_1 and α_3) are similar but not identical in composition.

Recently, Blumenfeld *et al.*[5,6], using hydroxylamine-treated tropocollagen, have been able to isolate sub-unit fractions which are homogeneous in size. These fractions fall mainly into two molecular weight classes of about 18,000 and 34,000, respectively. Considering that the integral sub-units are 17,000 to 18,000 in molecular weight, a single α-chain of molecular weight approximately 100,000 would be comprised of 6 sub-units. Within the chain, of course, these sub-units could be covalently bonded so that after particular chemical or physical treatments they could be found as multiples of the 17,000 sub-units.

From the cod-fish skin tropocollagen α-chain analyses various models were calculated subject to the following rules: (1) There are 3 different kinds of sub-units of equal size. (Attempts to calculate models based on occurrence of 2 sub-units failed when they were unable to rationalize the amino-acid compositional data.) (2) Each α-chain contains 6 sub-units. (3) In the composite 3 chains (that is, the tropocollagen unit), there are equal numbers of each of the 3 sub-units. (Without this rule a great number of combinations becomes possible.)

The *A,B,C* α-chain models then can be classified as (6,0,0), (5,1,0), (4,2,0), (4,1,1), (3,3,0), (3,2,1) either for all three chains in a unified manner (for example, $\alpha_1 = 4A+B+C$; $\alpha_2 = 4B+A+C$; $\alpha_3 = 4C+B+A$) or in combinations (for example, $\alpha_1 = 4A+B+C$; $\alpha_2 = 3B+2C+A$; $\alpha_3 = 2B+3C+A$) in a non-unified manner.

Calculations were made for all unified models and only one model was found which could fit the data for the 10 selected amino-acids. Other models are able to fit some of the amino-acid analyses; however, none could fit as well as the unified (3,2,1) model below:

$$\alpha_1 = 3A+2B+C$$
$$\alpha_2 = 3C+2A+B$$
$$\alpha_3 = 3B+2C+A$$

In Table 2 are tabulated the experimental and calculated α-chain compositions for this model with various integer assignments to the *A*, *B* and *C* sub-units.

A striking result of this analysis is that the several sub-units are considerably more dissimilar than are the different α-chains. For example, the model predicts that a *B* sub-unit of the cod-fish tropocollagen should have 8 residues of lysine in contrast to 1 residue in the *C* sub-unit and 5 residues in the *A* sub-unit. Also, it predicts that histidine should be absent from the *A* and *B* sub-units and present to the extent of 4 residues in the *C* sub-unit. Outstanding differences in the distribution of other amino-acid residues among the several sub-units are also predicted, as seen in Table 2. These predictions can be tested when the *A*, *B* and *C* sub-units are isolated separately and analysed.

A different sub-unit model has been proposed by Petruska and Hodge[7] on the basis of interpretation of the electron optical appearance of various precipitated forms of tropocollagen and, in particular, of the segmental long-spaced form (SLS). This model consists of 17 sub-units per tropocollagen molecule. The sub-units are of two different sizes and compositions. An α_1- or α_3-chain is considered to have 5 sub-units, and an α_2-chain to have 7 sub-units. This model, although having certain interesting features, is not in agreement with several important chemical and physical features of the collagen structure.

The 3,2,1 : *A,B,C* sub-unit model proposed here explains much of the chemical data, and agrees with the size units found after treatment of tropocollagen with hydroxylamine. The predictions that it allows concerning the composition of the different sub-units (*A,B,C*—each of a molecular weight approximately 17,000–18,000) can readily be tested by isolation and analysis of the sub-units. Experiments towards this end, utilizing various tropocollagens, are now in progress in this laboratory.

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Coacervate Behaviour in an Alternating Electric Field

Coacervates have been intensively investigated because of their presumed importance in the origin of life[1]; however, their electrical properties, representing many important processes, appear to have been relatively neglected. We

have continued work by Bungenberg de Jong[2] on the behaviour of coacervates in an alternating electric field, and effects similar to those reported by Heller and Texeira-Pinto[3] and Furedi and Ohad[4] on living cells have been observed.

Our work used gelatine–gum arabic and haemoglobin–gum arabic coacervates prepared by a method similar to that reported by Dervichian[5]. To prepare the gelatine coacervates, equal volumes of 1 per cent solutions of gelatine and gum arabic were mixed and brought to a *p*H range of 2·5–4·0 by adding sodium acetate-citric acid buffer. Coacervate formation could be seen by the appearance of a white turbidity as the mixture was acidified. The haemoglobin coacervates were formed by mixing two volumes of a 2 per cent haemoglobin solution (prepared by the lysis of red blood cells) with one volume of a 1 per cent gum-arabic solution and acidifying as above.

The electric field was applied to a simple assembly on a microscope slide. Brass strips 0·0125 cm thick separated from each other by a distance of 0·1–2 cm were used as electrodes. The brass strips were fixed to the slide by embedding them on melted 'Parafilm' sheets (manufactured by Marathon, Neeah, Wisconsin); the assembly was then allowed to cool, and a chamber was cut between the electrodes to enable direct contact to be made with the solution. The observations reported in this communication cover the frequency range from 4 c/s to 700 kc/s: the electric field intensity was a maximum of 500 V/cm.

Flattening of the gelatine–gum arabic coacervates, with the long axis perpendicular to the field, was observed throughout the frequency range. This response to the electric field was strongest during the first few minutes after the coacervates had formed. The minimum field strength necessary to obtain deformation increased at higher frequencies and higher *p*H ranges. After the field had been applied for several minutes, coalescence of these coacervates into streams perpendicular to the field occurred. When the coacervates were allowed to age for 15–60 min, the coacervate morphology changed and we could sometimes see a difference in the response to the electric field. The elongation became parallel to the electric field.

The haemoglobin–arabic coacervate behaviour in the electric field was also observed to be strongly dependent on time. When the coacervates were fresh, they flattened into discoidal shapes perpendicular to the field at all frequencies used and showed seemingly-random rotations. As the haemoglobin coacervates aged (about 1 min from the time of acidification) the perpendicular elongation disappeared and the only deformation observed was elongation parallel to the field and chain formation which occurred at the higher frequencies. These observations on the haemoglobin coacervates are very similar to those of Füredi and Ohad[4] on the living red blood cell. These authors found that an electric field of 120 Mc/s, applied to an isotonic sucrose suspension of erythrocytes, aligned the cell radii perpendicular to the field direction, and caused slow rotation. However, older cells (with regard to time of formation in the donor) became elongated in the direction of the field and formed chains parallel to the field. Our coacervates showed, to the living red blood cells, an analogous behaviour, when they first became elongated perpendicularly, then later parallel, to the field and form chains.

Füredi and Ohad explained their results by considering the electric field behaviour of elastic charged spheres. If the young cells had a lower dielectric constant than the medium and bear a surface charge, then their behaviour was accounted for. Older cells, having a higher dielectric constant and a lower surface charge density, would become elongated parallel to the field and form chains. This explanation would seem to be the most appropriate to explain the coacervate behaviour. We also expect that the frequency variation of deformation is connected with the finding of Schwan[6], who reported extraordinarily large dielectric constants of colloid suspensions at low frequencies.

Our observations as described here indicate that coacervates exhibit similar electrical behaviour in an alternating electric field to that of some living cells. The coacervates are, therefore, of interest as a model system for the study of the behaviour of living cells in electric fields.

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BIOCHEMISTRY

Effect of Vitamin E Deficiency on Uptake of Amino-acid and Incorporation in Isolated Rabbit Diaphragm

One of the most striking effects of vitamin E deficiency is the acute muscular wasting seen in young rabbits raised on a tocopherol-free diet. Although this nutritional muscular dystrophy has been known for more than thirty years, its mechanism remains obscure. If impaired muscle protein synthesis were the cause of the wasting, one should expect that vitamin E-deficient animals would incorporate less of a given dose of amino-acid labelled with carbon-14 into muscle proteins when compared with vitamin E-supplemented animals. Surprisingly, Dinning *et al.*[1] have found that dystrophic muscle incorporated more glycine-1-^{14}C.

This does not necessarily mean that muscle protein synthesis is increased in this condition. Radioactivity from an injected dose of glycine-1-^{14}C or of α-aminoisobutyric acid-1-^{14}C (AIB) reaches the free amino-acid pool of muscle of dystrophic rabbits faster than that of vitamin E-supplemented animals[2,3]. Higher radioactivity of the amino-acid precursor should be expected to lead to a higher radioactivity of the proteins. *In vivo* investigations are further complicated by possible effects of the dietary deficiency on plasma volume, blood supply and capillary permeability. In order to exclude such complications, I have investigated the effect of vitamin E deficiency on amino-acid uptake by isolated rabbit diaphragm, and amino-acid incorporation into diaphragm proteins *in vitro*.

Two groups of four-week-old White New Zealand rabbits received a vitamin E-deficient semi-synthetic diet. One group was given oral supplements of α-tocopherol acetate. The composition of the diet and the manner of vitamin E supplementation and other details of animal care were the same as in previous experiments[3]. When symptoms of beginning muscular dystrophy (urinary creatine excretion and muscular weakness) became evident in the vitamin E-deficient group after approximately three weeks, animals were killed and diaphragms were quickly excised. Conditions of the incubation of hemidiaphragms with ^{14}C-labelled amino-acids are indicated in Table 1. At the end of the incubation periods the hemidiaphragms were briefly rinsed in ice-cold saline, blotted, weighed and homogenized. Total proteins of diaphragm incubated with glycine were precipitated with hot trichloroacetic acid (TCA) and lipids removed in the usual manner. Radioactivity was determined in the proteins and in the TCA-soluble extract.

Table 1 shows that vitamin E-deficiency caused a more than two-fold increase of incorporation of glycine-1-^{14}C

into diaphragm proteins. The result obtained *in vivo* by Dinning *et al.*[1] has thus been confirmed with isolated muscle. This increase may well be due to a higher specific activity of glycine in the precursor pool, as indicated by the higher activity found in the TCA extract.

Glycine is not very suitable for an investigation of amino-acid uptake because of its participation in numerous metabolic pathways. Christensen *et al.*[4] have therefore suggested to use the non-metabolizable AIB as a transport model. This amino-acid is not incorporated into proteins and it resembles glycine in its transport properties[5]. In the work recorded here the effect of vitamin E deficiency on amino-acid accumulation in skeletal muscle has been investigated with the aid of AIB. After incubation of hemi-diaphragms of vitamin E-deficient and supplemented rabbits with AIB-^{14}C the tissue was homogenized and extracted with hot 0·01 N acetic acid and radioactivity was determined in the extract.

The results (Table 2) are in line with those obtained with glycine and differences between the two groups of animals are even greater. After 1 h of incubation, diaphragms of vitamin E-deficient animals contained on the average 92 per cent more AIB-^{14}C. The observation that vitamin E-deficient muscle accumulated less glycine than AIB is probably due to the fact that some of the glycine is utilized for protein synthesis and carbon dioxide formation, the latter being greatly elevated in vitamin E deficiency[6]. Since distribution ratios in Tables 1 and 2 are expressed on a 'per gram of tissue' basis, one might suspect that a higher ratio could be caused by higher water content or greater extracellular space. However, earlier experiments have shown[3] that differences in water content and inulin space between skeletal muscle of vitamin E-deficient and of supplemented rabbits are small. They cannot account for the differences observed here. The same applies to diaphragm weights. Average hemi-diaphragm weight of vitamin E-supplemented animals was 644 mg, and of vitamin E-deficient animals 669 mg. This difference is not statistically significant. The content of TCA-precipitable protein was significantly lower ($P < 0·025$) in diaphragms of the deficient group: 10·2 per cent, versus 12·5 per cent in the supplemented group.

The experiments recorded here do not exclude the possibility that there is a higher rate of protein synthesis in skeletal muscle of vitamin E-deficient rabbits. They do indicate that the big increase in the rate of incorporation of glycine-1-^{14}C into muscle proteins of vitamin E-deficient rabbits is at least partly due to a higher rate of glycine uptake into the tissue amino-acid pool. The finding of increased amino-acid accumulation in vitamin E deficiency is of particular interest in view of the increased oxygen consumption of vitamin E-deficient muscle[7], and the reported linear relation between oxygen consumption and membrane transport in some tissues[8,9].

Table 1. Uptake of Glycine-1-^{14}C by Isolated Diaphragm and Incorporation into Proteins

(*A*) Specific activity of diaphragm proteins (c.p m./mg protein)

	1 h	2·5 h
Vitamin E-supplemented (9)*	69 ± 20†	188 ± 63
Vitamin E-deficient (9)	153 ± 75	474 ± 137

(*B*) Distribution ratio of TCA-soluble glycine (c.p.m./g diaphragm divided by c.p.m./ml. medium)

Vitamin E-supplemented (9)	0·63 ± 0·10	0 81 ± 0·20
Vitamin E-deficient (9)	0·91 ± 0 21	1·11 ± 0·23

* No. of animals in parentheses.
† Mean value ± standard deviation.
Statistical evaluation by the Student *t* test indicates that the differences between vitamin E-supplemented and deficient groups are significant ($P < 0$ 025) at both incubation times.
Conditions of incubation: Dubnoff metabolic shaker; 37° C; O_2–CO_2 atmosphere 95 : 5; 8 ml. Krebs-Ringer bicarbonate buffer *p*H 7·4, containing 4 μc. of labelled amino-acid. Specific activity of glycine: 4·84 mc./mmole.

Table 2. Uptake of α-Aminoisobutyric Acid-1-^{14}C by Isolated Diaphragm

	Distribution ratio (c.p.m./g diaphragm divided by c.p.m./ml. medium)	
	0·5 h	1 h
Vitamin E-supplemented (6)	0·46 ± 0 04	0·66 ± 0·12
Vitamin E-deficient (6)	0·66 ± 0 07	1·27 ± 0·46

See legend to Table 1 for explanation of terms and conditions of incubation. The difference between the two groups is significant ($P < 0$ 025) at both incubation times. Specific activity of AIB: 10 03 mc./mmole.

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Determination of Serum Oxytocinase (*l*-Cystine-aminopeptidase) Activity

An increasing interest is being shown in serum oxytocinase (*l*-cystine-aminopeptidase (3.4.1.2.)) levels in pregnancy, yet the methods described in recent papers[1,2] for its determination seem unnecessarily laborious. We have for some time now been using the modification described here. It is based on the method used for the determination of *l*-leucine-aminopeptidase (3.4.1.1.) activity in serum[3–5], now used as a routine biochemical investigation for which commercial test kits have been developed, such as that of Boehringer, Biochemica, Mannheim, which we use.

The buffer is 0·05 M phosphate (KH_2PO_4 (Sørensen) and K_2HPO_4), *p*H 7·20. The substrate is *l*-cystine-di-beta-naphthylamide, 135 mg dissolved in hot 0·012 N HCl and made up to 100 ml. with H_2O. It is understood that preparations made with beta-naphthylamide are now known to be carcinogenic. Although opinions on the various concentrations required for harmful activity differ, we think that the particular concentration used here does not necessitate precautions other than usually employed in biochemical laboratories using often poisonous material. It is, however, the intention of the authors to replace the present substrate with a new preparation as soon as a manufacturer can be found to produce it routinely. The chromogen is diazotized 3-chloro-4-nitraniline (Echtrot '3 GL') 1 mg per ml. of H_2O.

The principle of this reaction of cystine aminopeptidase (CAP) is:

$$l\text{-cystine-di-beta-naphthylamide} + H_2O \xrightleftharpoons{\text{CAP}} l\text{-cystine} + \text{beta-naphthylamine}$$

The azo-dye produces a red colour with the enzyme-liberated naphthylamine which is extracted with ethyl acetate; its concentration is measured colorimetrically, and the CAP activity of serum is deduced. Since the liberation of naphthylamine is linear within the range of enzyme activity to be found in normal and abnormal human sera, a factor can be used for calculation of the enzyme activity per ml. of serum.

A mixture of 2·40 ml. of buffer and 0·01 ml. of substrate and 0·30 ml. of serum is incubated at 37° C for 1 h, and 2·50 ml. of trichloroacetic acid is added. After centrifugation, 1 ml. of supernatant is added to 1 ml. of chromogen, which then is mixed well and stood at room temperature for 10 min. 5 ml. of ethyl acetate is added and the tubes are shaken vigorously. The extracted colour intensity is measured in the ethyl acetate using a colorimeter at 546 mμ and a 1-cm light path rectangular cuvette against a blank prepared using H_2O instead of serum.

The absorbancy of the ethyl acetate is multiplied by the factor of 118 for calculating CAP activity per ml. of serum.

It should be pointed out that this factor needs to be corrected according to the variations in instrument sensitivity and other technical factors for individual laboratories by setting up an initial calibration curve of beta-naphthylamine.

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Effects of Propiolactone on Rat Fibrocytes

B-PROPIOLACTONE has been reported[1–4] to be a potent but slowly acting carcinogen *in vivo*. In an investigation of the effects of this agent on rat fibrocytes cultivated *in vitro*, treated cells were found to undergo a particular sequence of structural alterations which culminated in cytological malignancy. This sequence of changes appeared to be directly related to the process of carcinogenesis.

Fibrocytes were grown from Wistar strain rat embryo voluntary muscle tissue. They were cultivated as monolayers on No. 2 coverslips in hexagonal roller-tubes. Suspensions of fibrocytes in modified culture medium were prepared either directly by dissociating the parent tissue with 0·25 per cent commercial grade trypsin in 0·9 per cent sodium chloride solution at 4° C overnight, or by the trypsinization of recently derived stock cultures grown from muscle explants. The latter method gave cleaner preparations. The density of cell population was adjusted to give thin, incomplete monolayers at the beginning of treatment. This made provision for subsequent proliferation. Coverslip cultures were fixed in Heidenhain's 'Susa' fixative for 10 min and routinely stained progressively with Ehrlich's acid haematoxylin and alcoholic eosin. Stained cultures were mounted under matching No. 0 coverslips for study with oil immersion objectives.

The normal culture medium, as used for control cultures, was composed of 25 per cent of inactivated calf serum, 55 per cent of tissue culture medium supplement No. 199, and 20 per cent of Earle's buffered saline solution. It also contained lactalbumin hydrolysate and extract of autolysed yeast at final concentrations of 0·25 and 0·1 per cent, respectively. Mycostatin at 50 units/ml., penicillin and streptomycin at standard concentrations were also incorporated in the medium. The original cell suspensions were prepared in this medium, to which was added either 5 per cent of cell-free sarcoma 37 ascites tumour 'plasma', or 0·25 per cent of heparinized rabbit plasma. The addition of plasma promoted adhesion of fibrocytes to the coverslips.

Experimental media were prepared by the addition of propiolactone to the normal culture medium and serial dilutions. After a preliminary cultivation for several days, fibrocytes were treated daily with concentrations of propiolactone ranging from M/10 to M/100,000 in a standard volume of 4 ml. per roller-tube. The lactone was rapidly inactivated in culture medium. At concentrations of from M/10 to M/500 propiolactone quickly killed the cells during the first application. Depletion of basophilic material from both nuclei and cytoplasm was noticeable even when structural detail was virtually 'fixed'. This loss was not secondary to autolysis but due to a direct effect of the agent. One application of M/1,000 propiolactone irreversibly injured the fibrocytes; those in mitosis were most susceptible. Fibrocytes withstood 2–3 applications of M/2,500 and 5–6 applications of M/5,000 propiolactone, but almost no viable dividing cells were found at any time. M/10,000 and M/20,000 drug concentrations were slowly lethal and most mitoses were abnormal. The margin of safety was difficult to estimate because of the delayed toxic effects. Mitotic aberrations were widely varied. They included failure of chromosomes to move to the metaphase position, spindle failure, incomplete separation and coalescence of metaphase chromosomes, anaphase bridges and deletions, and faulty telophase reconstructions. The majority of mitotic aberrations occurred at prometaphase; the chromosomes remained entangled and degenerated.

After about eighteen successive applications of M/40,000 propiolactone cytologically malignant cells appeared in treated cultures. These cells were very hyperchromatic, with deeply basophilic cytoplasm and indistinguishable from typical sarcoma cells. They proliferated rapidly. At this drug concentration treated cells responded with over-compensation to the repeated injuries caused by propiolactone. M/100,000 drug concentration was usually less effective than M/40,000 in inducing malignancy.

Comparisons of interphase cells treated with the graded drug concentrations showed a common pattern of qualitatively similar lesions which varied from the infliction of rapid cell death to the transformation of normal fibrocytes to viable, dividing malignant cells by a progressive adjustment to repeated slight injuries. The initial aberrations of mitosis appeared to be less directly relevant than the alterations in interphase cells to the carcinogenic process.

Structural changes in interphase cells of the foregoing pattern were initially shown by the loss of basophilic material from the cytoplasm and nuclear organelles. This loss was relatively slight in cells exposed to M/40,000 propiolactone solutions. It was well marked in the nucleoli and chromocentres. Cytoplasmic basophilia is positively related to the presence of RNA-containing ribosomes in ergastoplasm[5,6]. The parallel disappearance of basophilic material from the matrices of nucleoli revealed their internal reticular structure. The typical mammalian nucleolus consists of such a reticulum embedded in an optically amorphous matrix[7,8]. The component threads of the intranucleolar reticulum or nucleolonema[9] were thicker than those of the nuclear reticulum itself, but the threads of the two networks were structurally continuous. These altered primary nucleoli had a variable reticular structure associated with different amounts of matrix substance.

The early loss of basophilic matrix substance was followed by hypertrophy of the nucleolonema structure. This was brought about by a preliminary thickening of nuclear reticulum threads continuous with those of the nucleolonema before they were incorporated into the structure of the enlarging nucleolus. This thickening usually began with the development of small discrete nodes along a thread. These enlarged and became confluent to form a generally thickened thread. The nodes were sometimes confluent from their first appearance. Modified nuclear reticulum threads became indistinguishable from the original threads of the primary nucleolus and laid down matrix material.

Sections of nuclear reticulum unconnected with nucleoli similarly hypertrophied. In some cells these altered threads appeared to give rise to surrogate secondary nucleoli. Altered nucleoli reached their maximum development in the induced malignant cells and were rich in basophilic matrix substance. They were usually irregular in shape through the protrusion of hypertrophied reticulum threads which tapered into strands of nuclear reticulum. Chromocentres enlarged and became more numerous parallel with the changes in reticulum threads. The process of nucleolar enlargement continued to the fully malignant stage at which basophilia was maximal. The malignant cells multiplied rapidly, unlike the altering cells. The complete sequence of alterations was readily followed in fibrocytes treated with M/40,000 propiolactone.

Comparison of sister cells in the reconstruction phase of the nuclei showed a detailed similarity in the pattern of altered reticulum threads of the twin nuclei. However, variation in pattern between different pairs of sister nuclei was great. This suggested that the changes were inherited. The latter continued to develop during interphase and probably gave rise to chromosome abnormalities during mitosis.

The nucleolus is regarded as the main site of development of ribosomal RNA (refs. 7, 8 and 10) and is positively correlated with protein synthesis and cell growth[5,6] which are dependent on the ribosomes of ergastoplasm. Replacement of cytoplasmic RNA in fibrocytes injured by propiolactone would seem, therefore, to be dependent on the restitution of nucleolar function, itself decreased by the carcinogen. The latter was achieved by conversion of sections of nuclear to nucleolar reticulum. This transformation of portions of nuclear reticulum implied the converse loss of their original functions. The nuclear reticulum and its associated organelles comprise the interphase equivalent of formed chromosomes. The carcinogenic action of propiolactone would therefore seem to be related to the progressive development of increased growth potential at the expense of losing specific chromosomal functions. The characteristics of malignant cells are in accordance with this interpretation.

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Methionine-activating Enzyme and Catechol-O-methyl Transferase Activity in Normal and Leukaemic White Blood Cells

It has been found recently in these laboratories that the leucocytes of patients with chronic myelocytic leukaemia have significant elevations of the important methyl donor, *S*-adenosylmethionine (SAMe) (ref. 1).

The original series of patients has been extended to include 40 leukaemic and 20 normal leucocyte preparations. Not only is there an elevation of SAMe in chronic myelocytic leukaemia, but there also appears to be a decline of this substance with remission of the disease[2]. The mechanism and significance of this abnormal biochemical finding are not clear and are now under investigation here. The work recorded here was designed to discover whether the SAMe-synthesizing enzyme or a methyl transferase can be detected in human leucocytes. In addition, since SAMe elevation could be due to increased synthesis or decreased utilization, the examination of these enzymes might help explain the elevations in SAMe noted.

Blood was obtained from 14 untreated patients with chronic myelocytic leukaemia at the National Cancer Institute. Our diagnoses were confirmed by haematological and cytogenetic examinations of peripheral blood and marrow. Total peripheral white counts ranged from 50,000 to 350,000 cells/mm^3, and all patients had elevations of immature peripheral white blood cells. Leucocytes were also obtained from 19 normal blood donors for comparison with leukaemic cells.

The white blood cells were isolated by plasmapheresis followed by dextran centrifugal sedimentation and osmotic stress at 4° C by the method of Fallon *et al.*[3]. The concentrated white cells were stored at −20° C until assayed for enzyme activity, usually within 48 h. With careful preparation, it was possible to obtain 1 g of white cells from 500 ml of normal whole blood. Aliquots of leukaemic cell preparations of the same quantity (1 g) were taken. Leucocyte homogenates were prepared by homogenizing aliquots of the frozen wet white blood cell preparations in 5 vol. of ice-cold isotonic potassium chloride and centrifuging at 0° C, 10,000*g*, for 30 min.

The activity of a typical and ubiquitous methyl pherase, catechol-*O*-methyl transferase (COMT) (ref. 4), was measured by a modification of a previously reported assay of the combined activity of methionine-activating enzyme (MAE) and COMT (ref. 5). Dihydroxy benzoic acid was converted to vanillic acid-methoxy-^{14}C with SAMe-^{14}C (New England Nuclear Corp., Boston, 40 c./mole) in tubes containing 1 μmole of substrate, 52 mμc. of SAMe-^{14}C, 50 μmoles of magnesium chloride, 125 μmoles of phosphate buffer (*p*H 7·8), and 0·5 ml. of the leucocyte preparations. The mixture was incubated for 30 min and ^{14}C-vanillic acid was extracted into ethyl acetate from the strongly acidified reaction products. The products, labelled with carbon-14, were assayed by liquid scintillation spectroscopy[6]. The extracted carbon-14-labelled product, in excess of a boiled enzyme blank, varied linearly with both incubation time and enzyme concentration.

The activity of methionine activating enzyme in the leucocyte homogenates was estimated by the method of Mudd *et al.*[7], utilizing the synthesis of SAMe-^{14}C from methionine-^{14}C and ATP, and isolation of SAMe-^{14}C on 'Dowex-50-NH_4^+' chromatographic columns. The carbon-14 eluted by ammonia varies linearly with incubation time and enzyme concentration.

Table 1. Enzyme Activities in Normal Human Leucocytes and in Chronic Myelocytic Leukaemia (CML)*

	Normal	CML	
N	19	14	*P*
MAE	3·59 ± 0·75	4·78 ± 1·06	0·3–0·4
COMT	6 05 ± 0·51	5·04 ± 0 97	0·3–0 4

* MAE = methionine activating enzyme, units ± *S.E.* COMT = catechol-*O*-methyl transferase, units ± *S.E.* Unit enzyme activity equals mμmoles of product formed in 30 min per gram of frozen wet leucocytes. *P* is probability by 'Student' *t*-test, *N* is the number of patients.

The results of these enzyme assays are presented in Table 1. They demonstrate that human leucocytes contain detectable quantities of methionine-activating and methyl transferase activity. In chronic myelocytic leukaemia, these enzyme activities are not different from normal.

The significance of methylation in leukaemia has not yet been adequately evaluated. In addition to the finding of increased levels of SAMe in leukaemic white cells[1], other facts are presently available to suggest that abnormal methylation may be of importance. Folic acid is increased in leukaemic cells[8], and antifolate agents have been used in leukaemia chemotherapy. Patients with chronic myelocytic leukaemia have increased amounts of vitamin B_{12} in their serum[9,10]. Finally, methionine activating enzyme and COMT appear to be normal in chronic myelocytic leukaemia leucocytes. Thus it is not clear whether SAMe is elevated in chronic myelocytic leukaemia cells because of increased synthesis or decreased utilization. There are many routes of utilization[11,12] which remain to be studied and which might be decreased. Elevations of SAMe have been demonstrated in the presence of normal MAE activity[13] when methionine availability is increased[14]. Methionine might be more readily available for SAMe synthesis if its entry into leucocytes were increased or its incorporation into protein were decreased. Assays of leucocyte methionine are thus in order. The suspected decrease in leukaemic white cell ATP degradation[15] is of uncertain significance for SAMe synthesis.

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Reduced Nicotinamide-adenine Dinucleotide and Pyruvate in Urea-denaturation of Lactic Dehydrogenase Isozymes

IT has recently been reported that lactate dehydrogenase isozymes differ from each other in denaturation caused by urea[1-4], probably depending on the different resistance of the sub-units in these isozymes against denaturating forces. The aim of this contribution is to show that the presence of coenzyme during urea incubation may help to differentiate isozymes composed of different sub-units, and that the increase of substrate molarity will further accentuate this differentiation.

The isozymes examined were LDH_1 (pig heart) and LDH_5 (rabbit muscle) received from Boehringer and Soehne, Mannheim. The determinations of LDH activity were made in duplicate as presented earlier[4] and were confirmed with repeated measurements. Urea concentration selected was 1·5 M, at which molarity we have noticed the greatest difference between these isozymes. Pyruvate concentrations used were $2{\cdot}5 \times 10^{-4}$ M and 10×10^{-4} M. NADH concentration was $1{\cdot}3 \times 10^{-4}$ M in all determinations.

In our first experiments we used $2{\cdot}5 \times 10^{-4}$ M pyruvate. As seen in Fig. 1*A* the incubation of LDH_1 in urea solution without pyruvate and NADH diminished the enzyme activity to 54 per cent. The presence of pyruvate during the incubation did not prevent denaturation (56 per cent), but when the enzyme was incubated with urea and NADH the activity remained at 64 per cent. The presence of NADH during incubation thus protected the activity of LDH_1 about 20 per cent. On the contrary the inactivation of LDH_5 in this urea concentration was not prevented by NADH. The activity of LDH_5 was already diminished to about 5 per cent in all incubation arrangements (Fig. 2*A*).

In order to see whether substrate concentration has an effect on the inactivation caused by urea the determinations were made with four times greater pyruvate concentration (10×10^{-4} M). The activity of LDH_1 incubated with urea solution in the absence of pyruvate and NADH was 70 per cent; in the presence of pyruvate, 69 per cent; but in the presence of NADH, 85 per cent, as shown in Fig. 1*B*. The protective effect of NADH against urea denaturation was the same (about 20 per cent) as in the lower pyruvate concentration. At the higher pyruvate concentration the increase of enzyme velocity was about 30 per cent in all incubation arrangements. As with the lower pyruvate concentration, the protective effect of NADH on LDH_5 was not evident at the higher concentration (Fig. 2*B*), and the enzyme velocity was not increased as compared with the lower pyruvate concentration.

The results observed here show that the presence of NADH during incubation favours the maximal difference between lactate dehydrogenase isozyme activities because it counteracts the inactivation of LDH_1 but does not seem to have this effect on LDH_5 at this urea concentration. The increase of pyruvate molarity also favours this effect. We have also noticed these same effects with human LDH isozymes, which is in accordance with our earlier study[4] where no major difference could be seen between the animal isozymes and human isozymes separated electrophoretically. Hardy[3] has recently presented a method for separation of LDH isozymes in clinical use. He incubated the enzyme with NADH during 2·6 M urea treatment and used $3{\cdot}1 \times 10^{-4}$ M pyruvate.

Coenzymes (NADH and its analogues) have earlier been shown to protect heart lactate dehydrogenase against various inactivating agents, and this protection has been thought to be mediated by the formation of

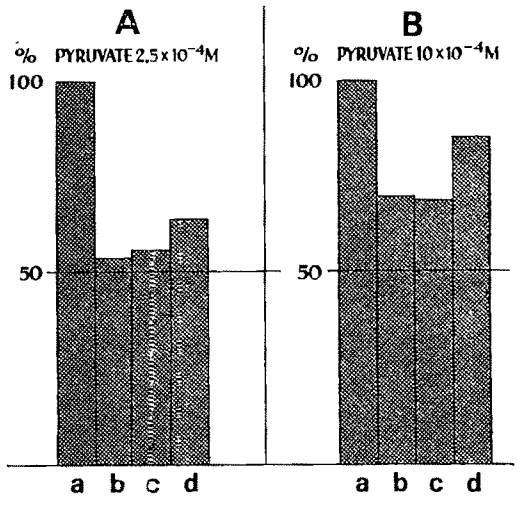


Fig. 1 *A*, Determinations made with substrate pyruvate concentration of $2{\cdot}5 \times 10^{-4}$ M. NADH concentration was $1{\cdot}3 \times 10^{-4}$ M in all determinations and the reaction was carried out in phosphate buffer of *p*H 7·4. (*a*), LDH_1 activity without urea inactivation. The activity of LDH_1 after 10 min incubation with 1·5 M urea when (*b*) the urea incubation was made without pyruvate and NADH, (*c*) the incubation in the presence of pyruvate, (*d*) the incubation in the presence of NADH. *B*, The diagrams are made as above but with pyruvate concentration of 10×10^{-4} M

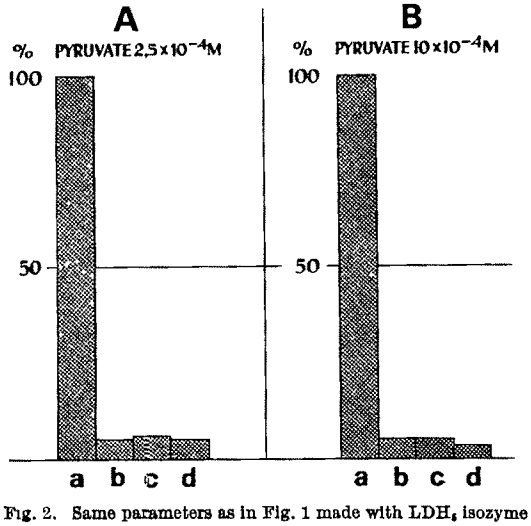


Fig. 2. Same parameters as in Fig. 1 made with LDH_5 isozyme

enzyme coenzyme complex[5-7]. If this is so the observations reported here allow the supposition that the coenzyme is bound more firmly to LDH_1 than to LDH_5, so protecting the tertiary structure of the enzyme molecule necessary to catalytic action.

The elevated pyruvate concentration increases the reaction velocity of urea-treated LDH_1. This consequently means that the affinity of the enzyme towards its substrate is lowered possibly as a result of changes in the conformation of enzyme molecule in its active centre caused by urea.

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Preparation of Endotoxin

When a watery suspension of Gram-negative organisms is heated at 80° C for 1 h, the endotoxin is liberated into the suspending fluid, giving it a dichroic appearance; a method of preparing endotoxin on a large scale by recovering it from such a supernatant was described earlier[1]. In that method concentration was effected by evaporation under reduced pressure. This method, improved and modified to avoid this, is described here.

A culture is grown in a medium containing commercial-grade ammonium sulphate 0·5 g, glucose 1·0 g, disodium hydrogen phosphate 0·2 g, sodium citrate 0·2 g, and magnesium sulphate 0·02 g in 100 ml. of distilled water. A still culture in this medium with the *p*H held at 7·5 yields 3×10^9 and an aerated culture 10^{10} viable cells per ml. The cultures are stored at 4° C, and when there is a convenient quantity available they are centrifuged to obtain the cells in a stiff paste; the cells are re-suspended in distilled water to about 10^{11} cells/ml. and the suspension is heated at 80° C for 1 h and centrifuged. The cells are discarded and the oil-like supernatant is dialysed against running tap-water for 72 h to remove amino-acids, and the endotoxin precipitated in 75 per cent alcohol and dried. It is a slightly greyish sugar-like powder.

Greater purity can be achieved by (*a*) re-suspending the living cells in chilled water and repeating the centrifuging, (*b*) continuing the dialysis against distilled water, and (*c*) re-precipitating in 75 per cent alcohol.

In a typical batch the yield of endotoxin from 10^{15} cells of *E. coli* was 7 g and the LD_{50} of this by intraperitoneal injection in 18–22 g mice was 0·5–1·0 mg.

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Depsides in Sunflower Leaves

This communication reports our finding of neochlorogenic, isochlorogenic, and 3-*O*-feruloylquinic acids in the leaves of sunflowers, *Helianthus annuus*, Russian Mammoth variety. The well-known chlorogenic acid (3-*O*-caffeoylquinic acid) has been previously found in sunflowers by Urban[1] and its presence there has been confirmed by Watanabe *et al.*[2].

Neochlorogenic acid, first isolated from peaches by Corse[3], has recently been proved to be 5-*O*-caffeoyl-D-quinic acid[4-6]. Isochlorogenic acid, as available commercially, has been shown to be a mixture of depsides[4-7]. Corse *et al.*[6] have identified one component as 4,5-dicaffeoyl-D-quinic acid, and Haslam *et al.*[5] have identified another component of the mixture as 3,5-dicaffeoyl-D-quinic acid. The isochlorogenic acid from sunflowers contains three components identical with three of the components, other than chlorogenic acid, of commercially available reference isochlorogenic acid. Neochlorogenic acid and isochlorogenic acid have previously been found in coffee beans, blueberry leaves, plums, and other fruits[8], but not in sunflowers. Recently, 3-*O*-feruloyl-D-quinic acid has been isolated as a crystalline material from unroasted coffee beans[9], but had not been found previously in sunflower plants.

Isolation and identification of neochlorogenic acid. Sunflower leaves (194 g) grown in the greenhouse of the Department of Botany, University of Oklahoma, were extracted by twice boiling the disintegrated leaves with 1 l. portions of 85 per cent isopropyl alcohol–water for 5 min. The extracts were filtered, concentrated *in vacuo* at 37° C to 250 ml. and extracted three times with benzene. The aqueous concentrate was poured on to a 4·5 × 10 cm chromatographic column which previously had been packed with 'Magnesol' (Food Machinery and Chemical Corp, N.Y.) and washed with distilled water under a pressure of 5 lb./in.^{2} pressure. The yellow zone on the column was developed with distilled water to produce two major zones, called fraction *A* and fraction *B*, which were eluted off separately. Fraction *A*, the faster moving zone, was streaked on to ten sheets of Whatman 3 *MM* paper and developed in *n*-butyl alcohol–acetic acid–water (6 : 1 : 2 v/v/v, here referred to as BAW). The broad zone at R_F 0·75 was cut out and eluted with methanol–water (1 : 1 v/v). The extracts were concentrated and streaked on ten sheets of paper and developed in butyl acetate–acetic acid–water (4 : 1 : 5 v/v/v) for 20 h. The broad zone at R_F 0·70 (chlorogenic acid) was separated from a narrow one at R_F 0·25. The latter zone was eluted and further purified by streaking the eluate on seven sheets of Whatman 3 *MM* paper and developing the chromatograms in BAW. This procedure was repeated on nine sheets of Whatman 3 *MM* paper, and then on eight sheets of Whatman No. 1 paper in isopropyl alcohol–formic acid–water (50 : 1 : 950 v/v/v, here referred to as IFW). The extract was taken to dryness *in vacuo*, dissolved in a small amount of methanol and poured on top of a 2·5 × 25 cm 'Magnesol' column previously packed under a pressure of 5 lb./in.^{2} with distilled methanol. The column was developed with one column length of methanol followed by methanol–water (1 : 1 v/v). The zone which fluoresced greenish blue under ultra-violet light (3660 Å) was collected. The absorption spectrum of the eluate showed a maximum at 326 mμ, shoulders at 242 and 300 mμ, and a minimum at 265 mμ. The eluate was taken to dryness and hydrolysed with 10 ml. of 5 per cent sodium hydroxide for 20 min at room temperature. The solution was then acidified with concentrated hydrochloric acid and extracted three times with ethyl acetate. The ethyl acetate extract was chromatographed on Whatman No. 1 paper to prove the presence of caffeic acid. After the evaporation of the aqueous phase, the dry crystalline residue was extracted with 5 ml. absolute ethyl alcohol. The alcohol extract on chromatography showed the presence of quinic acid. The hydrolysis products, the R_F values (Table 1), and the ultra-violet spectra on comparison with authentic neochlorogenic acid showed that the compound extracted was identical with the reference compound.

Isolation of isochlorogenic acid. Fraction *B*, the slower moving zone from the 'Magnesol' column, was concentrated *in vacuo* and streaked on 20 sheets of Whatman 3 *MM* paper and developed in IFW to give three zones. The broadest and most intense one under ultra-violet light at R_F 0·22 was cut out and eluted with 70 per cent isopropyl alcohol. The eluate was concentrated to a

low volume and extracted twice with ethyl acetate. The extracts were dried over anhydrous sodium sulphate and taken to dryness *in vacuo*. The absorption spectrum of an alcoholic extract of the residue showed maxima at 245 and 330 mμ, a shoulder at 300 mμ and a minimum at 265 mμ.

In all but one of the solvent systems used for paper chromatography (Table 1) the sunflower isochlorogenic acid produced only one zone on the chromatogram, and in each case this was identical with the isochlorogenic acid zone of the reference sample used. In 2 per cent acetic acid, however, the isochlorogenic acid from sunflower separated into zones with R_F values of 0·08, 0·16 and 0·30. The reference isochlorogenic acid (Fluka, Buchs *S.G.*, Switzerland) on the same chromatograms had R_F values of 0·09, 0·15 and 0·29, in addition to the chlorogenic acid zones.

Hydrolysis of the isolated acid was carried out by the procedure previously described for neochlorogenic acid. The R_F values of reference samples of quinic acid, quinide and caffeic acid were identical with the hydrolysis product zones in the solvent systems tried (Table 1).

Isolation of 3-O-feruloylquinic acid. Sunflower leaves (2,840 g) were extracted in a Waring blender with 10 l. of hot isopropyl alcohol. The extracts were filtered, concentrated *in vacuo* at 37° C to remove most of the organic solvent, and extracted three times with benzene to remove green pigments and lipids. The aqueous concentrate was then concentrated further to 140 ml. A column (3 cm diam.) was packed to a depth of 26 cm with 'Ultramidpulver' (Badische-Anilin und Soda-Fabrik, Ludwigshafen am Rhein) in distilled water under gravity, washed with dimethylformamide–acetic acid–water–ethyl alcohol (1 : 2 : 6 : 4, v/v/v/v) and then with water. The aqueous sunflower concentrate (50 ml.) was poured on the column and developed with distilled water. The yellow zone that was readily eluted off was concentrated *in vacuo* to 30 ml. and streaked on 110 sheets of Whatman No. 1 chromatography paper and developed in isobutyl methyl ketone–formic acid–water (14 : 3 : 2 v/v/v). The zone at R_F 0·60 which fluoresced faint blue under ultra-violet light (3660 Å) was cut out and eluted with 50 per cent methanol–water. The extract was re-chromatographed on fourteen sheets of Whatman No. 1 paper, and then on eight sheets in butyl alcohol–ethyl alcohol–water (20 : 5 : 11 v/v/v). The zone at R_F 0·74 was cut out and eluted with 50 per cent methanol–water. The ultra-violet absorption spectrum of the eluate showed a maximum at 323 mμ, a shoulder at 300 mμ, and a minimum at 263 mμ, the same as given by the authentic reference sample. Half this eluate was hydrolysed in the same manner as described for neochlorogenic acid to produce ferulic and quinic acids. The R_F values of the isolated depside and reference 3-*O*-feruloylquinic acid were identical in the solvent systems tested (Table 1).

This work was supported by a grant, *GB*–181, from the National Science Foundation. Dr. J. W. Corse supplied the authentic neochlorogenic acid, and Dr. E. Sondheimer supplied the reference 3-*O*-feruloylquinic acid.

Table 1. R_F VALUES OF ISOLATED AND REFERENCE COMPOUNDS

Compounds	Solvent systems* 1	2	3	4	5	6
Chlorogenic acid	0 68	0·49	0·03	0·59 0·73	0 49	0·56
Isochlorogenic acid	0 80	0 74	0 02	0·08 0·16 0 30	0·53	0·83
Neochlorogenic acid	0·57	0 49	0 03	0·64 0 75	0 42	0 34
3-O-Feruloylquinic acid	0 78	0 28	0 16	0 62 0·74	0·57	0·74
Caffeic acid	0 84	0·57	0·30	0·32 0 63		
Ferulic acid	—	—	0 80	0 36 0 64		
Quinic acid	0·24	0 08	—			
Quinide	0 51	0·70	—			

* Solvent systems: (1) *n*-butyl alcohol–acetic acid–water (6 1 : 2 v/v/v); (2) *n*-butyl alcohol–pyridine–benzene–water (5 3 1 : 3 v/v/v/v); (3) benzene–acetic acid–water (125 . 72 . 3 v/v/v); (4) 2 per cent acetic acid, (5) *n*-butyl alcohol–ethyl alcohol–water (14 . 3 : 3 v/v/v); (6) isobutyl methyl ketone–formic acid–water (14 : 3 · 2 v/v/v).

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PHYSIOLOGY

Metabolic Differences between Starch and Sucrose

SINCE 1957, papers from this department have stressed that the dietary factor involved in the aetiology of ischaemic heart disease is likely to be sugar (sucrose) rather than fat[1]. The evidence incriminating dietary sugar—now quite strong—is chiefly epidemiological. Investigations of populations show that the rise in the incidence of the disease coincides with the rise in the consumption of sugar and not of fat[2–4]. Again, during investigations of individuals we have shown that the disease is most likely to occur in those that have been eating a great deal of sugar[5].

Our suggestion that dietary sugar is a cause of ischaemic heart disease has sometimes been criticized on the grounds of implausibility (for example, Kinsell[6]). Sugar, we are told, is rapidly hydrolysed and rapidly absorbed as glucose and fructose, and the fructose is rapidly converted into glucose; in what way then can sugar act differently from the other major dietary carbohydrate, starch, which also rapidly appears in the blood as glucose? Are not all digestible carbohydrates interchangeable, so that they have identical metabolic effects?

In seeking for a possible mechanism by which sugar may induce ischaemic heart disease, we have therefore taken as our first task the discovery of differences in the metabolic effects of dietary sucrose and dietary starch. Our second task will be to see whether we can demonstrate which of these differences may be responsible for the involvement of sugar in the aetiology of heart disease.

A great deal of evidence contradicts the common belief that all forms of dietary carbohydrates are interchangeable. Macdonald[7], and Antar and Ohlson[8], have summarized some of the differences induced in man and in experimental animals when sugar is substituted for starch in the diet. The effects include an increase in serum cholesterol, changes in depot fats and liver fats, and diminished growth. In addition, Cohen and Teitelbaum[9] have found that sugar-fed rats exhibit an impaired glucose tolerance.

During the past year, we too have found several differences between starch-fed and sucrose-fed rats. We are now reporting these briefly with the purpose of combating the view that all dietary carbohydrates are equal, and particularly that starch and sugar are interchangeable.

All experiments were carried out on rats given purified diets and water *ad libitum*. The diets contained about 70 per cent carbohydrate, either as corn starch or as sucrose; except where noted, they were adequate in all known nutrients.

Diets containing sucrose produced a lower growth-rate in weanling rats (Fig. 1), or a loss of weight in adult rats, compared with diets containing starch. The effect was more noticeable when the diets were low in protein. The lower rate of growth was not due to a lower food intake, nor was there any difference in absorption. The effect must therefore have been due to less efficient utilization of the food.

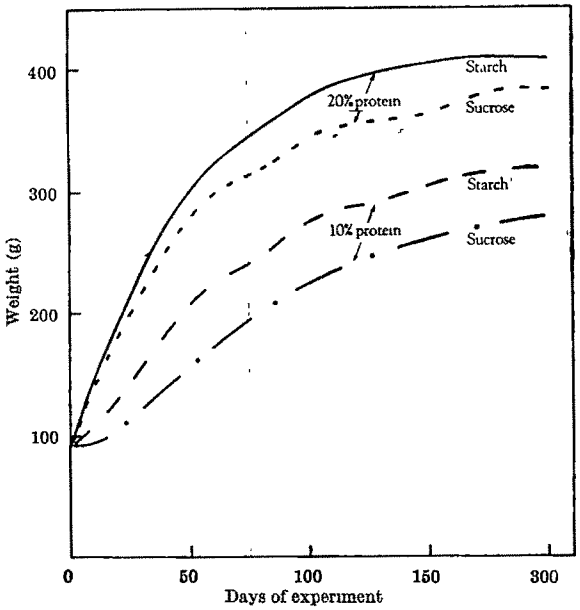


Fig. 1. Growth of male rats from the age of 28 days on purified diets containing two levels of protein, and 69 per cent carbohydrate in the form of starch or sugar. Each group consisted of four male rats

Measurements of oxygen uptake and carbon dioxide output were made in rats over periods of 24 h, about six months after they had been placed on the diets with starch or with sugar. The rats on the diet with starch gave the expected respiratory quotient of about 0·87. However, the rats on sucrose gave a respiratory quotient of about 0·75; this figure is far lower than that expected for a high carbohydrate diet, and similar to that expected (and, in fact, found) for animals on a high fat diet. We have at present no explanation for the low respiratory quotient of the rats on sucrose.

After feeding for six months, the level of serum cholesterol was higher in rats on the sugar-containing diet than in those on the starch-containing diet. The total body fat was significantly less in the sugar-fed rats. The liver fat was less in the sugar-fed rats when they were given the experimental diets from weaning; it was higher in the sugar-fed rats when they were given the diets from the age of three months (Table 1).

Table 1. Body Fat and Liver Fat in Rats fed Diets containing Two Levels of Protein, with 69 per cent Carbohydrate as Starch or as Sugar

Groups of four male rats fed on experimental diets for 6 months, from age 28 days (weaning) or age 3 months. Fat as percentage of dry weight of body or of liver

Young rats				Older rats			
Diet		Fat content		Diet		Fat content	
Carbohydrate	Protein	Body	Liver	Carbohydrate	Protein	Body	Liver
Starch	20%	35	12	Starch	10%	35	7
Sugar		30	9	Sugar		26	9
Starch	10%	32	9	Starch	5%	32	18
Sugar		32	7	Sugar		27	46

The major carbohydrate components of current human diets, especially in affluent countries, are starch and sugar (sucrose). Experiments have now shown conclusively that the substitution of one for the other in the diet of rats causes several metabolic changes. There is no justification for the view that starch and sugar are metabolically identical.

The effects of altering the carbohydrate in the diet stand in remarkable contrast to the effects of altering the fat in the diet. For example, Thomasson *et al.*[10] have given rats diets containing 54 per cent of the calories from fat, and compared natural soyabean oil with various hydrogenated soyabean and other oils. Some of these contained more than half of the fatty acids in the 'unnatural' *trans* form. They found no difference in body growth, body fat, kidney and liver function, and several other measurements.

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Proton Transfer Processes in Muscle: Pressure, Temperature and Isotope Effects

Investigations of muscular contraction in heavy water have shown that the rate-determining process involves proton transfer[1]. The magnitude of the effect suggests that more than one proton is involved. Varga[2] has observed that fibres under isometric tension in the presence of adenosine triphosphate are relaxed when the temperature is lowered, an effect which has been attributed to the endothermicity of actomyosin formation. On the other hand, increasing the hydrostatic pressure results in contraction of intact muscle[3–5].

At 25° C the limiting equivalent conductivity of the proton is nearly ten times greater than that of the similarly charged and hydrated lithium ion[6]. This anomalously great mobility of the proton has been attributed to the Grotthuss mechanism[7,8]. This mechanism entails three steps: (1) the rotation of adjacent water molecules to form a transitory H-bonded chain; (2) proton flips along this chain; (3) dissolution of the chain and rotation of water molecules to return to a random distribution. Huckel[8] and Conway, Bockris, and Linton[9] maintain that one of the rotational steps is rate-determining, while Bernal and Fowler[10] and Stern and Eyring[11] believe that the proton flip is the slow step. Our more recent studies have shown that the rotational step is not only rate-determining, but also that protonic conduction by the Grotthuss mechanism is restricted to the 'free', rotatable, water monomers between the structured Frank-Wen clusters[12–14].

The Grotthuss conductive process avoids areas of order because the activation energy required to rotate a bound water (as in ice) is greater than that required to rotate a 'free' water molecule; 12 compared to about 3 kcal/mole (the latter at 10° C)[14,15].

Some time ago Klotz[16] proposed that certain long-range biological redox reactions might involve electron transfer via a Grotthuss-like mechanism. Studies of the Fe(II)—Fe(III) electron-exchange reaction in ice have substantiated such a possibility[17]. Thus the Grotthuss mechanism can sustain both transport of electrical energy by protonic conduction and transport of chemical energy by electron exchange[18].

Recent nuclear magnetic resonance investigations by Bratton, Hopkins, and Weinberg[19] have yielded evidence that in relaxed muscle fibre the solvation sheath water molecules are bound tightly to the protein material in an orderly manner, but that on contraction the waters are released and set 'free'. From this information Horne[20] drew the inference that the waters are released so that they can rotate and thereby transport electrical or chemical energy by the Grotthuss mechanism. The purpose of this communication is to examine whether the observed pressure, temperature and isotope effects support this hypothesis.

The application of either thermal energy or hydrostatic pressure tends to break up the structure of liquid water, the rotation of water molecules is facilitated, and, as a consequence, the activation energy of protonic conduction decreases. This is shown in Fig. 1 where the temperature dependence of the activation energy of electrical conduction of aqueous hydrochloric acid solutions, which is to say, the activation energy for water molecule rotation, is calculated from the results of Owen and Sweeton[21] and where the pressure dependence is from Horne, Myers, and Frysinger[12]. (It should be noted that the former is for molar, the latter for molal conductances.) Hydrostatic pressure also breaks up the local structure around ions in solution[22]; one expects by analogy that it also breaks up the local structure in the solvation sheath of biopolymers. If such is the case, then water rotation and the Grotthuss transport process is facilitated and pressure should be associated with muscular contraction, as observed[3-5]. By the converse of the same argument temperature reduction should be associated with muscular relaxation, as observed[2]. Notice that the endothermicity of actomyosin formation is not relevant unless that process is rate-determining.

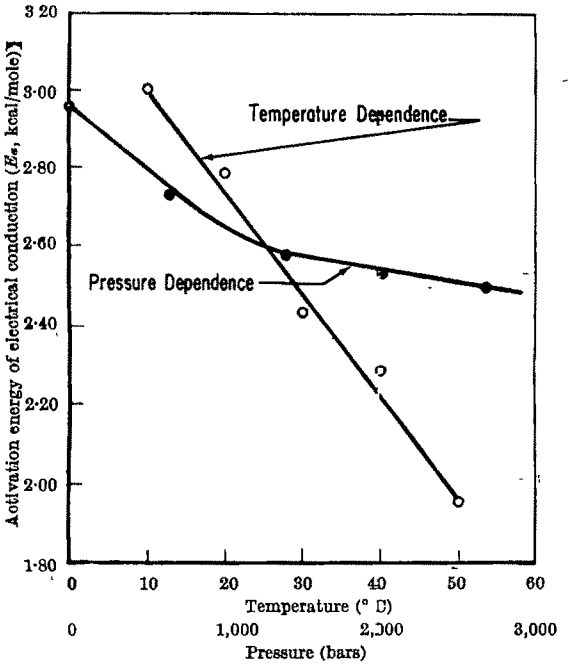


Fig. 1. Temperature and pressure dependence of the activation energy of electrical conduction of an aqueous 0·10 M hydrochloric acid solution

The isotope effect for electrical conduction by the Grotthuss mechanism is 1·1–3·0 (in water) or 2·2 (in ice) while that of electron exchange by the Grotthuss mechanism is 1·5–2·5 (ref. 18). The H_2O/D_2O effect for muscular contraction is about 3 (ref. 1). This value is commensurate with the values for the Grotthuss mechanism but may be slightly higher. Hydrophobic bonding plays a part in the hydration of biopolymers, unlike ions in solution or the bulk water structure. The disruption of such bonding involves the co-operative movement of more waters than does the disruption of electrostatic bonding, and this could easily give rise to a somewhat greater isotope effect.

Higher pressures, like higher temperatures, result in more profound and irreversible chemical reactions in proteins[23,24]. We visualize that at modest pressures (or temperatures) the waters of the biopolymer's solvation sheath are released and rendered 'free' to rotate in place. As pressure (or temperature) is further increased the sheath is totally disrupted and the water molecules may even stray away. The structured hydration sheath stiffens and stabilizes the biopolymers: when it is destroyed, both inter- and intra-molecular reactions occur resulting in denaturation and coagulation.

The bits of evidence discussed above all point to the conclusion that muscular contraction is associated with a release of the bound waters in the biopolymer's solvation sheath and with the transmission of electrical and/or chemical energy by the Grotthuss mechanism. However, the position of these processes in the temporal sequence of muscular contraction is far from clear. Are they cause or effect? In particular it would be most interesting to know the nature of the signal and of the processes in the protein which result in the release of its waters of hydration.

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Fine Eye Movements during Inattention

Recording by numerous observers[1] during steady visual fixation has shown a highly typical pattern of fine eye movements (Fig. 1) invariably present, beyond voluntary control, and consisting of three components: rapid saccades (flicks); slow drift and waves; and fine fast tremor. While these movements are usually related to a control mechanism for keeping the eyes on target[2], some feel that they play a greater part in visual function[3], especially since an artificially stopped retinal image disappears to perception[4], and since there is an evoked occipital response correlated with saccadic movements[5].

An important question about visual function is: What is happening in the visual system when the subject is not

attending to vision? The question can be asked at a number of levels—introspectively, in psychological experiments, or in physiological experiments correlating electroencephalogram, electroretinogram, etc. This communication reports findings correlating types of fine eye movements with attention and inattention.

During routine experiments recording fine fixation eye movements by standard techniques it was discovered that when the mind of one subject wandered a new pattern of fine eye movement (Fig. 2) occurred. If the subject was afterwards instructed to attend to the task of fixation the eye movements would again revert to the normal pattern and the subject became retrospectively quite aware of his inattention. This happening occurred several dozen times, always identified by the changed characteristics of the eye movements, and the retrospective awareness of inattention, so that the relationship to inattention was invariable. These eye movements were oscillatory, without finite periods of steadily maintaining relative stability on a comparatively small area of the retina. such as is true of the ordinary movements.

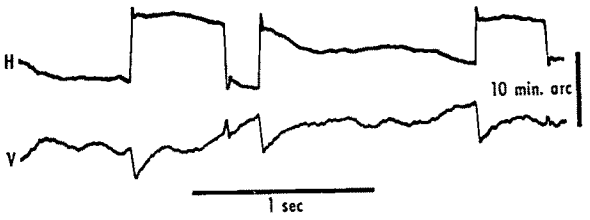


Fig. 1. Typical recording of fine eye movements with subject attending to task of fixating a small point. *H* is horizontal eye movement. *V* is vertical eye movement. Saccades, drift and slow waves and fine fast tremor can all be seen

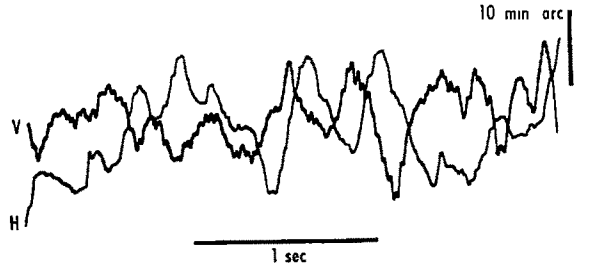


Fig. 2. Eye movements of the same subject during inattention several minutes later. *H* is horizontal eye movement *V* is vertical eye movement. Note infrequent saccades Note shifting phase relations of the traces, necessarily resulting in varying Lissajous figures describing the retinal image—retina relation over time

A feedback control system of fine eye movements might be assumed from this evidence to operate differently during attention and inattention. Most parsimoniously, it could be assumed that during attention there is a closed-loop feedback functioning with stability of the system, while during inattention the loop is opened, resulting in typical instability oscillation as recorded in artificially opened loops by Fender and Nye[2]. Thus, inattention would physiologically involve the opening of feedback loops which are ordinarily closed and operative. Although parsimonious, these assumptions should be tentative.

Another issue concerns the state of organization of visual information transmitted during attention and inattention. Introspection does not readily allow asking if what we see during inattention is different from during attention. The different space-time relationship of the retinal image to the retina during attention and inattention suggests that the organization of information transmitted by the optic nerve might be markedly different in the two states.

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PHARMACOLOGY

Drosophila-tests in Pharmacology

In pharmacology the possible genetical effects of drugs have so far been neglected. One reason for this is that the test organisms used by pharmacologists are not convenient for genetical tests. A similar problem was faced by geneticists and developmental physiologists up to the 1930's. Through tests with *Drosophila*, *Neurospora* genetics was developed and later the whole branch of biochemical genetics.

The increasing use of drugs of various kinds might, in addition to secondary somatic effects, also cause genetic effects. Among these are included mutagenic effects of various kinds and effects on segregation of the chromosomes—non-disjunction. The latter will, in man, either cause the death of the foetus before birth, or might cause severe somatic and mental deficiency, for example, Down's syndrome.

For this reason it will be necessary to develop tests whereby drugs can be tested for such possible genetic effects.

The use of mammals—mice—in such tests would be both expensive and time-consuming, and might slow down even more the development of new drugs. Thus, compared with the normal time lapse in their introduction, the benefit of such tests could be small. It occurred to us that *Drosophila* might be used in such tests, as we there have the most refined genetical methods. The problem is whether or not we can conveniently observe any somatic effects which are dependent on dose.

This communication presents some experiments which show the possibilities of *Drosophila* tests.

As somatic effects I decided that developmental time and survival would be the best characters to observe. The normal developmental time for *Drosophila melanogaster* at the optimum temperature of 25° C is: egg stage slightly less than 1 day, larvae 4 days and pupae 4 days. In conditions that are not overcrowded the developmental time is thus approximately 9 days.

As the larval period is one of very active feeding I thought that the best method of introducing the drug was to allow newly hatched larvae to live on a medium in which it was mixed.

Normal *Drosophila* medium consists mainly of agar–agar, syrup, corn-meal and water. To such a medium were added various drugs which were then thoroughly mixed with the medium.

When various concentrations were to be tested I started with the highest concentration and to half of that added an equal amount of normal food and mixed it thoroughly. Half of this was then taken to obtain the next lower concentration, etc. The medium was poured into vials into which 25 newly hatched larvae were placed.

In order to obtain a great number of young larvae, flies were bred in plastic boxes in which there was a hole covered by a Petri dish containing a blackened medium of agar–agar, syrup and water. The Petri dish could be easily exchanged. Egg-laying was allowed for only 4 h. Twenty hours after the beginning of egg-laying the Petri dishes were inspected and larvae which had already hatched were discarded. Within the next 4 h the larvae were sampled in groups of 25 which were placed in the test vials. In order to get uniformity, 25 larvae were added to one vial from each concentration for each drug and their controls before the second vials were used. There was a total of 8–11 vials in each group. From the 8th day onward the vials were inspected and emptied every 4th or 6th hour, with the longer intervals at night. The number of adults ecloding in each period was recorded.

The developmental time was taken as the time from the mean of the egg-laying period until the time of inspection. For each concentration total mean developmental times for males and females were calculated. These are given in Figs. 1–3.

From these figures it is evident that in most cases there is a linear relationship between the concentration of the drugs in the medium and the time of development. For one drug there is a plateau and in two others seemingly no effects at all.

Table 1. ADULTS OUT OF 25 LARVAE PER VIAL

Conc.	*A*	*B*	*C*	*D*	Conc.	*E*	*F*	Conc.	*G*
4 g	17·5	22·9	23·5	17·1	2	18·3	19·2	1	21·8
2	19·2	21·4	21·2	22·5	1	21·1	18·6	1/2	23 3
1	19·2	22·0	22·5	23·7	1/2	22·4	19·6	1/4	22·6
1/2	21·4	23·7	22·0	23·2	1/4	22·4	20·0	1/8	23·1
1/4	22·6	22·0	23·2	21·8	1/8	21·5	21·4	1/16	23·5
Control	23 2	23 2	23·2	24·0		23·2	23 2		24·0

A, Pentothal sodium; *B*, hexobarbitone; *C*, *cyclo*barbitone; *D*, sodium-salicylate; *E*, phenobarbitone; *F*, diallylbarbituric acid; *G*, glutethemide.

As an example of the effects on survival I have used the same groups as presented in Figs. 1–3. The mean number of adults per vial with originally 25 young larvae are given in Table 1. It is obvious that there are some toxic effects at the higher concentrations of some drugs. The effects on survival are, however, not at all as distinct in relation to the concentrations as are the developmental times. In many cases there might be a correlation between the effects on development and survival.

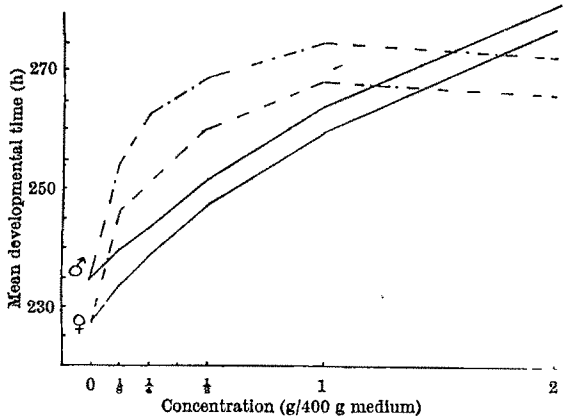


Fig. 1. —, Phenobarbitone; — · —, diallylbarbituric acid

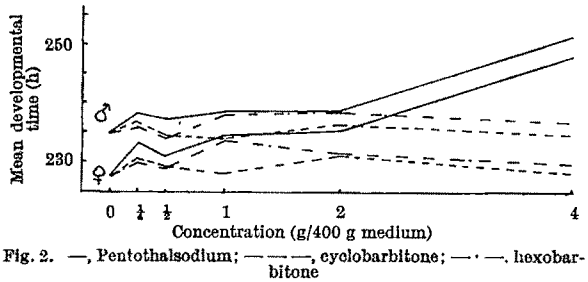


Fig. 2. —, Pentothalsodium; — — —, cyclobarbitone; — · —, hexobarbitone

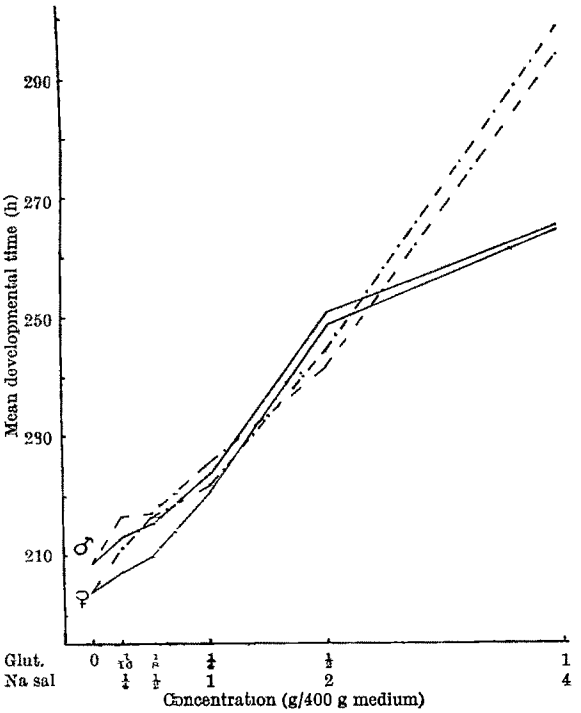


Fig. 3 —, Glutethemide; — · —, sodium salicylate

From these results we can conclude that lengthening of developmental time might be a fairly good indication of somatic effects. Furthermore, there is good evidence that individuals which have had a prolonged developmental time have been in one way or another affected by the drug, and thus may be used for further genetical tests.

In *Drosophila melanogaster* the first meiosis occurs in late larval stages of males, and during the pupal stage further cells pass meiosis.

Hence, possible effects of drugs could be manifested as chromosomal or genic disturbances in spermatozoa. We have chosen one of the drugs (glutethemide) used in the previous tests in order to see whether or not we could find any effects such as non-disjunction of the sex-chromosomes or chromosome breakage.

In order to perform such a test one needs stocks with convenient marker genes. The simplest method in this model test, namely, *y w sn* females and *y w sn* : sc^8Y males, was used. The males carry in their *Y* chromosome a very small piece from a normal *X*-chromosome with the wild-type allele of yellow. Thus the males are grey-brownish while the females have yellow bodies. If a female receives an extra *Y*-chromosome (*XXY*) they, too, will be grey-brownish, and if the males have but one sex-chromosome (*XO*) they will be yellow. By always using yellow-females and grey-brownish males one will be fairly certain that they are of the original normal types, *XX* and *XY* respectively. Non-disjunction will give *XXY* females which are phenotypically easily seen. The complementary class, *XO* males, may also result from other causes than non-disjunction, namely, chromosome lagging, or breaks resulting in the elimination of the broken chromosome. More *XO* offspring than *XXY* will therefore be expected. These types of aberrant individuals appear in normal stocks at low rates and for that reason one must run parallel controls on the experimental series.

In one preliminary test there was a significantly higher rate of non-disjunction in the glutethemide-fed group than in the control. It was then decided to perform two tests to confirm or refute this first observation. This time the material was divided into two groups according to the developmental time: the first half (early) and the second half of eclosions (late). The control group was bred at

room temperature (20° C) in order to cause a lengthening of developmental time for reasons other than drugs. The results of tests of males that had had their larval and pupal development on a medium containing 1 g glutethemide per 400 g (*D*), and the controls (*C*), are given in Table 2. As is seen, there is some variation but no clear trend. A comparison between the combined 4 glutethemide groups versus the controls gives no significant difference for any of the two types of aberrant individuals. We can therefore in this case conclude that the drug under consideration, although giving appreciable delay of development, has no detectable effects such as non-disjunction, lagging or breakage.

Table 2

Ser.	Total offspring	*XXY*	*XO*	10^{-4} *XXY*	10^{-4} *XO*
*D*1 *E*	47,144	25	117	5 3	24·8
*D*1 *L*	74,245	37	135	5·0	18·2
*C*1 *E*	86,139	35	133	4·1	15·4
*C*1 *L*	86,711	20	194	2·3	22 4
*D*2 *E*	51,822	19	90	3·7	17·4
*D*2 *L*	63,536	30	124	4 7	19·5
*C*2 *E*	45,718	31	67	6 8	14·7
*C*2 *L*	32,395	17	53	5 2	16 4
Total					
D	236,747	111	466	4·7	19·7
C	250,963	103	447	4·1	17·8

D, glutethemide; *C*, control; *E*, the early half of eclosion; *L*, the late half.

These tests are presented in order to show the possibilities in using *Drosophila* techniques to detect agents with genetical effects. Although in this particular case we happened to test a drug which did not show any secondary effects on the chromosomes the method might prove successful with other drugs.

There might be objections to the use of an invertebrate in pharmacological tests since its physiology differs so much from man. It is true that we may in some cases have quite other types of effects in mammals, especially man. On the other hand, a potent agent might manifest its effect on an invertebrate as well as in mammals. Thus one could have some better ground for the detection of undesirable secondary effects on the genetic side rather than, as at present, continuing to neglect the problem by doing no studies at all.

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Maintenance of Pregnancy in Spayed Rats with 20α-Hydroxypregn-4-ene-3-one and 20β-Hydroxypregn-4-ene-3-one

The two epimers 20α-hydroxy-pregn-4-ene-3-one (20α-progesterol) and 20β-hydroxy-pregn-4-ene-3-one (20β-progesterol) are in metabolic equilibrium with progesterone in ovaries[1–5], in the placenta[5,6], and probably in the adrenals[7]. They have also been isolated from ovarian and adrenal venous blood[3,8–13], their relative concentrations and their distribution varying from one species of animal to another[14]. 20α-Progesterol and 20β-progesterol possess certain progestational properties[5,15,16]. The purpose of the experiment described here was to determine to what extent and under what conditions the two epimers are able, like progesterone, to maintain pregnancy in the spayed rat.

Adult virgin female rats of the Sprague–Dawley strain weighing 200–250 g were placed in cages with adult male rats of the same strain on the evening of pro-oestrus. The following day, insemination was confirmed by the presence of spermatozoa in the vagina (1st day of pregnancy). The progestational steroids, in the form of a solution or a fine suspension in sesame oil, were administered subcutaneously twice daily (doses henceforth expressed in mg/kg daily) from the 8th to 19th day of pregnancy, either alone or in combination with oestradiol (1 μg/kg subcutaneously twice daily in sesame oil). On the 9th day, laparotomy was performed, pregnancy confirmed, and the animals (except for one group of intact controls) bilaterally ovariectomized. On the 20th day, all the animals were submitted to autopsy, during which the absence of ovaries was confirmed, the number of live and dead foetuses counted, and the two horns of the uterus examined for implantation sites.

Effect of the progestational compounds administered without oestradiol (Table 1). The two compounds 20α-progesterol and 20β-progesterol in doses up to 200 mg did not exert any pregnancy-maintaining effect. Progesterone, on the other hand, maintained pregnancy to some extent in a dose of only 12·5 mg. Following this dose, 33 per cent of the rats had 1–3 live foetuses; and the uteri of the rest of the animals either contained dead foetuses or showed implantation sites. A somewhat better effect was observed following 25 mg progesterone, while a dose of 50 mg maintained pregnancy in all the animals. In the latter case the number of live foetuses in the uterus was equivalent to that found in intact controls.

Effect of the progestational compounds administered together with oestradiol (Table 2). A partial response was elicited with 20α-progesterol. In response to a dose of 50 mg, a few dead foetuses were found in 25 per cent of the rats. With doses of 100 mg and 200 mg, foetuses—all of them dead—and implantation sites were found in 40–50 per cent of the animals.

Given in a dose of 50 mg, 20β-progesterol had only a limited effect: a few foetuses survived in 33 per cent of the rats, and dead foetuses were found in 22 per cent. A dose of 100 mg, on the other hand, resulted in complete maintenance of pregnancy, all animals containing a normal number of live foetuses.

Following a dose of only 3 mg progesterone, a certain number of foetuses survived in 66 per cent of the rats, and dead foetuses were found in all animals. The number of live foetuses found at autopsy in all animals treated with 6·25 mg, 12 mg, or 25 mg progesterone was comparable to that in intact controls.

Discussion. The finding that 20α-progesterol and 20β-progesterol show no ability to maintain pregnancy in the spayed rat when administered without oestrogen tallies with similar observations in the castrated mouse[17]. Under these conditions, the two epimers are less than 1/15 as active as progesterone.

Oestradiol, like oestrone in other experiments[18,19], reduced the dose of progesterone required to maintain pregnancy in the spayed rat to one-half or even one-quarter. Its synergistic effect appears to be even more marked with 20α-progesterol, and particularly with 20β-progesterol. The partial effect observed with 20α-progesterol probably indicates that maintenance of pregnancy was limited to a certain period of time after castration. When administered together with oestradiol, 20β-progesterol—in contrast to 20α-progesterol—produces qualitatively the same effects as progesterone, but is 15 times less active than progesterone.

The superiority of 20β-progesterol over its epimer is also apparent in the Hooker-Forbes test[5,16], in which it is twice as active as progesterone, whereas 20α-progesterone is two times less active. In the Clauberg test[5,16], by contrast, 20β-progesterol is 20 times less active than progesterone, whereas 20α-progesterol is only two times less active. The activity ratios between the two epimers and progesterone, as established in the course of these various tests, show that, in comparison with progesterone, 20β-progesterol and particularly 20α-progesterol have a weaker progestational effect on the maintenance of pregnancy than on the uterine mucosa. Hence, there is a marked dissociation between the various types of effect exerted by 20α-progesterol and 20β-progesterol. In order to explain this dissociation, it would be necessary to determine how

Table 1. PREGNANCY MAINTENANCE IN OVARIECTOMIZED RATS WITH 20α-PROGESTEROL AND 20β-PROGESTEROL

Treatment	Dose mg/kg s.c. daily	Total No of rats	No. and % of rats with live foetuses	Av. No. of live foetuses per rat having live foetuses	No. and % of rats with dead foetuses*	Av. No. of dead foetuses per rat having dead foetuses	No of rats with implantation sites only
Intact controls, sesame oil	—	10	10 100%	11·6 ± 1·1†	1 10%	1·0	0
Castrate controls, sesame oil	—	16	0 0	0	0 0	0	0
20α-Progesterol	200	4	0 0	0	0 0	0	3
20β-Progesterol	200	5	0 0	0	0 0	0	0
	100	5	0 0	0	0 0	0	1
	50	5	0 0	0	0 0	0	0
	25	5	0 0	0	0 0	0	0
Progesterone	100	6	6 100%	10·6 ± 0·9	2 33%	1·0	0
	50	10	10 100%	10·2 ± 0·5	1 10%	1·0	0
	25	6	3 50%	9·0 ± 1·3	4 66%	2·0 ± 0·4	2
	12·5	6	2 33%	2·0 ± 0·6	2 33%	3·5	2

* Also includes rats with some live foetuses together with dead foetuses.
† Mean ± *S.E.*

Table 2. PREGNANCY MAINTENANCE IN OVARIECTOMIZED RATS WITH 20α-PROGESTEROL AND 20β-PROGESTEROL TOGETHER WITH OESTRADIOL

Treatment	Dose mg/kg s.c. daily	Oestradiol dose mg/kg s.c. daily	Total No. of rats	No. and % of rats with live foetuses	Av. No. of live foetuses per rat having live foetuses	No. and % of rats with dead foetuses*	Av. No. of dead foetuses per rat having dead foetuses	No. of rats with implantation sites only
Intact controls, sesame oil	—	—	10	10 100%	11·6 ± 1·1†	1 10%	1·0	0
Castrate controls, sesame oil	—	—	16	0 0	0	0	0	0
Castrate controls, oestradiol	—	0·002	17	0 0	0	0	0	0
20α-Progesterol	200	0·002	4	0 0	0	2 50%	3·5	2
	100	0·002	5	0 0	0	2 40%	1·5	1
	50	0·002	8	0 0	0	2 25%	3·5	3
	25	0·002	4	0 0	0	0 0	0	0
20β-Progesterol	100	0·002	9	9 100%	10·4 ± 0·8	2 22%	1·0	0
	50	0·002	9	3 33%	3·6 ± 1·6	2 22%	1·5	0
	25	0·002	5	0 0	0	0	0	0
Progesterone	50	0·002	6	6 100%	11·3 ± 0·9	0	0	0
	25	0·002	6	6 100%	10·3 ± 1·3	1 17%	1·0	0
	12·5	0·002	6	6 100%	11·0 ± 0·9	3 50%	1·0	0
	6·25	0·002	5	5 100%	9·0 ± 1·7	4 80%	3·0 ± 0·7	0
	3·0	0·002	6	4 66%	5·2 ± 1·2	6 100%	5·0 ± 0·6	0
	1·5	0·002	5	0 0	0	0	0	0

* Also includes rats with some live foetuses together with dead foetuses.
† Mean ± *S.E.*

much of the activity of each of the two epimers is due to an intrinsic effect, and how much is due to metabolic conversion into progesterone.

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Excitatory Action of Prostaglandin E_1 on Brain-stem Neurones

PROSTAGLANDINS (PG) were first identified chemically in 1960 by Bergström and his co-workers[1–3] in extracts of sheep prostate gland, and since then they have been found in many other tissues. They are biologically active and stimulate or relax a variety of smooth muscle preparations[4]. Recently, evidence has been obtained for an action on the central nervous system following intraventricular or intravenous injection in small doses[5,6]. $PGF_{2\alpha}$ has been isolated from bovine brain[7] and evidence has been obtained that PGE_1 or PGE_2 is released from the cerebral cortex into a superfusate following afferent or cortical stimulation[8]. Krnjević, however, was unable to detect any action of PGE_1 on cerebral cortical neurones when it was released by iontophoresis[9].

Since some of the effects observed following the intraventricular or intravenous injection of prostaglandins[5,6] might have been due to an action on the brain-stem, it was decided to test for an action of PGE_1 on neurones in this region. A sample of PGE_1 (95 per cent purity) was supplied by Dr. D. A. van Dorp and initially a solution of 1 mg in 0·2 ml. adjusted to *p*H 8·2 with sodium hydroxide was used. In later experiments, this concentration was doubled and the *p*H was 6·7. The PGE_1 solution was placed in one barrel of three-, four-, or five-barrelled micropipettes. These were inserted into the medulla of unanaesthetized decerebrate cats and the spontaneous activity of single neurones was observed before, during and after the iontophoretic application of PGE_1. The methods used have been described elsewhere[10–12]. Histological controls showed that most of the neurones studied were in the medial reticular formation of the medulla.

Out of 265 neurones studied, 75 (28 per cent) were excited and 184 (70 per cent) were unaffected by PGE_1. The active ions were expelled from the electrode by a negative current and an equivalent current was passed through a saline-containing barrel as a control. A negative current through the control barrel sometimes caused excitation, but an effect of PGE_1 could be distinguished by a comparison of the magnitude and time course of the two effects, or by adjusting the current strength until it had no action when applied via the saline barrel. With some neurones, repeated application of PGE_1 led to a progressive reduction in effect whereas with others its magnitude remained relatively unchanged. The effects observed could not have been due to the expulsion of hydroxyl ions since the activity of PGE_1 was the same at both *p*H 6·7 and 8·2.

In some experiments, the effects of two related substances were compared with those of PGE_1. Approximately half the neurones excited by PGE_1 were similarly affected by the precursor, homo-γ-linolenic acid[13]. On the other neurones, it had only a slight effect or no effect at all. Linolenic acid was also active on some of the neurones excited by PGE_1 but inactive on others.

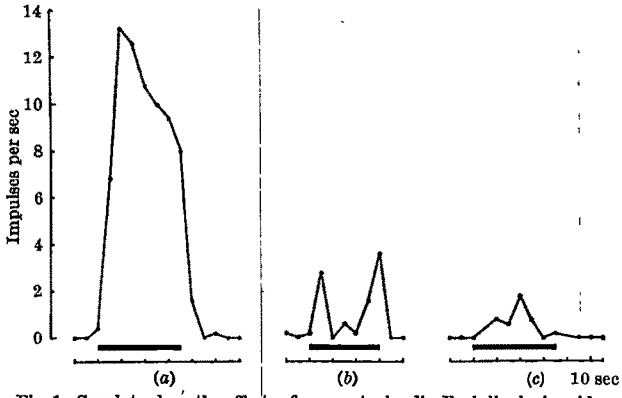


Fig. 1. Graph to show the effects of: *a*, prostaglandin E_1; *b*, linolenic acid; *c*, current, each applied with a current of 100n amp, on the impulse frequency of a medullary neurone

The excitatory action of PGE_1 on a neurone in the medial reticular formation of the medulla is shown in Fig. 1. At rest, this neurone fired only occasionally, but it was markedly excited by PGE_1, while current and linolenic acid had relatively slight effects.

Thus PGE_1 not only has an action on the central nervous sytem when injected intraventricularly or intravenously in very small quantities, but also has a selective excitatory action on brain-stem neurones. Since a related substance (PGF_{2a}) has been found in brain, the possibility that one of the prostaglandins or a related substance has some function of biological importance in relation to neuronal activity must be considered.

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Liver Metabolism of Anticholinesterase Compounds in Live Rats: Relation to Toxicity

The insecticide 'Isolan' (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate) is more toxic to rats by the dermal than by the oral route[1]. This finding is interesting, because the opposite is true of most compounds. It was not known whether the unexpected difference in the toxicity of 'Isolan' by the two routes was caused by detoxification in the intestinal lumen, poor absorption from the gastrointestinal tract, detoxification in the liver, unusually efficient percutaneous absorption, or some other mechanism.

It is generally recognized that the liver is the main site of metabolism of foreign compounds in mammals.

Compounds may enter the body through a variety of routes: the gastrointestinal tract, skin and respiratory system being the most important. Absorption by any of these routes involves either the lymph or the blood as a primary transport medium. The lymphatic circulation and the capillary blood networks of the skin and lung discharge into the systemic blood circulation; the blood is then passed through the heart and on throughout the body. The liver receives about 6·5 per cent of the cardiac output directly through the hepatic artery and another 21 per cent of the cardiac output less directly by way of the mesenteric arteries and portal venous circulation. In contrast, the blood capillary network of the gastrointestinal tract discharges directly into the liver via the hepatic portal venous system, and essentially 100 per cent of this blood is delivered into the liver. Thus (if one ignores the possibility of a compound being sequestered by a given tissue or capillary bed) virtually all of a compound absorbed by the gastrointestinal tract goes directly to the liver; whereas, if a compound is absorbed from any other site, only 27·5 per cent reaches the liver before being distributed in the general circulation.

To test the effect of a single pass through the liver, 'Isolan' was injected directly into the hepatic portal system (intestinal vein) in one group of rats and into the general circulation (femoral vein) in another group. Similar investigations were conducted with two organic phosphorus compounds—dichlorvos, a direct inhibitor of cholinesterase, and parathion, an indirect inhibitor. It is well known that parathion is converted to the highly toxic paraoxon in the liver, but it is presumably in the liver also that it is eventually detoxified.

Adult female Sherman strain rats were paired according to age and body-weight and prepared for infusion by cannulating the intestinal vein of one rat and the femoral vein of the other. Surgery for installation of the cannulae was performed on the rats while under anaesthesia with methohexital sodium solution given by intraperitoneal injection at a dosage level of 80 mg/kg. The method of cannulation of both veins was the same as that described by Ross and Rappaport[2] for cannulation of mesenteric veins.

As soon as the cannulation was completed and the incision sutured, each rat was confined to an individual restraining cage[3]. The cannulae were each fitted over the tip of a 27-gauge hypodermic needle and attached to separate 5-ml. hypodermic syringes on a dual infusion pump. Each rat was infused with normal saline, at the same rate as the poison formulation was later infused, for at least 1 h or until both rats recovered from the anaesthesia if recovery took longer. Then the syringes of normal saline were replaced with syringes containing the poison, and the infusion of the poison was started simultaneously in both rats.

Preliminary investigations were conducted with each of the compounds in a deliberate effort to establish a concentration, infusion rate, and duration of infusion which would kill all the rats infused by one route and allow those infused by the other route to survive. Once

these conditions were determined for a compound, all the rats infused with that compound were infused at the same concentration and rate and for the pre-determined period of time, unless they died earlier. Based on the results of the preliminary tests, the compounds (all technical grade) used in this investigation were formulated and infused as shown in Table 1.

Each rat was observed continuously during the infusion of poison, and a complete record kept of time of the onset and course of symptoms and of the time of death. Except for the earlier detection of facial muscle fasciculation in some of the rats, the first symptom of poisoning was light generalized muscle fasciculation. Consequently, the time of onset of the latter was indicated as the time of onset of symptoms. Rats which survived the infusion were held for observation until recovery from symptoms, but not necessarily until recovery from cholinesterase inhibition.

The toxicity of 'Isolan', dichlorvos, and parathion infused into the hepatic portal system (intestinal vein) compared with their toxicity when infused directly into the general circulation (femoral vein) in rats is shown in Table 2. Based on time to onset of symptoms, time to death, and mortality in the poisoned rats, it is apparent that 'Isolan' and dichlorvos infused into the hepatic portal system undergo considerable detoxification during a single passage through the liver before they reach the general circulation. On the other hand, parathion infused into the hepatic portal system is more toxic than when infused by way of the femoral vein into the general circulation.

Time to onset of symptoms caused by 'Isolan' and dichlorvos was delayed about three times as long in rats infused into an intestinal vein as in those infused in the femoral vein. Six rats given a mean total dose of 0·73 mg/kg of 'Isolan' in the femoral vein were killed, but 5 of 6 rats given a mean total dose of 1·63 mg/kg by intestinal vein survived. A mean total dose of 5 0 mg/kg of dichlorvos killed 6 rats infused in the femoral vein, but 6 rats infused in an intestinal vein survived a mean total dose of 15·2 mg/kg.

The results for parathion presented in Table 2 indicate that the time to onset of symptoms in rats infused in the femoral vein was about twice as long as that for rats infused in an intestinal vein. However, 3 of the 6 rats infused in the femoral vein for 75 min never exhibited any signs of poisoning and none of the 6 rats died. All the 6 rats infused in an intestinal vein were killed in 57–75 min.

It is clear that efficient detoxification of 'Isolan' by the liver *in vivo* is one factor contributing to its relatively low toxicity by the oral as compared to the dermal route. However, one or more other factors must be important, for the detoxification of dichlorvos by the liver seems equally efficient, and yet dichlorvos is slightly more toxic by the oral than by the dermal route. Even though other techniques will have to be used to explain fully the pattern of toxicity of 'Isolan' when given by different routes, the technique presented in this report is a useful and relatively simple procedure for learning the net effect of the liver on the metabolism of chemicals in intact laboratory animals.

Table 1. Formulation of Compounds and Rate of Intravenous Infusion in Female Rats

Compound	Vehicle	Formulation Concentration of poison (mg/ml.)	Infusion rate (ml./rat/min)	Maximal period of infusion (min)
'Isolan'	Normal saline	1·0	0·0083	60
Dichlorvos	Modified 'Lipomul'*	12·5	0·0068	60
Parathion	Modified 'Lipomul'*	1·0	0·0068	75

* As described by Tullner and Hertz (ref. 4).

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Incompatibility of Synthetic Tetraicosapeptide Corticotrophin with the Present U.S.P. and International Corticotrophin Standard

Corticotrophin, unlike insulin or the octapeptides of the posterior lobe of the pituitary gland, is known to exist in different biological forms, due to inconsistencies in bioassays in which various routes of administration are used. The first hormone preparations used were of the same type as the first and second International Standard, that is, crude corticotrophin—a glacial acetic acid extract of pituitary glands. When in 1951 the oxycellulose adsorption method of Astwood *et al.*[1] yielded a highly purified hormone, the bioassays carried out by subcutaneous injections into hypophysectomized rats showed, on the average, potency estimates three times greater than the classical assay method (Sayers test) which involves intravenous injection. This phenomenon led to a type distinction of oxycel-purified ACTH from crude corticotrophin, the former being called corticotrophin *A* (Wolfson[2]). Another attempt to purify corticotrophin involving peptic or strongly acid hydrolysis yielded preparations of lower molecular size (about 28 amino-acid residues), which also differed biologically from crude corticotrophin[3]. In this case the ratio of potencies observed after subcutaneous and intravenous comparison with the latter was reversed and of the order of 1 : 4. This third biological ACTH type was designated corticotrophin *B* or actide (ref. 2). The foregoing ratios were obtained in rats but appear, in the main, to agree with those in man so far as crude corticotrophin and corticotrophin *A* are concerned. Bearing in mind that ACTH is mainly given subcutaneously (extravascularly), corticotrophin *A* may be regarded as the most important type of native ACTH due to its high degree of purity and

Table 2. Intravenous Toxicity of Pesticides infused into the Hepatic Portal System (Intestinal Vein) compared with their Toxicity when infused into the General Circulation (Femoral Vein) in Female Rats

Compound	Route of infusion	Body weight of rats (g)		Time to onset of symptoms (min)		Time to death (min)		Total dose (mg/kg)		Mortality (died/tested)
		Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	
'Isolan'	Femoral vein	237–392	322 ± 21·8	10–15	13 ± 0 9	18–35	28 ± 2·5	0·44–1·00	0·73 ± 0 08	6/6
	Intestinal vein	255–368	311 ± 18 6	30–55*	48 ± 4·6	One died		1 35–1·95	1·63 ± 0 10	1/6
Dichlorvos	Femoral vein	292–410	346 ± 16·6	5–9	7 ± 0·1	17–31	21 ± 2·1	4 4–6·4	5·0 ± 0·29	6/6
	Intestinal vein	300–408	339 ± 16·9	14–29	24 ± 2·3	Survived		12 5–17 0	15 2 ± 0 71	0/6
Parathion	Femoral vein	270–336	305 ± 10 0	55–70†	64 ± 4·5	Survived†		1 52–1·89	1·69 ± 0·06	0/6
	Intestinal vein	280–354	315 ± 10·9	26–39	34 ± 1·9	57–75	65 ± 2·9	1·27–1 60	1·41 ± 0·05	6/6

* One rat showed no symptoms of poisoning.
† Three rats showed no symptoms of poisoning.

Table 1. Eight Independent Bioassay Results comparing Synthetic Corticotrophin (Tetraicosapeptide-Ciba) with the Present International Corticotrophin Standard by the Method of Rerup and Hedner

	Assay day	No. of mice	Weight factor of assay = W_1	Log potency ratio = M	Potency ratio = antilog M	$P = 0.05$ confidence limits
(*a*) Subcutaneous injection	18.12.64	12	18·6	1·3370	21·7 (I.U./mg)	
	10.3.65	16	68·0	1·5686	37·0	
	11.3.65	16	99·7	1·5473	35·3	
	12.3.65	16	59·7	1·4721	29·7	
	Total	60	246·0	Weighted mean 1·5190	33·0	24·6–44·3
(*b*) Intravenous injection	12.1.65	16	85·0	2·4105	257	
	9.3.65	16	65·9	2·3943	248	
	16.3.65	16	74·3	2·4055	254	
	19.3.65	16	183·9	2·4146	260	
	Total	64	409·1	Weighted mean 2·4088	256·4	204–322

favourable efficiency when given extravascularly. It is partly for these reasons that the now official International and U.S.P. Corticotrophin Standard preparations are of the corticotrophin *A* type, a fact which has practically eliminated bioassay discrepancies (crude corticotrophin is now rarely used). The reasons for the foregoing bioassay discrepancies are unknown, but it is most likely that they lie in the different rates of inactivation of the different types of ACTH at the site of extravascular administration.

The recent introduction of synthetic corticotrophin (tetraicosapeptide-Ciba), which due to its lower molecular weight should belong to the corticotrophin *B* type, has created a new situation, since formerly this type was not in general use. We tested the hypothesis of a type-difference between the synthetic tetraicosapeptide and the International Standard for corticotrophin (corticotrophin *A*) by a method utilizing the increase in the corticosteroid-level of mouse plasma. This method, devised by Rerup and Hedner[4], has been shown to give valid potency estimates for corticotrophin *A* preparations. Typical results are shown in Table 1.

It will be noted that the synthetic tetraicosapeptide when given subcutaneously showed only about 13 per cent of the activity expected from the assay using intravenous injection. Thus there can be no doubt concerning the strong biological difference between the present (International and U.S.P.) standard and the synthetic peptide. From this it follows that, so long as the international unit is used as a reference for corticotrophic activity, labelling of the synthetic product in weight units (mg) is inappropriate, since there does not appear to exist a fixed constant of equivalency of weight unit to biological unit. The findings indicate the need for (*a*) a clear distinction between preparations for intravenous and subcutaneous use (U.S.P. 1955)[5]; (*b*) a parallel study in man and laboratory animals to assess the equivalent of 1 mg of the tetraicosapeptide in international units after both intravenous and subcutaneous injections.

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PATHOLOGY

Carcinoma of the Glandular Stomach in Rats given Diets containing Aflatoxin

Spontaneously occurring carcinoma of the glandular stomach in the rat is exceedingly rare. In a large series Bullock and Curtis[1] reported a single case, while Stewart *et al.*[2] in a review of the literature failed to find a convincing example of adenocarcinoma of the stomach in the rat. Experimentally, Hare *et al.*[3] produced a low incidence of glandular carcinoma on intramural injection of the carcinogenic polycyclic hydrocarbons. The same substances on feeding failed to induce carcinoma of the stomach. Stewart *et al.*[2] have since demonstrated that oral administration of *N,N'* 2,7-fluorenylene*bis*acetamide will induce a low incidence of stomach adenocarcinoma (4 in 47 rats), and Schoental[4] has reported the induction of malignant adenocarcinoma with *N*-nitroso-*N*-methylurethane (NMU). It is the purpose of this communication to describe the incidence of carcinoma of the glandular stomach observed in a series of experiments in which the toxicity of groundnut meal containing aflatoxin was investigated.

In our original experiments to examine the toxicity of groundnut meal we reported two definite adenocarcinomas of the stomach while a third animal had multiple metastases from a histologically similar adenocarcinoma. These experiments were started when the rats were about 5 weeks old and the animals were killed 66–76 weeks after the start of the experiment[5].

In further experiments rats have been given a diet made up with 50 per cent of the same batch of groundnut meal. The level of aflatoxin in the diet was 3–4 p.p.m. In one experiment 6 male rats 1 year old were given the diet to test its effects on old rats. Five of the 6 rats survived for more than 39 weeks, the sixth dying of middle-ear disease after only 9 weeks on the diet. Of these animals 3 showed anaplastic hepatocellular carcinomas. Two of these animals also had a further primary carcinoma, one an adenocarcinoma of the rectum and the other an adenocarcinoma of the stomach. The rat with the stomach carcinoma was 104 weeks old. In other experiments to be reported in detail elsewhere, the effects of short-term administration of aflatoxin were investigated.

Six young rats were given the diet for 3 weeks and then returned to a normal diet; one animal which lived for 106 weeks after return to the normal diet developed a carcino-sarcoma of the stomach. In this group of 6 animals only one developed a hepatic carcinoma.

Thus a total of four confirmed and one probable carcinoma of the glandular stomach have been seen in rats fed aflatoxin. Although the overall incidence is low, the tumours reported here are of considerable interest in view of the difficulty with which this type of carcinoma can be induced.

It has been suggested that aflatoxin may play a major part in the aetiology of human liver carcinoma in some parts of the world[6]. Carcinoma of the glandular stomach also shows an interesting geographical distribution, and it is possible that this type of food contaminant may be a factor in the aetiology of carcinomas in organs other than the liver.

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Experimental Pyelonephritis in the Rhesus Monkey

Experimental work on pyelonephritis has been confined to rabbits, rats and mice[1]. The investigation on rhesus monkeys recorded here was undertaken because this species is phylogenetically close to man and, therefore, has better chances of developing the various characteristics of the human disease rather than any other labora-

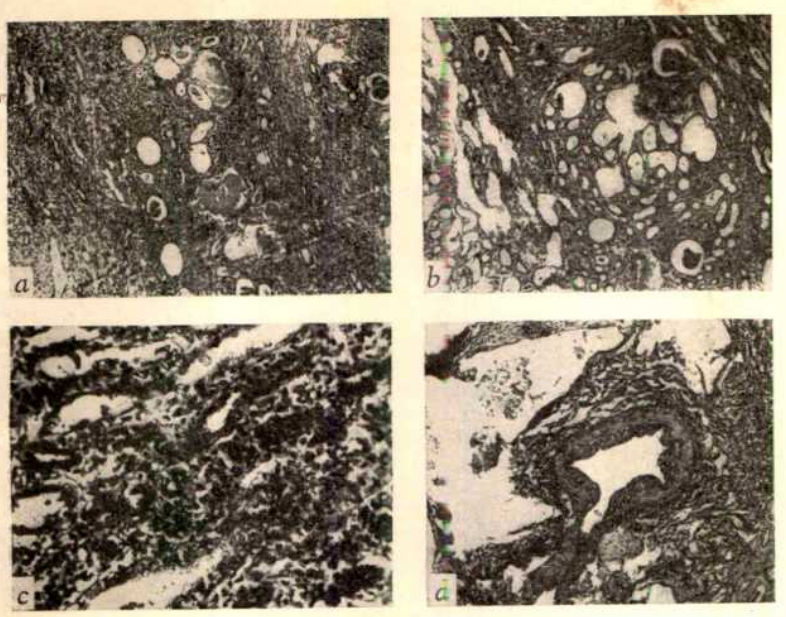


Fig. 1. *a*, Section of left kidney showing diffuse infiltration of interstitial tissue with inflammatory cells, destruction of renal tubules, casts in dilated and cystic tubules and shrunken glomeruli as a result of chronic pyelonephritis (× *c*. 70). *b*, Section of left kidney showing markedly dilated and cystic tubules with atrophic epithelial lining along with diffuse cellular infiltration of interstitial tissue (chronic pyelonephritis) (× *c*. 70). *c*, Section of left kidney showing destruction of renal tubules and interstitial cellular infiltration with mononuclear histiocytes, plasma cells and lymphocytes (× *c*. 250). *d*, Section of the pyelonephritic left kidney showing intimal elastosis of a medium-sized interlobar artery (× c. 70). Figs. *a–c*, haematoxylin and eosin; Fig. *d*, Verheeffs' stain

tory animal. This preliminary communication describes the techniques employed for the production of chronic pyelonephritis.

Twelve healthy animals weighing 2–2·5 kg were caged individually and were given a diet made up of soaked grain, green vegetables and bread. After adapting them to the laboratory conditions for a fortnight they were subjected to laparotomy aseptically under sodium pentothal anaesthesia. The left ureter was exposed and partially ligated by silk thread 2 cm below the hilum of the kidney, thus producing hydronephrosis. The right kidney served as control. 15 days after this operation, two doses of 18-h broth culture of haemolytic and coagulase positive *Staphylococcus pyogenes* representing $9{\cdot}76 \times 10^8$ organisms per ml. were injected into the femoral vein at a week's interval. The optimum dose of the culture which was found to avoid mortality was 0·5 ml. initially followed by 1 ml. after a week.

The animals were killed after 8 weeks of the second injection and autopsy performed. Culture of heart blood, liver, spleen, both kidneys and of urine was performed. The tissues were examined grossly and blocks fixed in 10 per cent neutral formaldehyde saline for histopathological studies. Paraffin sections 5μ thick were cut and stained with haematoxylin and eosin. Special histochemical techniques were used whenever required.

Although they became thinner, the test animals kept well throughout the experiment. Bacteriological examination of specimens at autopsy yielded growth of *Staphylococcus pyogenes* from the left kidneys of two animals which resembled the original culture morphologically and biochemically. The tissues of the remaining animals were found to be sterile.

The left kidneys were enlarged and showed hydroureter and hydronephrosis. The renal calyces were ill-defined, and the pelvic surface was shaggy. In one animal, the left kidney was markedly shrunken and the cut surface showed fibrous bands running irregularly in the renal parenchyma. The right kidney showed congestion. The urinary bladder was unaffected.

The microscopic examination of the left kidney showed inflammation and degeneration of tubular epithelium and cystic dilatation with protein and leucocyte casts. In the interstitial tissue oedema and capillary dilatation with diffuse infiltration by mononuclear histiocytes, lymphocytes, plasma cells and occasional polymorphs were seen. Early fibrous proliferation was also noted. The glomeruli appeared normal. Some of the renal arterial branches had fibro-elastic intimal thickening (Fig. 1*a–d*). The pelvic epithelium showed atrophy and the sub-epithelial zone was markedly inflamed. Examination of the right kidney showed a normal appearance in all the animals except two, in which interstitial inflammation with slight tubular degeneration were noted.

The tissues of the control group of monkeys did not show any pathological process.

The work recorded here has provided for the first time new information regarding suitability of rhesus monkeys for research on pyelonephritis. The morbid changes observed had many features of the human disease, for example, isolation of causative organisms from the kidney, presence of internal hydronephrosis, diffuse interstitial nephritis and vascular changes. It was also found that kidneys are particularly prone to localization of the pathogenic organisms if obstruction to urine flow is achieved. In other laboratory animals also, this point has been emphasized[2–5]. In this work, however, no advanced pictures of pyelonephritis or scarring of kidneys or inflammatory changes of urinary bladder were detected. This could have been due to the short period of observation.

We thank Dr. Mohini Malhotra for her help in standardizing surgical techniques in this experiment.

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Is there a Cortex–Medullary Cord Barrier in Lymph Nodes?

This communication discusses the evidence for a barrier between the cortex and the medullary cord of rat lymph nodes, as suggested by the regional distribution of lymph-borne particulates within such a node, and also by the cytological pattern of its reaction to intense antigen stimulation.

A useful method of examining particle distribution is to inject 0·1 ml. of iron–dextran ('Imferon', Benger) into the right hind footpad of the 80–100-g rat. 3-μ paraffin sections stained by Prussian blue are then used to plot the distribution of iron-marked macrophages in the medullary structures of the corresponding para-aortic lymph node, at graduated time intervals after this footpad injection.

From 0·5 to 24 h afterwards, medullary sinus macrophages are heavily outlined, but little iron is found in the cords. After 24 h, iron-marked macrophages in cord and sinus regions can be easily observed. After 2 days, relatively less iron is found in the sinuses; but in the cords closely packed groups of macrophages are seen to be laden with Prussian blue-positive material. Adjacent to this

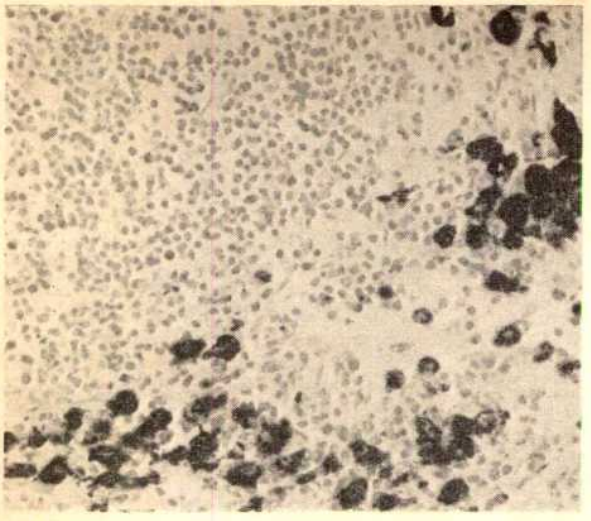


Fig. 1. View of cortex, top left, and medulla, bottom right, of para-aortic lymph node of rat 4 days after one footpad injection of iron-dextran. The maximum concentration of iron-marked macrophages is in the medullary cords. The cortex contains no visible iron. In terms of marked macrophages, there is a sharp cortico-medullary transition. Formol-fixed paraffin sections cut at 3μ, stained by Prussian blue-nuclear fast red (× *c.* 95)

heavy cord marking, the macrophages of the inner cortex contain little or no iron (Fig. 1). Especially in 4-day preparations, this gradient can be very abrupt.

Para-aortic lymph nodes have been observed for up to 9 weeks after a footpad iron–dextran injection. At the end of this time, some scattered cortical macrophages have become Prussian blue positive. However, the general exclusion of iron from the inner cortex remains unchanged. These regional peculiarities of macrophage iron–dextran distribution are duplicated by lymph-borne carbon used under similar conditions ('Pelikan' indian ink, Gunther-Wagner, *C*11/1431*a*).

The behaviour of lymph-borne particles thus suggests a barrier between the medullary cord and the cortex, in spite of the fact that collagen and reticulin stains do not support such a suggestion, nor does the adherent carbon-frozen section technique, as described in a previous communication[1], show any obvious organization of macrophage processes at this site (Fig. 2).

Yet certain cytological observations support a cortico-medullary barrier concept. Antigen stimulation by TAB vaccine (0·1 c.c. Commonwealth Serum Laboratories vaccine twice daily for 5 days) produces a sequence of blast-plasma cell change in the medullary cords, while the adjacent cortex shows remarkably little alteration of its predominantly small lymphocyte population. The line of separation between the lymphocytes of the cortex and the blast-plasma cell population of the adjacent medullary cord can be visually abrupt on the low-power view (Fig. 3) although higher magnification usually shows a zone of transition rather than a sharp boundary line.

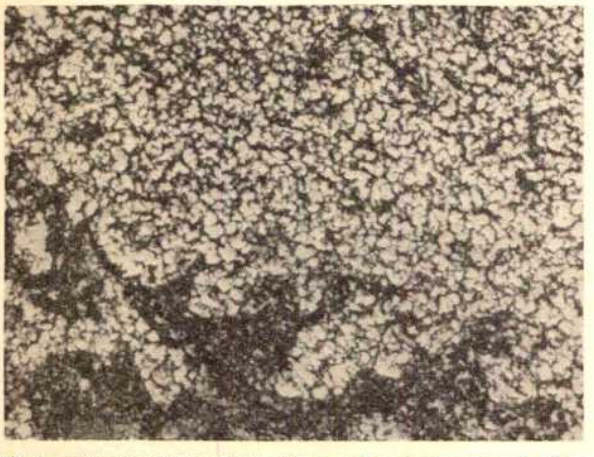


Fig. 2. View of cortex, top right, with macrophage processes marked by adherent carbon. Bottom left, the medullary sinuses are dark because of their dense macrophage concentration. Between the sinuses, the medullary cords can be seen in the region of the cortico-medullary junction. There is no special macrophage organization in this zone to suggest a cortico-medullary barrier. 10-μ frozen section, unfixed. Stained by adherent indian ink (× *c.* 130)

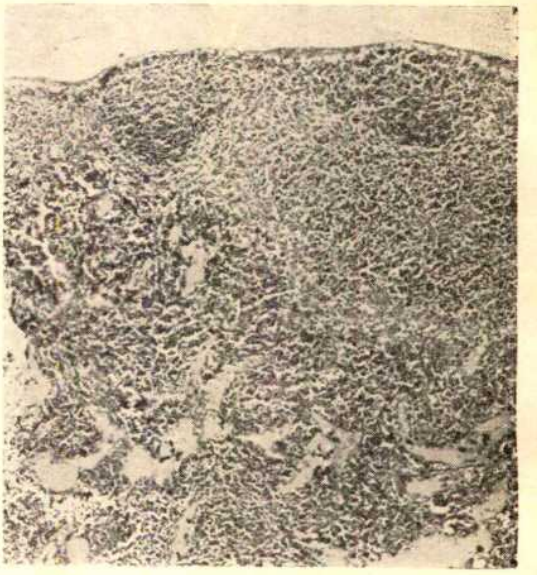


Fig. 3. View of cortex, top right, with medullary cords and sinuses, bottom left, at the end of a TAB antigen course by footpad injection, as described in text. The cortex maintains its small lymphocyte population; the medullary cords are full of maturing plasma cells. Visually, these two populations show an abrupt cortico-medullary transition. Formol-fixed paraffin sections cut at 3μ, stained by haemalum (× 50)

Electron microscopic observations will be necessary to confirm objectively the presence of this cortico-medullary barrier, which has so far been inferred only by indirect means. Further investigation into this point is now in progress.

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RADIOBIOLOGY

Radiation-induced Mitotic Delay in Spermatogenesis of *Drosophila melanogaster*

Welshons and Russell[1] demonstrated in their histological observations on the testis of X-irradiated adult *Drosophila* males that secondary spermatogonia and young primary spermatocytes are easily killed by X-rays and that their destruction results in a period of temporary sterility of the males. These data were tabulated by the authors in a semi-quantitative way. If one presents the results graphically (Fig. 1) it becomes evident that, besides cell killing, mitotic delay has been induced by the exposure to X-rays.

The authors find that the minimum of curve *A* at day 2 is caused by radiation-induced degeneration of secondary spermatogonia and young primary spermatocytes. The minimum of curve *B* is explained by assuming that the old primary spermatocytes develop into sperm without degeneration and without being immediately replaced from the spermatogonia. However, the minimum of curve *B* is lower than that of *A* and—after curve *B* has gone through this minimum—the frequency of *B*-cells temporarily exceeds the control value. These two facts can easily be explained by postulating mitotic delay of at least a part of the *A*-cells. If these *A*-cells

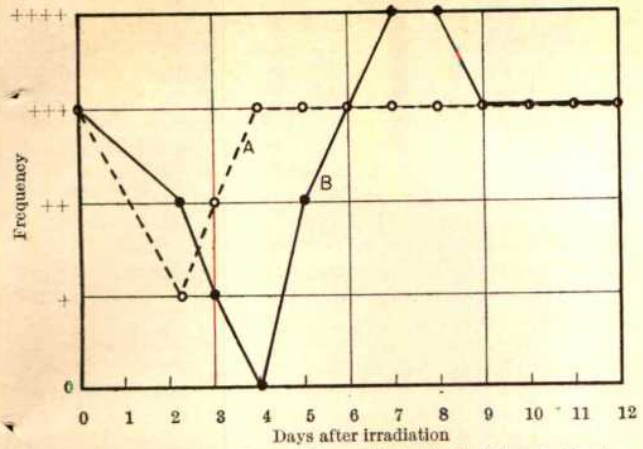


Fig. 1. Histological condition of *Drosophila* testis of adult males irradiated with 4,000r X-rays (after ref. 1). The normal condition of the testis is designated by + + +, conditions characterized by radiation induced decrease of the number of germ cells are designated by + +, + and 0. *A*, Secondary spermatogonia and young primary spermatocytes; *B*, old primary spermatocytes

interrupted their development the minimum of curve *B* might become lower, as if it were determined only by the frequency of killed *A*-cells. When the younger *A*-cells, which were not delayed in their development, together with the delayed *A*-cells, formed *B*-cells, the frequency of *B*-cells was raised above its normal value by this process of synchronization. One wonders why there is no corresponding excess of *A*-cells; perhaps this increase was not detectable by this semi-quantitative investigation.

Though the authors do not discuss mitotic delay in their paper, we feel that their observations do support the postulate that radiation-induced mitotic delay occurs in the testis of *Drosophila melanogaster*. If so, mitotic delay, as well as cell killing and dominant lethality, contributes to the sterile interval observed in brood pattern experiments utilizing irradiated *Drosophila* males.

I thank Prof. D. W. Dittrich and Dr. S. Bhaskaran (Münster) and Dr. W. J. Welshons (Oak Ridge) for their advice.

Note added in proof. The symbols *A* and *B* used in this communication have nothing to do with the classification of mouse spermatogonia into type *A*- and *B*-cells.

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BIOLOGY

Root Cap and the Perception of Gravity

During investigations into the fine structure of apical meristems under the electron microscope, one of us (S. G.) discovered that it was possible, in maize and barley, to detach the intact root cap cleanly from the rest of the root tip. This is shown in Fig. 1. The roots of grasses are characterized by having a discrete cap meristem and, as can be seen in Fig. 2, a very well marked boundary between the cap and the root proper. The fracture takes place through the unstrengthened walls of the meristematic region of the cap as indicated in Fig. 2. We have not so far succeeded in removing the cap intact from other species such as broad bean or pea.

Removal of the cap has no effect whatever on the growth in length of the root as a whole, whatever the orientation of the root may be. This is in strong contrast to the early claims[1], on which much of the theory of root growth control has been based, that decapitation accelerates extension rate. On the other hand, removal of the root cap (which we shall henceforth call decapping) completely prevents the perception of the gravitational stimulus, and decapped roots will continue to grow horizontally for up to 30 h without check or curvature.

Regeneration of the root cap follows decapping and is complete at 28° C in about 36 h. An increase in temperature accelerates the regeneration and we have some evidence that young roots replace their root caps more rapidly than do older roots. The quiescent centre[2] of the root is exposed on its distal margin by the removal of the cap, and it is the cells of the quiescent centre, stimulated into rapid meristematic activity, which replace the cap meristem and consequently the root cap itself. Towards

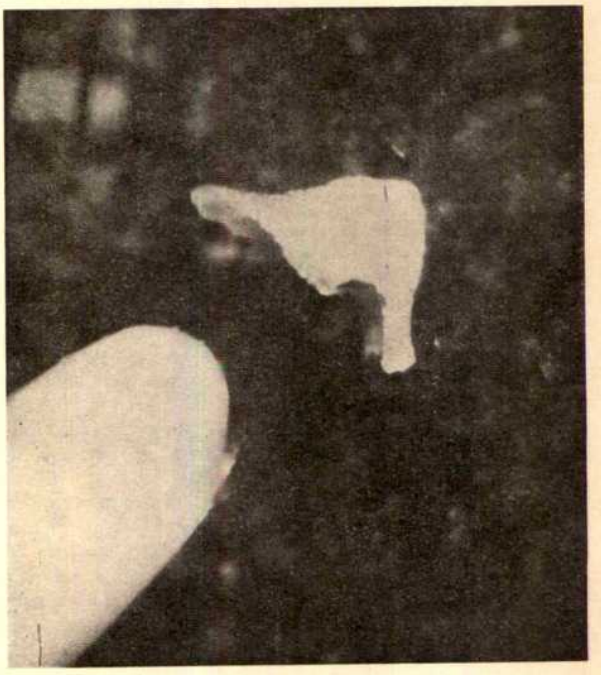


Fig. 1. A decapped maize root showing the clean undamaged surface of the meristem

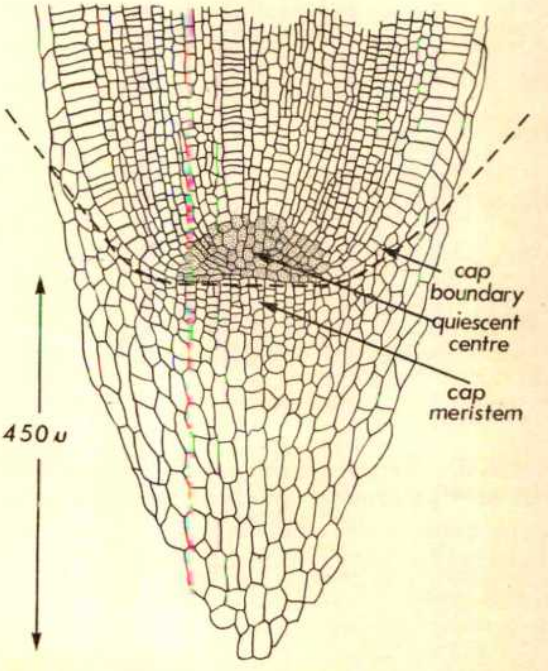


Fig. 2. Cell outline drawing of a median longitudinal section through a root tip of maize

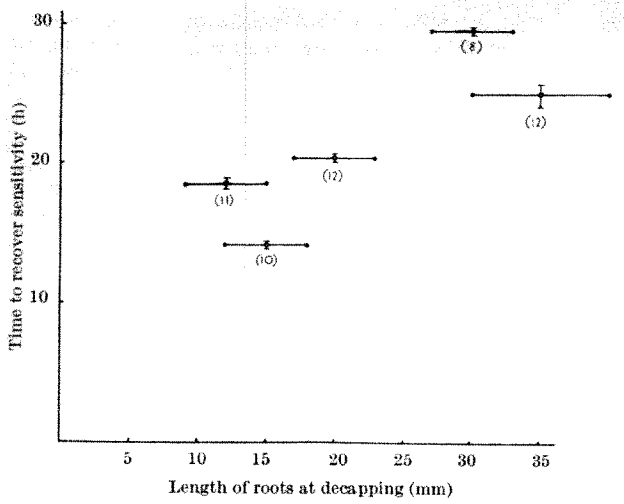


Fig. 3. Graph showing relationship between root length at decapping and time taken to recover geotropic sensitivity in maize roots at 20° C. The lengths of the lines through the means represent ± the standard error of the recovery times and the range of the root lengths in the samples. The numbers in brackets record the number of roots observed per sample

the end of regeneration the cap boundary is reformed and the quiescent centre re-established.

The ability to perceive gravity is regained somewhat abruptly and is correlated, so far as can at present be judged, with the regeneration of a major proportion of the root cap. Fig. 3 shows the relationship between root-length at decapping and the interval between decapping and the recovery of geotropic sensitivity in maize roots. The more rapid recovery of younger roots is correlated with the more rapid cap regeneration noted previously. In other experiments one of us (B. S.) has been able to remove a completely regenerated cap and to observe the regeneration process and recovery of geotropic sensitivity for the second time in the same root; this demonstrates clearly the lack of any damage to the root meristem by the decapping process.

The implications of these results are far-reaching. Clearly the root cap has no direct influence on root elongation and hence is unlikely to be the centre of root growth regulator production. But since it is the site of the perception of gravity it must in some way control growth regulator production in or movement from the tip meristem itself. Our results so far give no clue as to the nature of this control, and work on this problem is continuing.

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Metabolic Activities of Isolated Heterocysts of the Blue-green Alga *Anabaena cylindrica*

Knowledge of the nature of the heterocysts of blue-green algae has been limited by the lack of suitable techniques for investigation of the physiology and biochemistry of these puzzling structures. The association of the presence of heterocysts with the ability to fix free nitrogen and the findings[1,2] that ammonia suppresses both nitrogen fixation and heterocyst formation have led to the suspicion that these cells might be involved in the nitrogen-fixation process. In this communication we report a successful technique of 'differential cell disruption' for the isolation of heterocysts of *Anabaena cylindrica*. We also present the first results concerning the metabolic activities of isolated heterocysts.

In making cell-free extracts of *A. cylindrica*[3] by smashing filaments in a French press[4], it was noticed that some of the heterocysts resisted breakage even at pressures exceeding 2,100 atm. At lower pressures (760 atm.) it was found that most of the heterocysts remained intact while all the vegetative cells were broken. Since then it has proved possible to obtain by this means a pure preparation of heterocysts, free of cell fragments and soluble cell material.

The experimental material was prepared from an exponentially growing culture[3] with developing heterocysts[1,5]. It was important to use young cultures free from both akinetes, which survive the smashing process, and extracellular mucilage, which interferes with the purification of the heterocyst fraction. The heterocyst frequency was about 6 per cent. The algal material was collected by centrifugation and forced through the pre-cooled French press at 760 atm. The suspension was homogenized a little at a time, in ten volumes of distilled water, using a Potter homogenizer. The suspended material was bulked and centrifuged at 1,300*g* for 10 min. Most of the soluble material and mucilage remained in the supernatant. The pellet was resuspended in distilled water, homogenized and centrifuged at 100*g* for 2 min, which precipitated the heterocysts but left most of the cell fragments in suspension. The supernatant was discarded and the heterocyst residue treated twice more as before. A heterocyst fraction practically free of soluble and particulate cell material was obtained. The main remaining contaminant was cell walls of broken heterocysts. The control fraction was prepared by identical treatment of algal material passed through the French press at a pressure not exceeding 95 atm., which caused the breakage of the filaments into short lengths but left the majority of the cells intact. This preparation is referred to as 'filament'. Both the final fractions were suspended in standard medium[3] and used immediately for experimental investigations.

Nitrogen fixation by heterocysts was tested by exposure to an atmosphere containing nitrogen-15 enriched nitrogen gas. Uptake of nitrogen-15 was measured by the method of Burris and Wilson[6]. The results obtained in the two experiments and shown in Table 1 demonstrate that no significant fixation occurred in the heterocyst fraction while the filaments fixed nitrogen vigorously.

Table 1. Fixation of ^{15}N-Nitrogen in Heterocyst and Filament Fractions of *Anabaena cylindrica*

Fraction	Atom per cent nitrogen-15 excess Exp. I	Exp. II
Heterocyst	0·004 (0·003)	0·000 (0·002)
	0·001	0·004
Filament	1·538 (1·616)	0·338 (0·368)
	1·693	0·397

Five-ml. suspension samples were incubated in Warburg flasks at 600 ft.-candles and 30° C with continuous shaking for 2 h. Gas phase: 0·1 atm. N_2 (containing 50 atom per cent ^{15}N in Exp. I and 20 atom per cent ^{15}N in Exp. II), 0·1 atm. oxygen; 0·02 atm. carbon dioxide; and the rest argon. Means in parentheses.

The photosynthetic capacity of heterocysts was investigated by exposing the fractions in the light to ^{14}C-labelled carbon dioxide. Carbon dioxide fixation was measured by radioactivity assay of samples collected on membrane filters. The results of the two experiments shown in Table 2 show that the heterocysts had no appreciable photosynthetic activity in contrast to the filaments, which became highly labelled.

Respiratory activity was measured by the direct manometric method of Warburg, and the results (Table 3) indicate that the heterocysts had greater respiratory activity than the filaments.

Table 2. Fixation of ^{14}C-Carbon Dioxide in Heterocyst and Filament Fractions of *Anabaena cylindrica*

Fraction	Radioactivity of cells (counts/min/μg cell N) Exp. I		Exp. II	
Heterocyst	12	(11)	0	(85)
	10		169	
Filament	11,040	(11,460)	58,750	(52,575)
	11,880		46,400	

Six-ml. suspension samples were incubated in Dickens-Simer flasks at 600 ft.-candles and 30° C with continuous shaking for 2 h. In each sealed flask ^{14}C-labelled carbon dioxide was generated by tipping 0·5 ml. 0·1 N hydrochloric acid placed in the side-arm into 0·5 ml. $NaHCO_3$ solution (containing 1·9 mg $NaHCO_3$) placed in the trough of the flask, thus providing 1 per cent carbon dioxide concentration (plus 2 μc. activity in Exp. I and 10 μc. activity in Exp. II) in the gas phase of each flask. Means in parentheses.

Table 3. Respiratory Activity of Heterocyst and Filament Fractions of *Anabaena cylindrica*

Fraction	μl. O_2 uptake/mg cell N/h	
Heterocyst	218·8	(230·2)
	241·3	
Filament	170·4	(159·4)
	148·4	

Four-ml. suspension samples were incubated in Warburg flasks, each provided with 0·5 ml. 12 per cent potassium hydroxide in the side-arm, in the dark at 30° C with continuous shaking. Means in parentheses.

Electron microscope investigations[5,7] with *A. cylindrica* and other blue-green algae have shown that the development of heterocysts from vegetative cells involves extensive changes in the fine structure, the most striking of which is the disorganization of the lamellar system. The paired lamellae (thylakoids), which are considered to be the structural units of the photosynthetic apparatus, the structure of which seems to be essential for the retention of the photosynthetic pigments, become open to the heterocyst and the unit membranes run singly, surrounding the vesicles or forming an elaborate branching system. Presumably the absence of photosynthetic activity in heterocysts results from the disorganization of the photosynthetic apparatus. The interesting finding that nitrogen fixing capacity is also absent in heterocysts is in good agreement with our previous report[3] that the site of nitrogen fixation in this alga is located at the photosynthetic lamellae. Our findings are in agreement with histochemical investigations[8,9] which indicate that heterocysts possess an active respiratory mechanism. Further investigations regarding the physiological and biochemical changes which occur during heterocyst formation are in progress.

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Use of Lipase and Strepzyme for the Isolation of 'Protoplasts' from Fungal Mycelium

The isolation of protoplasts from bacteria[1], vegetative fungal cells[2-4] and plant tissues[5], following the use of enzymes digesting the cell wall, suggested that protoplast-like bodies might be obtained from resistant spore forms of fungi. It has recently been shown in this laboratory that free 'protoplasts' are also obtained when conidia of *Trichothecium roseum*[6] and *Fusarium culmorum*[7] are incubated in a solution containing the lytic enzyme preparation. Age of the spores seemed to be important for the digestion of the cell walls; under appropriate conditions, using young spores (3–4 days old), more than 90 per cent of the suspended conidia were transformed into 'protoplasts'. It was then suggested that spore protoplasts represent a satisfactory starting material for obtaining suspensions of free uniform protoplasts.

It was previously shown that Actinomycetes possess marked bacterial[8] and fungal lytic[9] properties. Following an investigation undertaken to explore the possibility of using enzyme preparations from actinomycetes in the digestion of fungal cell-walls, we found some interesting results. Cell-free shake flask culture filtrates of *Streptomyces RA* grown for 6–8 days at 28°, according to the method of Rodriguez Aguirre *et al.*[4], produced a lytic enzyme (strepzyme) which was very useful for the preparation of hyphal protoplasts of several species, including *Fusarium culmorum* mycelium.

It was noteworthy that protoplasts were not formed from cells of the more resistant forms, the fusaroid conidial cells, which in contrast were very susceptible to other strepzymes, for example *Micromonospora chalcea* enzymes. These results encouraged us to make a comparative study not only of the cell wall composition of the hyphal and spore cell walls but also of some of the enzymes present in the strepzyme preparations from the lytic organisms *Streptomyces RA* and *Micromonospora chalcea*. It was found that the conidial cell walls of *F. culmorum* contain less lipids than the mycelium cell wall[10]. However, it is well known that the total lipid content of fungus mycelium is so strongly affected by cultural conditions and age that any set of figures might be misleading. On the other hand, when the lipase activity was examined in the two cultures of actinomycetes mentioned here it was detected as very low, or practically no lipase activity in the strepzyme preparation from *Micromonospora AS* which is active on conidia and high lipase activity in the *Streptomyces RA* preparation.

These results may in part explain some of the previous observations on the digestion of the hyphal and spore cell walls and on the liberation of protoplasts.

Furthermore, in order to test and confirm the previous observations a commercial lipase preparation was used. The *Fusarium* mycelium was previously incubated for a short time (30 min) with 200 μg/ml. of pancreatic lipase enzyme (Sigma, U.S.A.) and then washed by centrifugation with a stabilizing buffered solution. The mycelium was again resuspended in the same solution and treated with the strepzyme preparation as already described. Surprisingly, and confirming the previous results, it was found that the combined action of lipase and the strepzyme from the *Micromonospora RA* successfully gave rise to the rapid formation of large numbers of mycelium protoplasts.

These findings, in addition to those described by various other workers when using combinations of chitinases and glucanases[11-13], may clearly speak in favour of the possibility of more frequent use of combinations of commercial and crude or partially purified microbial preparations for digestion of fungal cell walls and subsequent liberation of protoplasts, as well as for examination of the structural components of the cell walls released during degradation. Breakdown of cell walls by enzymes of one kind or another has been studied extensively in bacteria. Our experience of lytic activities suggests that combination of enzymes often shows a synergistic action, and these synergistic effects are apparent not only on living cells but also on dead cells and on their isolated cell walls. However, as has been suggested for the bacterial cell walls, a further assessment of the sites of action and relative specificities of the many enzymes which attack fungal cell walls

must await more information about chemical structure of their substrates—the walls themselves.

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Semi-fossil Lichen Fungi in Scottish Hill Soils

Recent investigations[1] into the micromorphological features of hill soils in north-east Scotland have revealed the presence, within the soil profile, of what appear to be perithecia containing dark-coloured muriform spores. These perithecia are apparent in profiles from Meall-an-t-Slughain, Aberdeenshire (at heights of 2,050 ft. and 2,300 ft.), Ben Rinnes, Banffshire (2,500 ft.), and Morven Aberdeenshire (2,025 ft., 2,800 ft. (two) and 2,850 ft.). The profiles are developed on parent materials derived from both granite (Meall-an-t-Slughain and Ben Rinnes profiles) and basic igneous rocks (Morven profiles). Peat cover is either absent or amounts to no more than 2–3 in., except on one of the 2,800 ft. profiles on Morven, which has a peat cover 14 in. thick, the base of the peat being pollen dated within the Sub-Boreal period.

In many Ascomyceteae the whole ascus undergoes digestion at maturity, thus setting free the ascospores[2], and it is likely that this has occurred in the perithecium shown in Fig. 1. The size of the ascospores (Fig. 2) is rather variable, and though the majority are of the order of 40μ × 20μ some are considerably larger, up to 60μ in length. The size of the perithecia is also rather variable; the majority are approximately 160μ in diameter, but one from the 2,800 ft. Morven profile without peat cover was approximately 270μ in diameter. Some of the perithecia exhibit well-developed ostioles (Fig. 1).

Dr. T. D. V. Swinscow of the British Lichen Society and Mr. P. James of the Department of Botany, British Museum (Natural History), have confirmed (personal communication) our opinion that the structures described are the fungal components of a pyrenocarpic lichen, probably a species of *Polyblastia*[3], although it is impossible to recognize algal tissue in the soil sections.

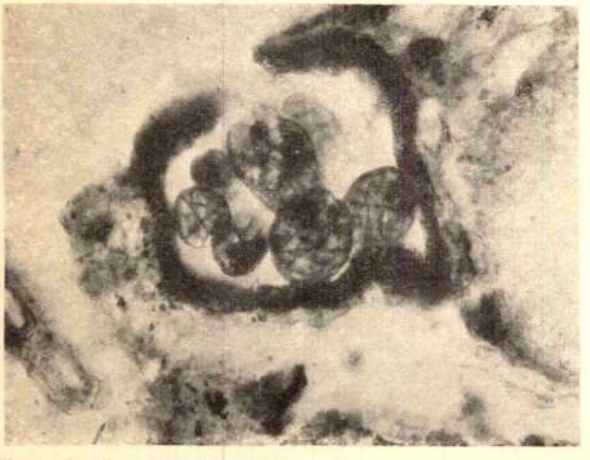


Fig. 1. Perithecium containing ascospores in thin soil section (× 270)

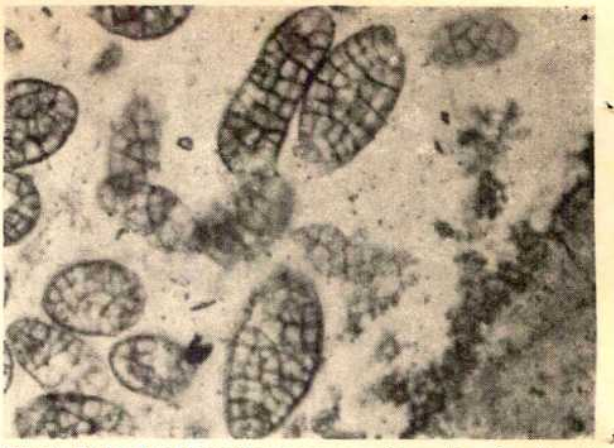


Fig. 2. Dark-coloured muriform ascospores with portion of perithecial wall in thin soil section (× 375)

Within the soil profile, the presence of either perithecia or ascospores has been noted to depths of 9 in. below the mineral surface, though in the case of the 2,800 ft. Morven profile not covered by peat they are present to a depth of 19 in. The Morven profile with peat cover has a thin iron pan at 8–10 in. below the mineral surface, and above this ascospores have been found in association with a pollen assemblage of Atlantic age.

This appears to be the first record of fungal components of a lichen, in a semi-fossil state.

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Nitrogen Fixation by Lichens of Arid Soil Crusts

The nitrogen budgets for natural ecological systems are poorly understood, and the contribution of particular microflora in these associations has been only tentatively assessed[1–3]. This applies especially to arid zones where soils sometimes have a surface crust of lichens, some blue-green algae, and other cryptogams covering as much as 30 per cent of the total soil area. It is of considerable interest therefore to determine to what extent these lichens fix atmospheric nitrogen, since they might contribute appreciably to the maintenance of a favourable nitrogen balance in these areas.

About forty species of lichens are known to occur in the southern central arid zone in Australia, and twelve of the commonest were exposed to a gas mixture containing nitrogen enriched with nitrogen-15. Thus each lichen was incubated in a Warburg flask for 6 days at 21° C under artificial light, using the following gas mixture (in atmospheres): oxygen 0·2, nitrogen 0·2 (enriched with 35 atom per cent excess nitrogen-15), and helium 0·6. After this

period the contents of each flask were digested in acid, and converted to ammonia by distillation in a Markham apparatus and then to nitrogen gas which was analysed in an A.E.I. MS_2 mass spectrometer. Nine lichens, including *Parmelia adhaerens* Nyl, apud Cromb., *P. australiensis* Cromb., *P. semiviridis* (F.v.M. ex Nyl.) P. Bibby, *Caloplaca citrina* (Hoffm.) T. Fries, *Caloplaca* T. Fries sp., *Dermatocarpon hepaticum* (Lam.) Th. Fr., *Diploschistes scruposus* (Schreb.) Norm., and *Lecidea decipiens* Ach., with one unidentified species, showed no significant fixation. There was a significant incorporation of nitrogen gas, however, into *Collema coccophorus* Tuck. (0·97 and 0·69 atom per cent excess in duplicate samples), whereas *Lecidea crystalifera* Tayl. (0·022 atom per cent excess) and *Parmelia conspersa* (Ehrh.) Ach. (0·0073 atom per cent excess) had a marginal fixation only.

Collema coccophorus has a blue-green algal symbiont (*Nostoc* sp.). The lichen is minute but very widely distributed in the soil lichen crust. Shields, Mitchell and Drouet[4] have suggested that this species might fix nitrogen in the arid soil-crust in California. *Lecidea crystalifera*, which is also widespread in this area, has a green algal symbiont (*Protococcus*), hence the fixation associated with this lichen is of interest and will be investigated further. The lichen has a small squamulose thallus with long rhizoids penetrating up to 1 cm into the soil. *Parmelia adhaerens* similarly has a green algal symbiont (*Protococcus*), and occurs on rocks where it is fairly widespread.

This preliminary work suggests that the lichen crust may contribute appreciably to the nitrogen balance in Australian arid soils.

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Measurement and Control of the Rate of Carbon Dioxide Assimilation by Glasshouse Crops

In recent experiments on the effect of environment on glasshouse crops, the net assimilation rate[1] and other indices of growth are found by measuring the dry weight and leaf area of plants sampled from the crop at intervals of the order of 10 days. During this time the intensity of the solar radiation reaching the plants fluctuates over the whole range between zero and the maximum value, so that a series of different combinations of light intensity and plant temperature occurs even if the air temperature and content of carbon dioxide are successfully maintained constant. Such experiments are thus difficult to interpret, especially as the rate of uptake of carbon dioxide by individual leaves is not related linearly to light intensity at all levels of temperature and carbon dioxide concentration[2], and even when light and all other environmental factors are held constant the rate may vary with time of day[3].

Interpretation is easier if the rate of assimilation of carbon dioxide can be measured during an interval of the order of 10 min rather than 10 days. In apparatus designed for this purpose, air is made to pass at a known rate through an enclosure containing a leaf[2,4] or a whole plant[4] and an infra-red gas analyser is used to measure the concentrations of carbon dioxide in the incoming and outgoing air. With difficulty, it might be possible to make a glasshouse sufficiently airtight for this method to be used to measure the net rate of assimilation of carbon dioxide by the plants contained in it.

An alternative approach is to make no attempt to seal the glasshouse or to make air flow through it at a constant rate. Instead, carbon dioxide is injected from a weighed cylinder at a rate varied to maintain a constant concentration, $[CO_2]_1$, in the glasshouse air, which is stirred by fans to distribute the gas uniformly. In the course of a time interval, Δt, the loss in weight, Δw_c, of the cylinder of carbon dioxide represents the net amount supplied to the plants and soil plus that lost by ventilation. To estimate the loss by ventilation, nitrous oxide, which is absent from the outside air and is not absorbed or produced by plants or soil, is injected into the glasshouse air from a weighed cylinder at a rate varied to maintain a concentration:

$$[N_2O]_1 = b([CO_2]_1 - [CO_2]_0) \qquad (1)$$

where b is a constant and $[CO_2]_0$ is the concentration of carbon dioxide in the air outside the glasshouse. The density, ρ, of nitrous oxide happens to equal that of carbon dioxide, although the technique does not depend on this being so. If Δw_n is the loss in weight of the cylinder of nitrous oxide during the time interval Δt, the rate of carbon dioxide uptake by the plants is:

$$C = S + \frac{1}{\Delta t}\left\{\Delta w_c - \frac{\Delta w_n}{b} + v\rho\left(\frac{\Delta[N_2O]_1}{b} - \Delta[CO_2]_1\right)\right\} \qquad (2)$$

where S is the rate of carbon dioxide output from the soil and v is the volume of the glasshouse. $\Delta[N_2O]_1$ and $\Delta[CO_2]_1$ represent any small changes in concentration between the beginning and end of the time interval due to imperfections in the control system.

In preliminary experiments, infra-red gas analysers have been used to measure $[CO_2]$ and $[N_2O]$, as the two gases have well-separated infra-red absorption bands. By using the signals from these analysers to give proportional control of the concentrations of the gases in the air of a small ($v \approx 5 \times 10^7$ cm^3) glasshouse, the fluctuations in the control system have been small enough for $\Delta[N_2O]_1$ and $\Delta[CO_2]_1$ to be negligible when Δt is of the order of 10–100 min, and when $[CO_2]_1$ is about 1,000 vol. per million, which is near the level required to give maximum uptake by leaves in bright light[2]. Under most weather conditions, changes in $[CO_2]_0$ also remain negligible for long periods and it is only necessary to confirm this by measurements at the beginning and end of each time interval. It is then possible to make $b = 1$ in equation (1) and equation (2) can be simplified to:

$$C = S + \frac{\Delta w_c - \Delta w_n}{\Delta t} \qquad (3)$$

For ordinary agricultural soils, S is small and can readily be measured[5]. Although it depends on temperature, it changes only slowly because of the large thermal inertia of the soil. It can be minimized, for experimental purposes, by growing the plants in sand, when the only source of carbon dioxide will be the roots and their associated micro-organisms.

Equations (2) and (3) thus provide a basis for measuring the rate of assimilation of carbon dioxide by all the plants in a glasshouse over an interval of time so short that the intensity of the solar radiation and the other components of the environment remain nearly constant. So also will the relevant physical properties of the plants, such as the leaf area index, the stomatal resistance and the diffusion pressure deficit in the tissues, and the chemical properties, such as the concentration of assimilates accumulated in the leaves.

By conducting a long series of these brief experiments at various intensities of solar radiation and at various levels of the environmental factors influencing the uptake of carbon dioxide, it would be possible to find the relation between this uptake and the factors on which it depends.

However, the complexity of this problem is such that, although the results might be of great botanical interest, solutions of practical value to horticulture may emerge only slowly, albeit faster than is possible using only the present techniques of growth analysis. The risk will remain that before the problem has been solved for one genotype, the plant breeders will have offered the horticulturists something new.

But a better solution is now in sight. If $C - S$, the net rate of uptake of carbon dioxide by the plants and soil, can be measured over a period of a few minutes, it is possible to feed back this information to a control system which then adjusts the environment until it produces the maximum possible value of $C - S$ attainable at the prevailing intensity of solar radiation. As S changes only very slowly, it is not necessary to measure it for the present purpose. At first, the control system could probably be designed to adjust the air temperature and $[CO_2]_1$, and in order to comply with equation (1) it would also be necessary to adjust $[N_2O]_1$.

The method could be used as an experimental tool to find the combinations of solar radiation, air temperature and concentration of carbon dioxide which produce maximum assimilation of carbon dioxide in various circumstances; but its most interesting potential use is in practical horticulture, where it could simply be used to ensure that during the hours of daylight a crop was at all times assimilating carbon dioxide at the maximum possible rate. This appears to be a promising basis for obtaining maximum crop production, provided the best levels of certain other factors, such as night temperature, are known from the results of orthodox experiments.

It is likely that the system would best be operated by a simple form of digital computer, which would call alternately for an adjustment of the temperature or $[CO_2]_1$ and $[N_2O]_1$, and for a measurement of $C - S$, with sufficient delay between the operations to allow for steady conditions to be reached.

The apparatus at present available is too large and expensive for use in practical horticulture, but computers are likely to become very much more compact[6] and a relatively cheap and simple device for the continuous measurement of $[CO_2]$ is at present being developed[7].

The control systems for the preliminary experiments were designed and made by G. E. Bowman and D. A. Browne, and the work was stimulated by discussions with L. G. Morris.

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Selective Herbicidal Activity of Iodides

Investigations carried out here with the selective herbicide ioxynil (3,5-diiodo-4-hydroxybenzonitrile)[1] have shown its mode of action to be complex. The compound inhibits the Hill reaction in isolated chloroplasts, strongly uncouples oxidative phosphorylation, inhibits photosynthetic phosphorylation and when exposed to ultraviolet light there is evidence that free radicals are produced with the loss of iodine from the molecule[2,3]. In further investigations of the breakdown of ioxynil in soil and plant we have found that hydrolysis to 3,5-diiodo-4-hydroxybenzoic acid occurs with the amide as an intermediate product. The liberation of iodine in these systems has also been demonstrated.

In a separate investigation, solutions of the sodium salt of 3,5-diiodo-4-hydroxybenzoic acid applied to the soil caused little damage to most plant species but were very damaging to dwarf bean plants (*Phaseolus vulgaris*, var. 'Canadian Wonder'). As the acid became degraded in this plant and also in soil with liberation of iodide, experiments with iodide solutions were carried out. These showed that dwarf bean is very susceptible to iodide toxicity; foliar applications of sodium iodide (0·2 per cent) caused damage to the leaf tissues (see also ref. 4) and when the chemical was applied to the soil at the rate of 15 mg/3-in. pot (about 30 lb./acre) the seedlings were destroyed. Many other species of plants, however, were unaffected by iodide at this rate of soil treatment, thus indicating the possibility of selective herbicidal action. Experiments with some 50 weed and crop plant species from 20 families have confirmed this. Among weeds, annual nettle (*Urtica urens*) and fat hen (*Chenopodium album*) were found to be very susceptible to iodide, as was red and white clover (*Trifolium* spp.) and all other legumes tested. We have also achieved eradication in the field of well-established stinging nettle (*Urtica dioica*) with sodium iodide applied to the soil at rates of the order of 0·5 oz./yard^2 in one gallon of water. *Brassica* plants have been found to be very resistant to iodide, and a striking control of annual nettle growing in a cabbage crop can be achieved by application of sodium iodide to the soil at 20 lb./acre. We have also found that, while clover is susceptible, most species of grasses are tolerant, thus providing a simple means of removing clover from lawns. Such uses of iodides on the field scale, however, are likely to be uneconomic.

While the toxicity of iodides to certain plants is well known[5,6], the specific herbicidal selectivity here reported is a new finding. This might arise from differential uptake, though the results of our preliminary experiments carried out with two 'susceptible' and two 'resistant' species do not support this. In considering the chemical basis for this selectivity, it would appear that the fate of the iodide ion in susceptible and resistant species is different. In the former case, iodide could perhaps be oxidized more readily to iodine by peroxidase enzymes in a manner analogous to that thought to occur in the animal[7]. The iodine thus liberated within the tissues might then lead to the iodination of certain plant constituents. Reaction with tyrosine[8] might effectively deplete the level of this essential amino-acid in the amino-acid pool, and this, together with the simultaneous production of iodo derivatives, could have drastic effects on protein metabolism.

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Correlation between Induced Extension-growth and Regeneration in Horseradish

Previous work has shown that initiation of bud and root primordia on roots of intact horseradish plants is influenced by the presence of a rosette of leaves, and that regeneration in isolated root segments is controlled by a growth factor produced during the extension of the buds first-formed following excision[1].

During an investigation of the effects of gibberellic acid on regenerating horseradish, further light was thrown

Table 1. Mean Number of Primordia formed per Lateral Root Scar

Month during which segments grown	Time from last spray treatment to excision of root segment (weeks)	Sprayed 4 times with water*	Sprayed 4 times with gibberellic acid (Na salt) 1 mg/ml. in water*
January	16	(2) 1·46†	(6) 1·59 ± 0·15
June	6	(5) 1·90 ± 0·26	(5) 1·01 ± 0·11
April	2	(4) 1·10 ± 0·26	(5) 0·09 ± 0·04

* Figures in parenthesis indicate the number of replicates.
† The standard error is not given where insufficient material was available.

on the nature of the correlation between stem extension-growth and regeneration. The main stem has been induced to grow in clonal horseradish plants treated with fortnightly sprays of an aqueous solution of the 75 per cent sodium salt of gibberellic acid. Plants were sprayed four times with 5 ml. of the solution, which contains 1 mg of the salt. Control plants were sprayed with the same quantity of distilled water. All seven treated plants elongated, whereas four out of five untreated plants remained as rosettes. Root segments 2 cm long and 2–5 mm thick were excised from some of the plants and were grown in the dark at 25° C on moist sand in Petri dishes. Segments from treated plants showed different regeneration patterns from the control segments; these results are expressed in Table 1 as mean number of primordia formed per lateral root scar. The regeneration pattern appears to be influenced by the length of time between the completion of treatment and the excision of the segments. It is also possible that these differences in root behaviour owe their origin to the use of clonal material of different age or to the different time of year in which experiments were carried out; seasonality in regenerating horseradish roots has been reported[2]; an age effect is suspected[3].

Gibberellic acid inhibits root and bud formation when applied directly to *Begonia* leaf disks[4], but it inhibits root initiation while promoting root extension-growth in pea and bean stem-cuttings[5]. It has also been reported to inhibit shoot formation in tobacco callus cultures at concentrations which stimulate callus growth[6]. The suggestion has also been made that gibberellic acid enhances the enzymatic destruction of indolyl-3-acetic acid[7]. In another series of experiments, unpublished results indicate that low concentrations (1 p.p.m.) of IAA can diminish the inhibition of root regeneration in treated horseradish plants. Table 1 indicates an improvement in regeneration ability in treated plants, the longer the interval between treatment and root excision. This would seem to indicate a possible recovery of a regeneration-controlling system from the inhibitory effect of gibberellic acid. As a working hypothesis, it could be suggested that gibberellic acid pre-treatment in causing extension-growth induces the formation by the elongating stem of either an antagonist to endogenous auxin, or, as seems more probable, the development of an auxin–oxidase mechanism in the roots. It is hoped that investigations in progress on indolyl-3-acetic acid oxidase mechanisms in horseradish will provide additional data which will explain these results.

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Uptake of Small Particles by *Moniliformis dubius* (Acanthocephala)

The adults of *Moniliformis dubius* Meyer live as parasites in the small intestine of rats. Because they do not possess an alimentary canal, acanthocephalans, like cestodes, must obtain all their food through the surface of

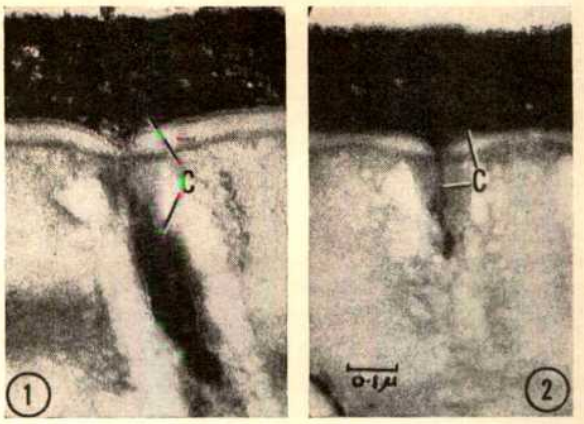


Fig. 1. Transverse section of cuticle of *M. dubius* showing an aggregation of particles of carbon and thorium dioxide within a vesicle below the cuticle (note layer of carbon and thorium dioxide (*C*) adhering to the outer surface of the cuticle)

Fig. 2. Transverse section of cuticle of *M. dubius* showing method of entry of the carbon and thorium dioxide particles (*C*)

their bodies. It is known that *M. dubius* is able to absorb directly through its surface a number of simple sugars[1,2] and some amino-acids, the latter sometimes against a concentration gradient[3,4].

Recent experiments carried out in this department have shown that *M. dubius* can also take up very small particles of solid matter in a manner that resembles pinocytosis. Figs. 1 and 2 are electronmicrographs of transverse sections of the cuticle of a male of *M. dubius* showing the uptake of a mass of small particles of carbon and thorium dioxide. The worm (aged 7 weeks) after its removal from the rat was immediately placed in a solution of 0·8 per cent sodium chloride containing carbon particles of diameter about 250 Å aggregating to 500–800 Å and thorium dioxide particles of diameter 70–250 Å. The temperature of the solution was maintained at 37° C. After 3 h the worm was removed, washed in warm saline, cut up into small pieces, fixed in 2 per cent osmium fixative and embedded in methacrylate. Sections were cut at 600 Å.

Confirmatory results showing that the uptake of small carbon particles occurs *in vivo* were obtained from electronmicrographs of the cuticle of worms removed from rats which had been dosed orally with saline solution containing carbon particles in the parasite.

We thank Dr. J. R. Casley-Smith, of the Electron-microscope Unit of the Departments of Botany, Microbiology and Zoology, for his assistance, and Prof. W. P. Rogers for his advice.

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Ubiquity of Melanin and the Effect of Phenylthiourea

Black or brown pigments are widespread among animals. Although they have been widely assumed to be melanin, they have not always proved to be so[1]. Their ubiquity is manifest in another way, by their widespread occurrence in a particular organism.

These pigments have been interpreted as protective, either by assisting concealment[2] or by acting as a screen against nocuous radiation[3–5], and in some cases they have been considered as energy transducers[6]. Such

adaptive significance is less evident in the case of the abundant black pigment that may occur in the viscera, and perhaps for this reason this pigment has been relatively neglected. Though an excretory significance has been proposed for melanin[6], some reluctance to accept the idea has been expressed[1]. Our investigations show that considerable quantities are indeed excreted, sometimes by surprising routes.

The ubiquity of such pigment is striking among amphibians. Some indication of this has already been reported in the case of *Xenopus*[7], but the account is incomplete, and further, the widespread distribution is more evident in the larvae of *Ambystoma*, *Rana pipiens* and *Rana temporaria*. In addition to the pigment that is obvious in the dermis, epidermis and eyes, it is present in the peritoneum, heart, walls of the major blood vessels, gut, liver, lungs, pronephros, mesonephros, spleen, pancreas, gills, neural tube and brain, membranous labyrinth, olfactory epithelium, the myotomes and in cartilage corpuscles.

The pigment in these sites is melanin, and is not to be confused with ommochromes or lipofuscins, in so far as it is insoluble in a wide range of solvents, including strong formic and sulphuric acids; it is readily oxidized and bleached, but not reduced; it reduces ammoniacal silver nitrate and appears dark green after staining with Nile blue under the conditions specified by Lillie[8].

Pigment in the epidermis, myotomes, gut and kidneys should not be confused with that contained in processes of dermal or peritoneal melanophores which insinuate themselves among the cells of these structures. Epidermal pigment in *Xenopus* is very scanty, being confined to the oral sucker and the tip of the tail, between stages 25 and 41 (Nieuwkoop and Faber); but it is abundant in the other species investigated, thus substantiating earlier observations[9–11]. Moreover, in *Rana pipiens* appearances strongly suggest that melanin granules are transferred from the processes of dermal melanophores into the epidermal cells among which they are insinuated (Fig. 1).

In the central nervous system, pigment is present not only in the meninges as previously recorded[7], but more significantly, we find that it occurs in the cerebral ventricles and neural canal as large clumps which appear to arise by compaction of granules cast off by the walls (Figs. 2 and 3).

The pigment in the myotomes appears as fine or coarse granules in the striated muscle fibres as well as in the myoblasts and in the surrounding connective tissue.

The effect of phenylthiourea is very significant. Its suppressive action on the melanin pigmentation of amphibians has been reported several times[7,12–16]. It is usually assumed to act directly on melanophores by inhibiting formation of their pigment and therefore its action in causing loss of colour has been held to show the existence of a considerable turnover of melanin[13]. This may appear surprising in a pigment that is chemically so notoriously inert. In *Xenopus*, it has been urged that there is no turnover[7,16], the pigment merely accumulating during normal development, so that the overt effect of phenylthiourea disappears by about stage 46. Its effect persists only in regenerating tissues that are still accumulating their complement of melanin and in which the melanophores consequently retain an active enzyme system.



Fig. 1. *Rana pipiens*. Transfer of melanin from a melanophore to an epidermal cell. From a larva 19 days after hatching. Two dark processes of a melanophore insinuated among epidermal cells appear on right. The upper process makes intimate contact with an epidermal cell into which it is apparently transferring two granules of pigment. Fixed 10 per cent formol; Mallory's triple stain. Scale, 5μ

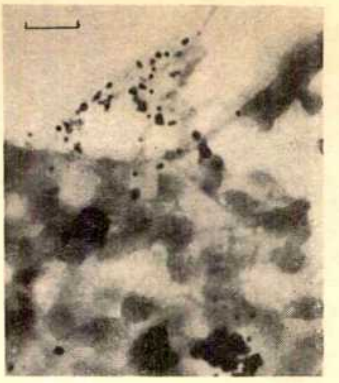


Fig. 2. *Xenopus laevis*. Discharge of melanin into the ventricle of the diencephalon. The lower portion of photograph shows melanin in the lateral wall of the diencephalon, from which secretion containing pigment may be seen passing off. Fixed 10 per cent formol. Stained by Masson's argentaffin reaction, counterstained in Mallory's triple stain. Scale, 8μ

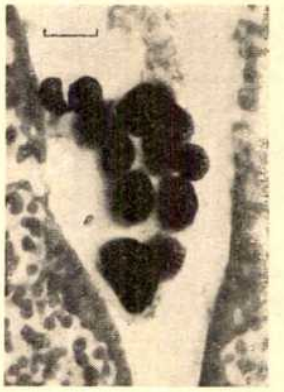


Fig. 3. *Xenopus laevis*. Compacted melanin in ventricle of medulla. Fixed 10 per cent formol; Mallory's triple stain. Scale, 18μ

The situation is not so easily explained. Our experiments on *Xenopus* confirm that the marked effect of phenylthiourea on pigmentation is relatively short-lived, beginning at room temperature at about stage 25 and persisting until about stage 41. Thereafter it progressively declines, although its effect on pigmentation can still be detected in the meninges and dermis until about stage 49. Nevertheless, melanin slowly accumulates up to the time of metamorphosis (the latest stage we have investigated) in most of the areas already listed here, with the notable exception of the gut and the brain.

Most significantly, however, from the very earliest stages the pigment in some situations is unaffected by phenylthiourea. This is not wholly due to the fact that some pigment is carried over from the egg, for the pigment that appears later in certain situations remains unaffected, or is even increased in amount by the drug. Thus, pigmentation of the eye, dermis, peritoneum, meninges and olfactory sacs is diminished by it, whereas that of the brain, gut, liver, myotomes and membranous labyrinth is unaffected or increased. The latter 'aberrant' effect appears very clearly in the two species of *Rana*; more-

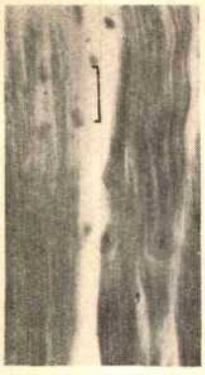


Fig. 4



Fig. 5

Fig. 4. *Rana pipiens*. Striated muscle and connective tissue of myotomes in a larva 19 days after hatching. Fixed 10 per cent formol; Mallory's triple stain. Scale, 15μ

Fig. 5. *Rana pipiens*. Melanin in striated muscle and connective tissue of myotomes as it appears in a larva 19 days after hatching, which has been treated from the time of hatching with 0·01 per cent phenylthiourea. Fixed 10 per cent formol; Mallory's triple stain. Scale, 15μ

over, here it also appears in the kidneys, particularly in the pronephros. In *Rana pipiens*, the amount of pigment appearing in the muscle fibres and surrounding connective tissue of the myotomes is strikingly increased after phenylthiourea (Figs. 4 and 5).

In the instance of the gut, central nervous system and kidney tubules, the histological picture presented is of granules of pigment being cast into the lumen from the free border of the surrounding epithelium. The same is seen in animals that have been starved, or fed on milk powder, so that in the case of the gut, absorption is an unlikely interpretation. Pigment appears to accumulate in the myotomes and epidermis, though in the latter case some will be lost by desquamation.

Much melanin is therefore being excreted and the process occurs particularly in the areas apparently unaffected by phenylthiourea or in those where pigmentation may be increased by it. The areas where melanin is diminished after phenylthiourea are those where pigment is being formed. During development active excretion is roughly contemporaneous with rapid pigment formation so that rapid turnover clearly occurs at some stages and the overall pigmentation will be determined by the amount of pigment excreted as well as by the amount formed and accumulated.

Phenylthiourea, therefore, not only affects formation but exerts other effects on melanin pigmentation. Its precise mode of action is still a matter of argument, so that considerable circumspection is necessary in interpreting its effects.

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A Protozoan Parasite of *Molgula manhattensis* (De Kay) (Urochordata)

During the course of an investigation into oogenesis in the ascidian *Molgula manhattensis* a protozoan was discovered parasitizing a few of the nearly mature and fully mature eggs of one gonad. The ascidian which contained the infected gonad was one of several of the same species taken from Plymouth Sound in June 1963. Although there was insufficient material to classify the parasite accurately, the similarity of the observed stages to certain stages in the life-cycles of the Haplosporidia suggests that it is allied to this group[1,2].

The infected gonad had been excised, fixed and sectioned before the parasite was discovered, and as the remaining parts of the specimen had been discarded it was not possible to discover whether any other organs were infected. Fixation was carried out in Lillie's acetic–alcohol formalin[3]. It was found after sectioning normal gonads from other specimens that the fixative caused some distortion of the egg contents; it is therefore possible that the structure of the parasite may have been similarly affected.

The Feulgen reaction followed by staining in fast green gave the clearest picture of the parasite's structure; therefore the following description has been made from sections subjected to this procedure.

The stages of the parasite comprised plasmodia, cysts which contained uninucleate spores, or spores and plasmodia, and structures intermediate in development between plasmodia and cysts.

Each plasmodium was spherical and surrounded by a thin unbroken membrane. The diameter varied from 5 to 20μ. The cytoplasm contained an eccentrically sited vacuole, and from 4 to approximately 64 nuclei, all of which were oval and were coloured evenly by the Feulgen reaction. Their number increased with the diameter of the plasmodia (Fig. 1).

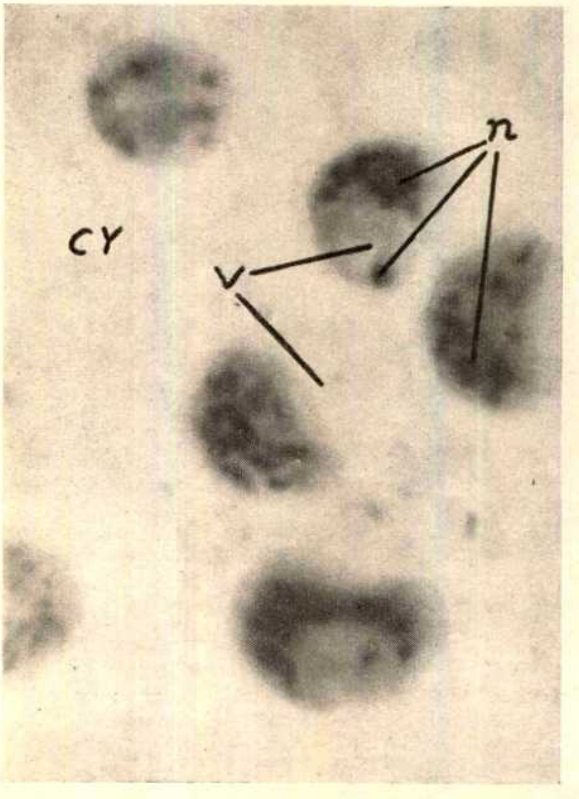


Fig. 1. Plasmodia in cytoplasm (*cy*) of mature egg. The nuclei (*n*) appear as dark oval bodies, the plasmodial vacuole (*v*) as a diffuse unstained area (× 3,400)

Stages greater than 20μ in diameter were encysted. Each cyst wall was formed from the thickened plasmodial membrane which had become irregular in outline. The greater portion of the cytoplasm had disappeared, and each nucleus together with a small portion of the remaining cytoplasm had developed directly into a spore (Fig. 2). The number of spores per cyst was estimated as being between 150 and 200. Each spore was oval and measured 3μ × 1·5μ. One end was coloured by the Feulgen reaction, the other was stained faintly by the fast green. No other structural details could be observed.

It was considered reasonable to assume that the Feulgen positive region of each spore contained the nucleus. Presumably a thin membrane surrounded each spore, but was so delicate that it was not demonstrable by the procedure used.

Some of the larger cysts contained a few small plasmodia as well as spores (Fig. 2), and a few of these cysts had ruptured membranes. The plasmodia within the cysts were spherical, 5–8μ in diameter and contained 4–8 nuclei. A plasmodial membrane was not observed.

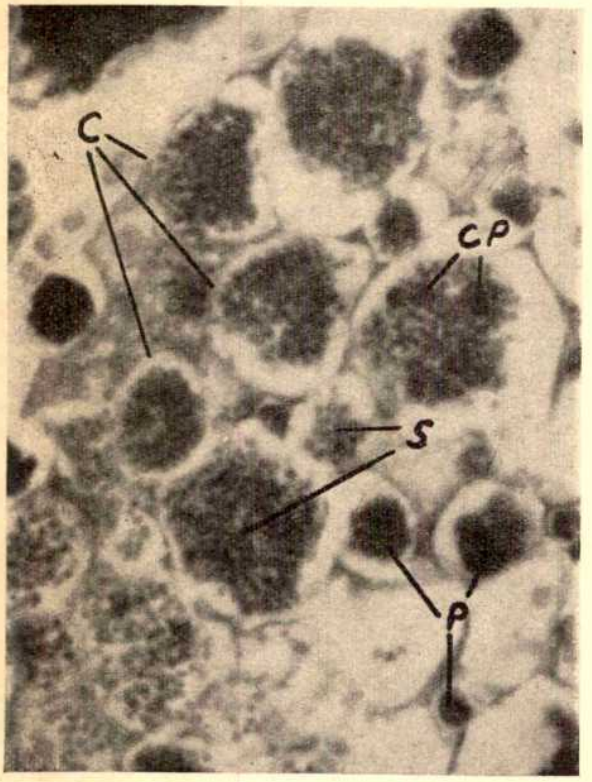


Fig. 2. Portion of heavily infected egg containing plasmodia (*p*) and cysts (*c*). Some of the cysts contain plasmodia (*cp*) as well as spores (*s*) (× 1,300)

The degree of infection varied considerably. A few infected eggs contained only plasmodia scattered between the yolk platelets, whereas the majority contained cysts, plasmodia and intermediate stages. These eggs were distended; the yolk platelets had either degenerated or perhaps been consumed by the parasite as a source of nourishment.

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Growth of Processes from Single Isolated Dorsal Root Ganglion Cells of Young Rats

Cavanagh[1], using the technique described by Nakai[2], has filmed the growth of isolated cells from chick-embryo dorsal root ganglia. By the application of a similar technique, growth of new processes has been shown in isolated cells from dorsal root ganglia of new-born rats up to 7 days old. The Wag-strain rats were decapitated, the spinal cord separated, and the ganglia teased out with needles under microscopic vision. The ganglia were torn open to release a large number of cells into the culture Medium 199 (ref. 3) in well slides. The slides were covered with cover-slips, sealed with petroleum jelly, and incubated at 37° C. They were examined and photographed under phase-contrast microscopy immediately on incubation (20 preparations), and then three times a week; in this series, cultures from 15 young rats, and 11 adult rats, were observed between 7 and 14 days after the beginning of incubation. No processes were visible in the freshly incubated cells, nor were they in the cells from the adult ganglia (Fig. 1). However, in 13 out of 15 preparations

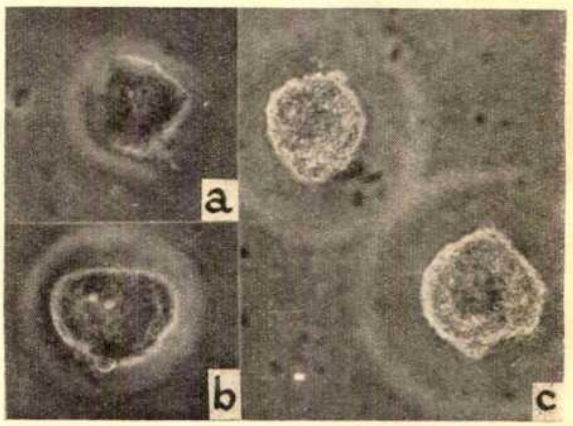


Fig. 1. Isolated dorsal root ganglion cells of rat. *a*, *b*, Freshly isolated from 4-day-old rat; *c*, incubated for 14 days from a 3-month-old rat. In both figures the cells are photographed under phase-contrast microscopy and are in Medium 199. The calibration is given by the horizontal bar in Fig. 2 (20μ)

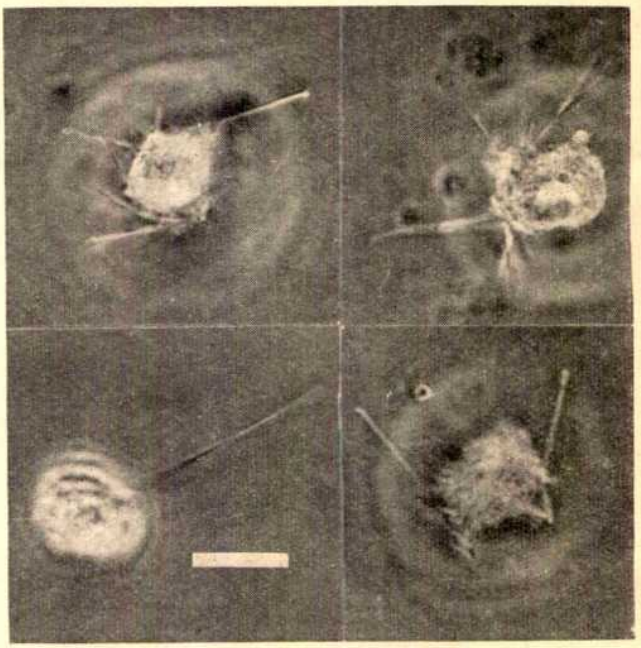


Fig. 2. Similar cells, isolated from 4-day-old rats and incubated for 14 days. Note the processes, with their cones

of cells from young rats, straight or angular processes of length equivalent to from one-quarter to two times the cell body diameter could be seen in about a quarter to a third of the cells in each preparation (Fig. 2). Growth cones could sometimes be seen either at the tips of the processes or where they changed direction.

I thank Dr. J. B. Cavanagh for his advice.

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Metabolism of Gammexane in Flies, Ticks and Locusts

THE insecticide gammexane is known to be converted into water-soluble compounds in a variety of invertebrates[1], but the nature of these metabolites is not known. Some indication of their structure has been given by Bradbury and Standen[2], who obtained a mixture of dichlorothiophenols after the alkaline hydrolysis of excreta from gammexane poisoned flies. It is also known that homogenates of flies require glutathione if enzymatic conversion of gammexane to water-soluble derivatives is to occur[2]. These results have suggested that a compound like *S*-(pentachloro*cyclo*hexyl)glutathione might be a metabolite, and this hypothesis is supported by the presence of highly active enzymes in a variety of insects which catalyse the condensation of other chloro-compounds with glutathione[3].

We have attempted to identify such a metabolite in ticks, houseflies or locusts dosed with gammexane or in enzyme extracts fortified with glutathione, but have found the major metabolic product in each case to be an aromatized molecule which appears identical with *S*-(2,4-dichlorophenyl)glutathione.

The synthesis of this compound was achieved by a variation of the general method of preparing *S*-aryl cysteines[4] in which the cuprous mercaptide of glutathione was treated with diazotized 2,4-dichloraniline. The product was isolated by isoelectric precipitation at *p*H 3 and crystallized from ethanol–water. The *S*-(2,4-dichlorophenyl)glutathione had m.p. 215° C decomp. and $[\alpha]_D^{20} = 0 \pm 5$ ($c = 1$ in 0·1 N sodium hydroxide). Found: C, 42·5; H, 4·6; N, 8·4; Cl, 16·0; S, 7·0 per cent. $C_{16}H_{19}N_3O_6Cl_2S$ requires: C, 42·5; H, 4·2; N, 9·3; Cl, 15·7; S, 7·1 per cent. This compound was also isolated in good yield from unsuccessful attempts to prepare *S*-(pentachloro*cyclo*hexyl)glutathione from gammexane and glutathione in liquid ammonia as described for other glutathione derivatives[5]. The *S*-(2,4-dichlorophenyl)glutathione had an absorption spectrum similar to that of the analogous cysteine derivative with λ_{max} 258 mμ, ε_{max} 6,600 in 0·1 N sodium hydroxide and λ_{max} 257 mμ, ε_{max} 5,600 in 0·1 N hydrochloric acid. After hydrolysis at 100° C with 2 N hydrochloric acid for 4 h, 2,4-dichlorophenylcysteine was isolated identical with a sample prepared according to Parke[4].

Gas chromatographic analyses of cattle ticks (*Boophilus decoloratus*) kept at 35° C overnight showed that only 77 per cent (64–96 per cent) of a 10 μg topical dose of gammexane was recoverable unchanged. In similar experiments with ^{14}C-gammexane 28 per cent (6–30 per cent) of the dose was found in the water-soluble fraction after partitioning the homogenized ticks between water and xylene.

Enzymatic experiments were carried out with homogenates of cattle ticks, houseflies, or locust fat body containing 5 mM glutathione at *p*H values between 5 and 8 and with sufficient ^{14}C-gammexane to give a concentration of 5 mM. Water-soluble radioactive products were only formed when both glutathione and enzyme were present. When the reaction mixtures were examined chromatographically (Table 1) or by ionophoresis at *p*H 2, 3, 5, 7 and 8, a major part of the metabolized material was identical in behaviour with reference 2,4-dichlorophenylglutathione.

Table 1. R_F VALUES OF POSSIBLE GAMMEXANE METABOLITES

	S-(2,4-dichlorophenyl)-cysteine	*S*-(2,4-dichlorophenyl)-glutathione
Collidine–lutidine–H_2O, 1:1:1	0·75	0·50
n-Butanol–acetic acid–H_2O, 4:1:5	0·70	0·60
Pyridine–*n*-butanol–H_2O, 1:1:1	0·80	0·70
n-Propanol–ammonia sg. 88, 7:3	0·80	0·65
2-Butanone–H_2O, 1:1	0·05	0·05

The characteristics of this enzymatic reaction differed from those associated with the same enzyme sources when 2,4-dinitrochlorobenzene or *p*-nitrobenzyl chloride were used as substrates[2]. While the reaction rate with these substrates was constant over several hours, the reaction rate with gammexane fell off to a negligible value in 2–5 min. Moreover, the conversion of gammexane to a water-soluble derivative was not significantly affected by 0·1 mM bromsulphonphthalein, which completely inhibits the enzyme-catalysed reaction between *p*-nitrobenzyl chloride or 2,4-dinitrochlorobenzene and glutathione[3].

The formation of an aromatic derivative from gammexane is in general agreement with Bradbury's observation[1] that 4–6 atoms of chlorine are lost from gammexane when it forms water-soluble metabolites. On the other hand, it is difficult to reconcile the formation of a single major metabolite with Bradbury and Standen's isolation[2] of substantial amounts of 2,5- as well as 2,4-dichlorothiophenols from hydrolysed fly excreta unless the corresponding isomeric *S*-arylglutathiones are identical in all our chromatographic and ionophoretic systems or unless 2,4-dichlorophenylglutathione is an artefact formed from a very labile pentachloro*cyclo*hexylglutathione.

It has been suggested[6], on the basis of a colorimetric estimation procedure, that pentachloro*cyclo*hexene is a major but transient metabolite of gammexane in resistant flies, though this has not been confirmed by isotopic techniques. We have therefore applied the Schechter-Hornstein[7] colorimetric procedure to 2,4-dichlorophenylglutathione to see if it would give a false indication of pentachloro*cyclo*hexene. The metabolite did not interfere significantly in the reaction, but it would be desirable to investigate its probable precursor, pentachloro*cyclo*hexylglutathione, as the 'apparent' pentachloro*cyclo*hexene found by Sternberg and Kearns[6].

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ENTOMOLOGY

Increases in the Incidence of *Atherigona indica infuscata* Emden Diptera (Anthomyiidae) on Sorghum due to Spraying

Central shoot fly, *Atherigona indica infuscata* Emd., is a serious pest of sorghum in the Old World. It is extremely common in East Africa and has been recorded from *Brachiaria brizantha*[1], *Sorghum verticilliflorum*, *Eleusine coracana*, *Pennisetum typhoides*, *Zea mays*[2], as well as cultivated sorghums. The fly is a pest of the young seedlings, and causes severe damage to the growing point. This usually leads to tillering; these tillers may or may not escape subsequent attack. If the former, the only effect is to lengthen the maturation period; if the latter, yield may be seriously affected.

Swaine and Wyatt[3] obtained some control of the pest using weekly applications of DDT/benzene hexachloride dusts in Tanzania. Ingram[4] has reported failure to control the pest at Serere, Uganda, with DDT, and was unable to correlate the degree of infestation with yields. Clinton[5] also found that insecticidal sprays failed to afford control of this Anthomyid in the Sudan. Wheatley[6], working in Kenya, has obtained control with DDT/benzene hexachloride dusts on late plantings.

In subsequent experiments at Serere, we tested three insecticides against a control treatment, namely, DDT (25 per cent ml.) at 1 lb. a.i. per acre, carbaryl (85 per cent sprayable) at 2 lb. a.i. per acre and fenitrothion (50 per cent ml.) at 1 lb. a.i. per acre. Sprays were applied 7 and 14 days from germination to sorghum planted at three dates separated by 4-day intervals. Two varieties were used, 'Serena' (*SB* 68) which shows field resistance to the pest, and 'Combine Kaffir' (*CK* 60) which is susceptible. The two experiments had different error variances and were analysed separately. Counts on random rows within harvest plots, protected by the appropriate sprayed guard rows, were made. Results are shown in Table 1.

Table 1. Mean Number of Shoots killed by *A. indica* at Fourteen Days

Planting date	Control	Carbaryl	Fenitrothion	DDT	Mean
		A. 'Serena'			
1	69	56	55	51	58
2	47	68	38	86	60
3	137	303	145	286	218
Mean	84	143	79	141	112
S.E. treatment mean, ± 27·3.					
		B. 'Combine Kaffir' 60			
1	64	60	34	48	52
2	70	72	66	132	86
3	87	171	127	199	146
Mean	74	101	76	126	94
S.E. treatment mean, ± 15·2.					

In both varieties late planting leads to an increase in the number of plants attacked ($P < 0.001$), but DDT and carbaryl also increase the pest attack compared with the control. Fenitrothion had no effect. The increased attack on the DDT and carbaryl treatments was significant at $P < 0.01$ on *CK* 60 and at $P < 0.05$ on 'Serena'. The effect of late planting has been observed previously, but the insecticidal effect was unexpected. DDT and carbaryl apparently control parasites or predators of *Atherigona* more efficiently than the pest itself, leading to an increase in crop damage.

There was no significant difference in the degree of infestation in the two varieties, although *CK* 60 shows less attack and the difference approaches significance. Analysis of yield data from both varieties showed that there was no significant difference between the insecticidal treatments for either variety. The regression of yield on dead hearts was significant at the 5 per cent level for *CK* 60, but not for 'Serena'. This confirms the apparent field resistance of 'Serena'. The mechanism of varietal resistance is probably a reduction in the level of attack on the secondary tillers of 'Serena'. Secondary tillers of *CK* 60 are heavily attacked, and yields are consequently affected.

Such resistance is probably possessed by many of the indigenous sorghum varieties of East Africa, and explains Ingram's result. Introduced varieties from areas where the pest does not occur are not so resistant.

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MICROBIOLOGY

Comparison of Oxygen Demand Rates and Oxygen Utilization Rates

The rate of oxygen consumption by a bacterial suspension can be measured by observing the change in concentration of oxygen in the air in contact with the culture or dissolved in the suspension. The rate of consumption is known as the oxygen demand rate which Gaden[1] defines as the maximum use of oxygen by a culture in an ideal environment. Oxygen utilization is defined by the author as the terminal use of oxygen in respiration, that is, the formation of water. In this investigation both oxygen demand and oxygen utilization of a culture of *Pseudomonas fluorescens* during respiration only were simultaneously measured.

To act as a hydrogen acceptor in respiration, oxygen must pass from the gaseous state to the dissolved state and then through a number of interfaces and obstacles all of which provide considerable resistance to its passage. Theoretically, then, oxygen utilization may occur at the same rate as oxygen demand, but should lag oxygen demand by a time factor which is dependent on the magnitude of the resistance to oxygen transfer. The purpose of the investigation reported here was to test this hypothesis.

To measure the rate at which water is formed in an environment that contains water it is necessary to use a tagged oxygen atom. The heavy isotope, oxygen-18, was used in this investigation. The method of using oxygen-18 to measure utilization rate, that is, the formation of $H_2{}^{18}O$, has already been described[2].

Manometric determinations of oxygen demand were made simultaneously with the oxygen utilization tests. The apparatus used automatically recorded the oxygen demand of the respiring culture[3].

The results of oxygen utilization and oxygen demand tests for two runs are shown in Fig. 1. It is apparent that oxygen utilization does not proceed at the same rate as oxygen demand, at least for the time period of these tests. It is evident, however, that the rate of utilization increases through the test. This is especially evident in run *B*, where the rate of utilization increases and the oxygen demand rate is constant. In run *C* the oxygen demand rate decreased during the final minutes of the run, and so the increase in oxygen utilization rate is not as

Table 1. Percentage Production of $H_2{}^{18}O$

Run	Sample No.	Rate of $H_2{}^{18}O$ produced / Rate of ^{18}O consumed (per cent)
B	2	22·4
	3	34·8
	4	42·7
C	5	25·1
	6	17·4
	7	44·0

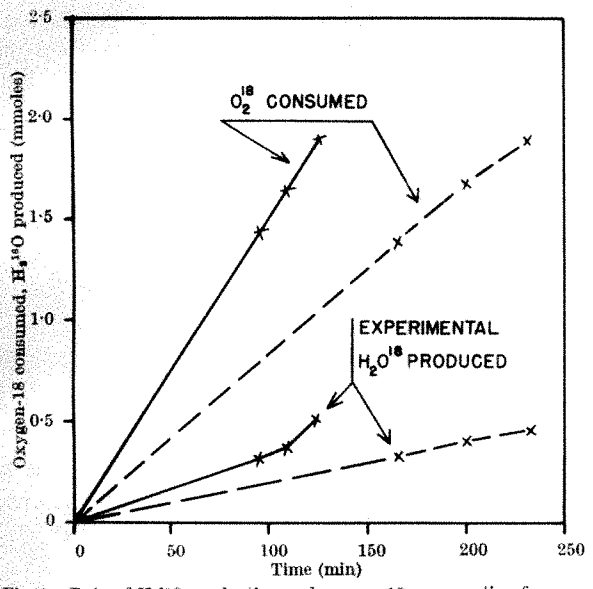


Fig. 1. Rate of $H_2{}^{18}O$ production and oxygen-18 consumption for runs *B* (——) and *C* (— — —)

obvious. However, if the rate of $H_2{}^{18}O$ production is divided by the rate of consumption of oxygen-18 and converted to a percentage as shown in Table 1, it is evident that the rate of utilization is increasing in both the runs. There is no apparent explanation for the reduction in the percentage for sample 6.

This exploratory work indicates that there is a lag between oxygen demand and oxygen utilization. As the bacteria were deprived of oxygen for approximately 2 h before the test was undertaken, it is possible that this lag is merely an indication that oxygen must first be used to replenish stored oxygen, after which it is then utilized as a hydrogen acceptor. Since oxygen utilization is closely associated with product formation, the lag determined here may be similar to the lag in product formation reported by Gaden[4] or Finn[5].

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Enhanced Growth of *Bordetella pertussis* in Dialysis Culture

THE propagation of phase-1 *Bordetella pertussis* for vaccine production has been the subject of considerable experimentation. Some investigations[1–4] dealt with a description of media which do not contain blood, yet few studies were directed to population development or to methods for achieving high-titred cultures. Pollock[5] observed that the growth of the *Pertussis* organism in liquid media was enhanced by the presence of a diphtheroid; and Holt[6] reported the recovery of high concentrations of *B. pertussis* from a solid-liquid biphasic system.

Recently, Gerhardt and Gallup[7] described a dialysis flask useful for the concentrated culture of micro-organisms. In this twin-chambered flask, designed with a dialysis membrane sandwiched between a sterile reservoir of medium and the inoculated culture medium, concentration ratios for some microbial species approached the theoretical limit for the reservoir-culture volume ratio (10 : 1) used. This communication reports experiments designed to test the possibility that the concentration advantages of dialysis culture might be coupled with symbiotic association to achieve highly concentrated populations of *B. pertussis*.

B. pertussis phase-1 (obtained from R. D. Wende, Houston City Health Laboratories, Texas) was seeded into a biphasic culture vessel[8] which consisted of a 250 ml. Erlenmeyer flask containing 48 ml. of Cohen and Wheeler[2] agar medium overlaid with 12·5 ml. of liquid medium. This culture was incubated at 37° C on a reciprocal shaker (Eberbach, Ann Arbor, Michigan) for 24 h, and 1·0 ml. was inoculated into 100 ml. of Cohen and Wheeler medium contained in the culture portion of a twin-chambered dialysis flask (obtained through the courtesy of Prof. P Gerhardt, University of Michigan, Ann Arbor). A cellulose membrane (Visking Co., Chicago) separated the culture portion of the vessel from 1 l. of Cohen and Wheeler medium contained in the reservoir below. The reservoir was inoculated with *Corynebacterium pseudodiphtheriticum* cultivated in a manner similar to that described for *B. pertussis*. In non-symbiotic experiments, either the reservoir of the dialysis flask was not inoculated (dialysis culture) or it was separated from the culture above by a disk of stainless steel (control culture). The flask was mounted on to a rotary shaker (New Brunswick Scientific, New Brunswick, New Jersey) operated at 200 r.p.m. through a 1-in. stroke and was incubated at 37° C for 60 h. Samples were removed periodically from the upper culture chamber for determination of viable cells of *B. pertussis*: appropriate dilutions in 0·1 per cent (w/v) tryptone were spread over prepared plates of Cohen and Wheeler agar medium, and colony counts were made after incubation at 37° C for 48 h.

Typical experiments are shown in Fig. 1. The results indicate not only that dialysis culture was successful for the concentrated culture of *B. pertussis* but also that the provision for dialytic symbiosis resulted in significantly higher cell yields than obtainable in dialysis culture alone. The nature of the symbiotic relationship between *B. pertussis* and *C. pseudodiphtheriticum* is not known. Pollock[9] suggested that growth stimulation in symbiotic culture might be explained by the removal, by diphtheroids, of toxic fatty acids released by *B. pertussis* during growth. However, when the reservoir of the dialysis flask contained only the filtrate from a culture of *C. pseudodiphtheriticum*, the growth of *B. pertussis* was equivalent to that found under conditions of dialytic symbiosis. The results also indicate the survival advantage gained by cells grown in dialysis culture: *B. pertussis*

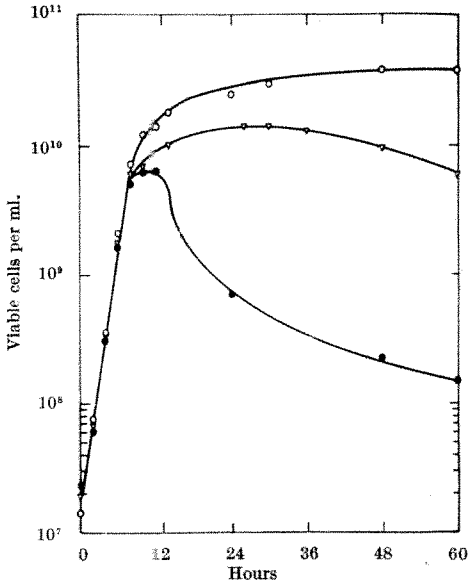


Fig. 1. Growth curves of *Bordetella pertussis* in liquid culture. ●, Control; ▽, dialysis culture; ○, dialytic symbiosis culture with *Corynebacterium pseudodiphtheriticum*

grown in the control culture reached a population peak at 12 h and then declined rapidly in numbers, while growth in the dialysis or dialytic symbiosis cultures continued to 24 h or more, then levelled off. Similar findings have been reported for *Serratia marcescens* grown in dialysis culture[7].

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Influence of Incubation Temperature and of Storage on the Viability of Fungal Spores damaged by Phenol

The evidence for an improved revival of damaged bacteria on incubation at temperatures below the optimal for the undamaged species applies mainly to irradiated cells (see, for example, refs. 1 and 2), but optimal revival at 27°–29° C of *E. coli* damaged by phenol has also been reported[3,4]. The storage of irradiated bacteria and mammalian cells at sub-optimal growth temperature for some hours leads to an increase in the number of survivors[5]. We find that storage has a beneficial effect, but not a lower optimal incubation temperature with fungal spores damaged by phenol.

Suspensions containing $6{\cdot}25 \times 10^6$ *Penicillium notatum* or 5×10^6 *Aspergillus niger* spores per ml.[6,7] were mixed with equal volumes of phenol solutions at 25° C. These were filtered through 'Oxoid' membrane filters after definite contact times; the spores were washed with water until the filtrate was free of phenol and then re-suspended in water. One-drop aliquots were added to equal volumes of double-strength 'Oxoid' Sabourand liquid medium on each of four microscope slides which, after placing in Petri dishes containing wet cotton wool, were incubated at the required temperature. Differential counts of 100 germinating and non-germinating spores were made on replicate slides after 8 h and, when hyphal growth was not too dense to permit accurate counting, at intervals up to 24 h.

To examine the effect of storage, spores were treated with 1 per cent phenol, separated, washed and re-suspended in water. Portions of these *A. niger* suspensions were stored at 5°, 23·5°, 30°, 36°, 40° and 50° C for 6–48 h, and *P. notatum* suspensions at 5°, 15°, 25°, 28°, 31° and 37° C for 5–25 h. Replicate slides were prepared and incubated at the optimal growth temperatures: *A. niger* at 36° C, *P. notatum* at 28° C. Controls were prepared by plating out suspensions directly after contact with phenol.

The optimal incubation temperature of spores damaged by phenol was the same as that for the undamaged spores (Fig. 1). Damaged spores, however, took longer to germinate than those not damaged; at optimal temperatures they took 2 h or more to reach maximum survival levels. At temperatures above or below the optima the differences between these times were even greater, but the respective maximum percentage survival levels were obtained with both damaged and undamaged spores on prolonged incubation. The rate of germination and the viability fell more rapidly at temperatures above than below the optima.

Storage of damaged spores in water for some hours at temperatures ranging from below the optima to a little above gave improved revival (Fig. 2). In general, an increase in the temperature of storage resulted in a shorter period being required for maximum revival. Storage at temperatures just below the optima for about 24 h gave some of the best recoveries. This effect may

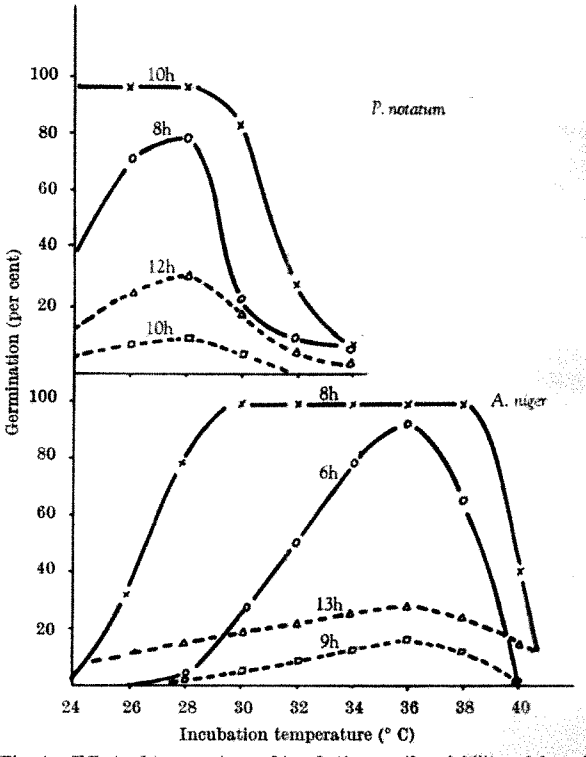


Fig. 1. Effect of temperature of incubation on the viability of fungal spores before and after treatment with 1 per cent phenol. ——, Untreated; - - - -, treated *P. notatum* for 20 min and *A. niger* for 15 min. Times are incubation periods before counting

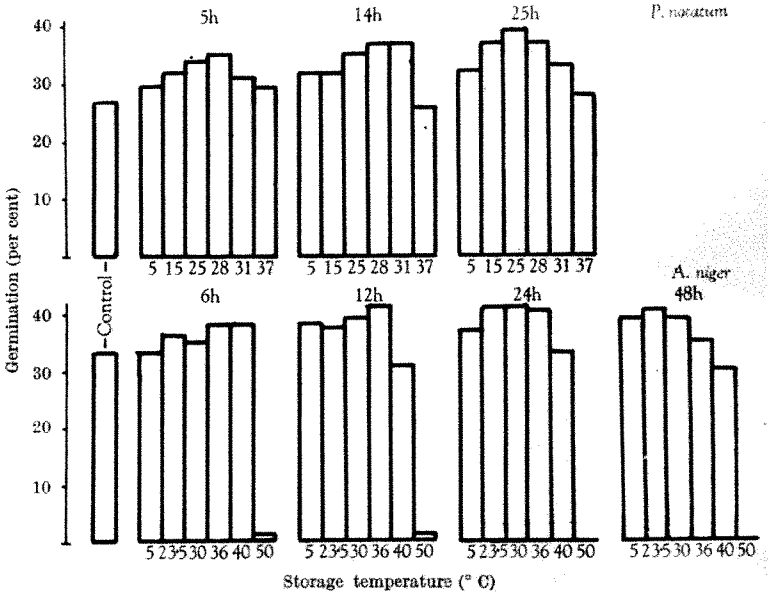


Fig. 2. Effect of duration and temperature of storage in water on the viability of fungal spores treated with 1 per cent phenol. *P. notatum* for 20 min and *A. niger* for 15 min. Times are duration of storage periods of spores in water after treatment with phenol, before aliquots of spore suspensions are added to double-strength Sabouraud medium and incubated at 28° for *P. notatum* and 36° for *A. niger* until maximum germination is obtained

be due to the utilization of residual energy for cellular repair and not for immediate spore germination, since water is an unsuitable growth medium. The transference of spores to culture medium may then permit such previously 'critically viable' spores to germinate. Desorption of the toxic agent from the spores during storage, as was suggested with triazine-treated *Neurospora sitophila*[8], seems an unlikely explanation since even after storage for 72 h supernatant fluids obtained by removal of spores by centrifuging had no ultra-violet absorption spectrum characteristic of phenol.

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CYTOLOGY

Position of Labelled Chromatids in Diplochromosomes of Endo-reduplicated Cells after Uptake of Tritiated Thymidine

ENDO-REDUPLICATION as defined by Levan and Hauschka[1] consists of a microscopically non-visible duplication of the chromosomes within the cell nucleus leading to the formation of so-called 'diplochromosomes' in the following mitosis. Endo-reduplication has been reported to occur regularly, although rarely, in human tissues *in vitro*[2,3]. In a previous paper[3], we showed that in cultured human fibroblasts diplochromosomes originate by two separate and successive chromosome duplications. Diplochromosomes of endo-reduplicated cells which took up tritiated thymidine during the second chromosome replication show all four chromatids labelled in the following metaphase, whereas when tritiated thymidine was given during the first chromosome replication the label was restricted to but one chromatid of each of the two chromosomes forming a diplochromosome. These observations lead to the conclusion that the chromatids of diplochromosomes are, like chromatids of ordinary human chromosomes[4], composed of two sub-units which are distributed in a semi-conservative mode during chromosome duplications. The duration of one endo-reduplication cycle is in the range of two cycles of a diploid cell.

In the work recorded here we induced endo-reduplication with α-mercapto-ethanol[5] in order to examine the formation of diplochromosomes in a greater number of cells. In autoradiographic investigations using tritiated thymidine our attention was drawn to some irregularities in the duration of induced endo-reduplication and to a regular pattern of the position of newly replicated chromatid sub-units in diplochromosomes.

Cultures of peripheral blood cells from healthy persons were set up in the usual way[6] with α-mercapto-ethanol added at the beginning of culturing in a final concentration of 0·01 vol. per cent. After 3·5–4 days, tritiated thymidine (Radiochemical Centre, Amersham, sp. act. 7·2 c./mmole) was added in a concentration of 1 μc./ml. medium for a period of 12 h; the cultures were then washed twice in Gey's solution and fresh medium (made up with autologous serum) without tritiated thymidine and without mercapto-ethanol was added. Chromosome preparations were made 40 and 52 h after the end of treatment with tritiated thymidine. Colcemid (2 μg/ml.) was given 4 h previous to preparation. Kodak emulsion *NTB2* was used for autoradiography; radiation time was 14 days.

In cultures thus treated with mercapto-ethanol many metaphases of endo-reduplicated cells are found. The diplochromosomes of these cells are somewhat different from those occurring spontaneously in fibroblast cultures. In the latter the two chromosomes forming a diplochromosome are only weakly connected at the centromeric regions and usually lie a small distance apart, probably due to the spreading process during preparation. The chromosome pairs of diplochromosomes in mercapto-ethanol treated blood cultures are more closely attached. No direct connexion, however, is visible between the centromere of the two chromosomes.

The labelling patterns of diplochromosomes in preparations made 40 and 52 h after the end of a 12-h treatment with tritiated thymidine indicate that most endo-reduplicated cells are labelled during first synthesis and a few even during second synthesis, that is the synthesis preceding mitosis. Diploid cells in the same preparations show chromatid labelling, indicating that they are in second or third mitosis after uptake of tritiated thymidine. Thus diplochromosome formation induced by mercapto-ethanol may take longer than two cycles of a diploid cell.

In diplochromosomes of endo-reduplicated cells which took up tritiated thymidine during first replication and therefore have one chromatid of each of the two paired chromosomes labelled, quite frequently the two outward situated chromatids are the labelled ones (Fig. 1). Exceptions from this behaviour are rare, mostly when chromatids or their arms seem to be bent and folded around each other or where true chromatid exchanges may have occurred. The relatively small size of human chromosomes and the rather poorly preserved morphology of diplochromosomes after mercapto-ethanol treatment have allowed no detailed analysis of frequency and type of chromatid exchanges so far.

The characteristic outward position of labelled chromatids in diplochromosomes escaped our attention when we examined spontaneous endo-reduplication in fibroblast cultures[3], since in this case diplochromosomes are seen with the labelled chromatids also in other positions. However, a count of 60 diplochromosomes from spontaneous endo-reduplicated fibroblasts labelled during first synthesis revealed that more than half the diplochromosomes show both the labelled chromatids in the outward position;

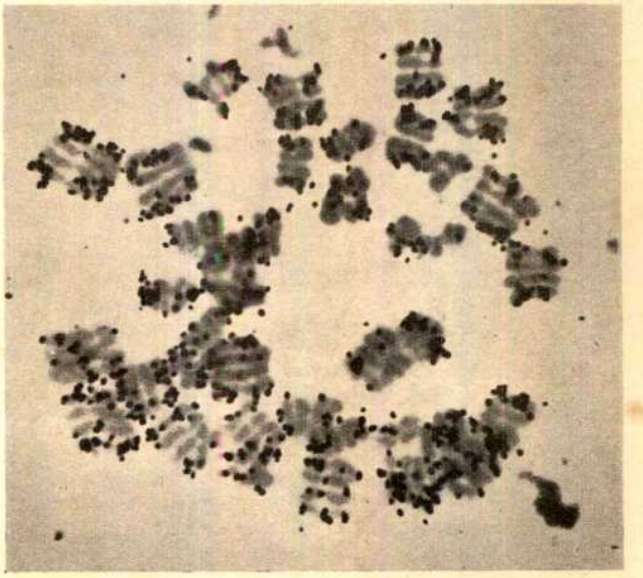


Fig. 1. Autoradiography of a metaphase of an endo-reduplicated cell which took up tritiated thymidine during first DNA-synthesis. In most diplochromosomes the grains are restricted to the two outward positioned chromatids (× 1,200)

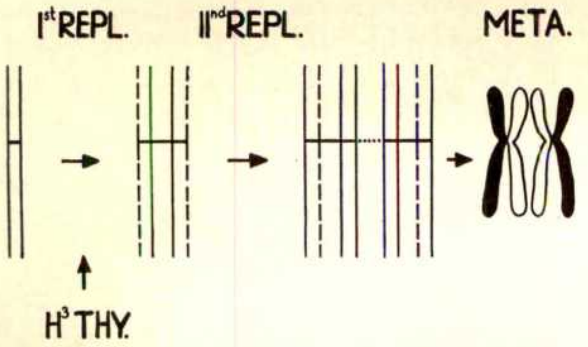


Fig. 2. Schematic representation of position of sub-units in endo-reduplication. Tritiated thymidine added during first replication. Broken lines: labelled sub-units, labelled chromatids of diplochromosomes in metaphase (indicated in black) in outward position

the rest show in most cases one labelled chromatid outward and one inward and only very rarely are both inward chromatids labelled. If the labelled chromatids are distributed at random, in 25 per cent of diplochromosomes both should lie outward, in 50 per cent one inward and one outward, and in the other 25 per cent both should lie inward. The actually observed preference of the outward position of both labelled chromatids indicates that in spontaneous endo-reduplication also this is the original position which can only be disturbed by secondary movements of the chromosomes such as, for example, those caused by spreading in the course of preparation.

The sub-units which were newly synthesized with first replication are seen on second replication to be old sub-units of the outward situated chromatids (Fig. 2). There must be a certain direction in replication bringing newly synthesized sub-units into a defined position. They are also forced to move outward, that is in a direction opposite to that of the centromeric connexion of the original sub-units, when the next replication takes place. It is possible that the centromere has some influence on the position of newly synthesized sub-units. Also chromosomal proteins or 'linkers' connecting DNA strands or segments[7,8] could serve as a frame which allows segregation and replication of DNA molecules only in a given direction.

Note added in proof. After this communication had been submitted for publication, K. Walen (*Genetics*, **51**, 915; 1965) reported observations on the position of chromatids in endo-reduplicated cells of *Potorous tridactylis*, quite similar to our findings in human material.

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Chromosome Abnormalities in Lymphatic Leukaemia in Cattle

The specific change in the chromosome complement reported by Gustavsson and Rockborn[1] in three cases of overt leukaemia in cattle is extremely interesting, but care should be taken in identifying the chromosome changes with the neoplastic condition. This may not have been the intention of the authors, but it is an important distinction for the following reasons:

(1) We have studied the chromosomes in more than twenty cases of lymphosarcoma (lymphatic leukaemia) in cattle, nine of which have been reported[2], and in only two cases, one described by us[2] and the other by Basrur *et al.*[3], have similar chromosomal changes been observed; namely, an additional submetacentric chromosome and a minute chromosome in place of one of the small acrocentric chromosomes.

(2) In the three cases reported by Gustavsson and Rockborn, all the mitoses from the peripheral blood leucocyte cultures showed the same chromosomal change. This would be unusual if the chromosomal change was associated with the neoplasia, because in our experience, even when chromosomal changes occur in the peripheral blood leucocytes of cattle with lymphosarcoma, a number of cells with apparently normal karyotypes are always present in cultures to which phytohaemagglutinin has been added.

(3) The finding of the same chromosomal change in cells from the bone marrow and kidney of the foetus of one of the cows and in peripheral blood leucocytes of the three cows would seem to offer further evidence that the chromosomal change was generalized and not confined to the cells of the lymphatic system or associated with the neoplastic condition. Chromosomal analyses of other cells from the cows, for example, bone marrow cells and fibroblasts, in addition to the peripheral blood leucocytes, would have helped to throw light on the significance of the change in the chromosome complement and on the possibility that it may have been inherited.

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GENETICS

Periclinal Nature of the Ivy-leaf Sport in Potatoes

The use of X-ray treatment[1] can produce from periclinal chimeras of potatoes (*Solanum tuberosum* L.) a small frequency (about 5–10 per cent) of plants which have at least the two outer layers at the growing point (L_1 and

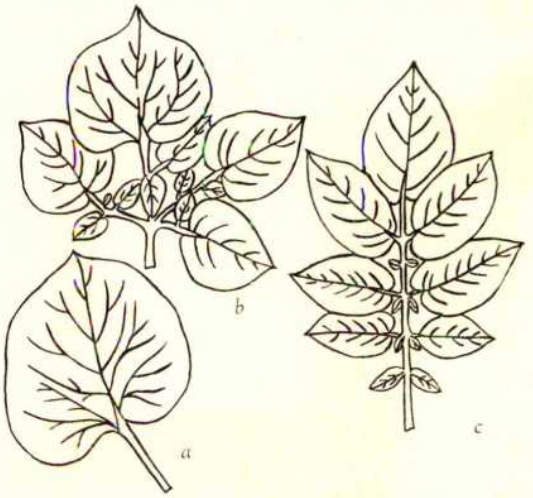


Fig. 1. *a* and *b*, Leaves of Ivy-leaf 'Doon Star' sport; lower, simple, and upper, compound respectively; *c*, pinnate leaf on shoot of plant produced by X-ray treatment of the sport. (All × *c*. 1/3)

L_2) with the constitution of L_1 in the chimera. Sports for large changes in leaf shape[2] probably always have a mutated L_2 and in many cases the mutation is also in L_3. Such sports cannot therefore be shown to be chimeras by either breeding or eye-excision experiments; this explains why the ivy-leaf sport (Figs. 1*a* and *b*) has not previously been shown to be a chimera.

Eye-excision experiments with the ivy-leaf sport of the variety 'Doon Star' produced only plants with ivy leaves. The cross of ivy-leaf 'Doon Star' × 'Ulster Prince' gave a family in which there were 23 seedlings with ivy leaves and 28 with normal pinnate leaves. In crosses of normal 'Doon Star' no seedlings with ivy leaves are produced. There is thus no doubt that ivy-leaf 'Doon Star' results from a dominant mutation for leaf shape in L_2.

Ten rose (apical) ends of tubers of ivy-leaf 'Doon Star' were given an X-ray dosage of 3,500 r., and all ten ends produced plants. On two of these plants shoots with normal pinnate leaves (Fig. 1*c*), in addition to shoots with ivy leaves, were found. It thus appears that L_1 in the ivy-leaf sport, like L_1 in the docken-(entire-) leaf 'Majestic' sport[3], has the unchanged genotype for normal, pinnate leaves.

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PSYCHIATRY

Correlation between Scores for Dementia and Counts of 'Senile Plaques' in Cerebral Grey Matter of Elderly Subjects

Small, discrete masses of argyrophilic particles, with a mean diameter of 50–60μ, were first seen in the cerebral cortex by Blocq and Marinesco[1] and named "senile plaques" by Simchowicz[2], who discovered large numbers of these lesions in cases of senile dementia. Since they may be present in the normal old person[3] and no close relationship between intensity of plaque formation and clinical changes was found by some workers[4], their relevance for senile dementia—the extreme form of intellectual decay of old age—has often been called into question. More recently Corsellis[5] has demonstrated a reasonably good correlation between clinical diagnosis and neuropathological changes in post-mortem studies of aged mental hospital patients. But the nature and extent of the relationship between plaque formation and mental deterioration in old age remained uncertain. An attempt has been made to shed further light on this problem by enquiries in a series of patients, aged 60 years and more, admitted with psychiatric or physical illness to mental and geriatric hospitals and subsequently studied at post-mortem. The aim was to carry out a quantitative assessment of the severity of dementia in individual subjects during life and to ascertain whether this bore any relationship with mean plaque counts in cerebral grey matter. At this stage of the enquiry cases with gross cerebrovascular lesions were excluded as there was no available technique for making a quantitative estimate of their extent. The degree of dementia was expressed in quantitative form by carrying out the two following assessments.

(1) Since dements show an increasing range and number of defects as their disorder progresses, a list of defects was drawn up with special emphasis on those involving everyday habits and skills. At assessment, the patient's current ability with regard to each item on the list was ascertained, and a positive score of 1 given for each item present. The sum of scores for the individual defects gave a 'dementia score' which might lie anywhere from 0 (indicating absence of dementia) to +28 (indicating very severe dementia).

(2) Simple psychological tests were given which had been previously shown to differentiate groups of demented and non-demented aged persons[6,7]. Scores were allocated for correct performance in these tests, the summed scores lying between 0 and +37. In this case the scoring was in the opposite direction.

These two assessment have been repeated at six-monthly intervals on survivors.

In the neuropathological investigation, sections were taken from the same areas of the frontal, parietal, temporal and occipital lobes of each cerebral hemisphere and stained by the Von Braunmühl method. Plaque counts were carried out in each of 60 low-power fields chosen at random from these sections by one of us (B. T.) who had no knowledge of the dementia or test scores. Product moment correlation coefficients were computed between the scores for dementia and psychological test performance recorded during life, and the mean plaque count estimated in each of 37 patients.

Item	Coefficient of correlation	Significance
Age	+0 184	N.S.
Dementia score	+0 777	V.H.S. $P < 0{\cdot}01$
Test score	−0 4734	V H.S. $P < 0$ 01

In Fig. 1, the mean plaque count has been plotted against dementia score. It will be seen that there is an approximately linear correlation between them. Plaque distribution was on the whole remarkably uniform. The significance of variations in the density of plaque distribution is under examination. Although the distinction between demented and non-demented subjects appears in clinical practice to be relatively sharp, the neuropathological difference between them would seem, in the light of this study to be a quantitative rather than a qualitative one. Thus, if we take a cut-off line at a mean plaque count of ten, 23 of the 24 subjects with a mean count below this figure had a dementia score of less than ten, while only two out of thirteen subjects with a higher mean count had a dementia score of less than ten. Dementia was present in a clinically obvious form in all subjects whose score exceeded approximately eight to nine, and the majority of those with clear-cur dementia are therefore placed to the right of the cut-off line (mean plaque count, 10) while nearly all of those showing no deterioration clinically were to the left of the line. In the only striking example of a discrepancy between mean plaque count (5) and dementia score (17), the neuropathological picture was in certain respects an unusual one, differing from that of the remaining cases with a dementia score of more than ten. In the latter group, the appearances conformed closely with those described in cases of senile dementia. The overlap in respect of plaque counts between those with low and those with high dementia scores is therefore small. It may arise from the margin of error attaching to the technique for estimating mean plaque

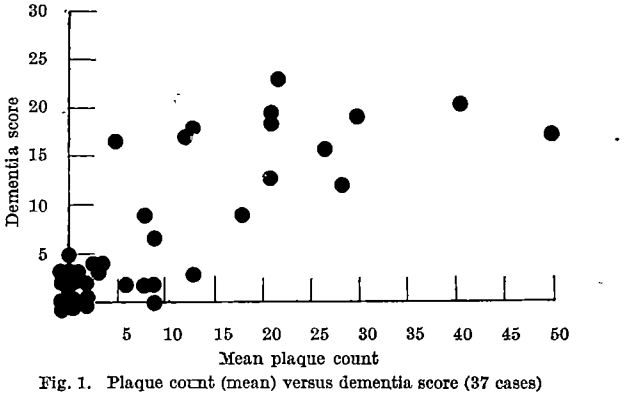


Fig. 1. Plaque count (mean) versus dementia score (37 cases)

counts, or of measuring dementia, from individual variation in respect of threshold for mean plaque count beyond which dementia becomes manifest, or more than one of these factors. It seems unlikely that the accumulation of plaques in large numbers could have been a preterminal phenomenon since several cases dying of carcinomatous metastases had very low plaque counts.

The following tentative conclusions may be drawn from the findings:

(1) Far from plaques being irrelevant for the pathology of old-age mental disorder, the density of plaque formation in the brain proves to be highly correlated with quantitative measures of intellectual and personality deterioration in aged subjects.

(2) The establishment of a valid measure of cerebral damage in one group of organic psychoses opens up the possibility of defining and measuring the psychological defects that show the best correlation with measures of cerebral damage.

(3) Senile dements have a markedly reduced expectation of life[8]. As the brain is an organ with a fixed cell population and such organs have long been known to be particularly liable to the changes of senescence, the facts suggest that senile plaque formation and related processes may also deserve investigation with the aid of more precise techniques than those employed in this study, for their possible relevance to the problems of ageing in man.

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G. Blessed

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PSYCHOLOGY

Tranquillizers in Pregnancy and Behavioural Effects on the Offspring

In 1963 we published the third[1] in a series of reports[2,3] concerned with the administration of tranquillizers to gravid rats and their postnatal effects on the offspring. The data of this last report[1] indicated that meprobamate and not reserpine or chlorpromazine resulted in significant deficits in learning a Lashley III maze. This report generated much editorial comment[4,5] and additional experimental findings[6,7].

I wish to comment on these last two reports[6,7], not because they failed to replicate our findings but rather because their alterations in procedure implicate important and significant methodological questions concerning this area of research. Kletzkin *et al.*[6] administered meprobamate at a dosage of 20 mg/kg as compared to 60 mg/kg/day reported by Werboff and Kesner[1]. Furthermore, they[6] stated that the drug was injected for four days of gestation on days 5–9, 10–14, or 14–18 (these represent five days). Thus, the total dosage of meprobamate administered was 240 mg/kg in Werboff and Kesner's[1] experiment and either 80 or 100 mg/kg in the report by Kletzkin *et al.*[6]. Their inability to find differences between meprobamate-treated and control offspring in either body-weight, activity on the inclined plane, activity and emotionality on the open-field, and learning ability on the Lashley III maze could readily be explained by these differences in levels of dosage administered. These discrepancies then suggest the need for more detailed analysis of the dose-response characteristics of pregnant animals to meprobamate administration.

Hoffeld and Webster[7], in a more complete replication, compared the effects of meprobamate, reserpine, chlorpromazine, or water on body-weight, learning a Lashley III maze and a conditioned avoidance problem. The major contention of these authors is that our previous report failed to demonstrate effects related to the stage of pregnancy in which the drugs were administered. Stage-dependent results were found only in neonatal mortality[3]. The fact that no consistent stage-dependent results occurred on several unrelated measures[1-3] of maturation and behaviour requires that a more critical evaluation be made of the methodology of the Hoffeld and Webster[7] report. Two factors are immediately apparent. These authors purchased their animals already pregnant and thus such animals probably experienced severe stress from shipping in the early stages of pregnancy; secondly, the offspring were colony-housed from weaning at 21–80 days of age. During this time, they experienced whatever stresses are concomitant to a populated colony cage, and they did not experience the handling and testing on the battery of behavioural measures used by Werboff *et al.*[2,3]. Thus, the Hoffeld and Webster[7] animals had a variety of other prenatal and postnatal conditions which could easily have masked and altered the effects of the particular drugs administered in pregnancy on the behaviour of the offspring.

Hoffeld and Webster[7] deal specifically and critically with the Lashley III data from the Werboff and Kesner[1] report. It should be pointed out that Hoffeld and Webster[7] misquote these findings[1]: only meprobamate and not reserpine was found to produce learning deficits. Furthermore, Hoffeld and Webster[7] state that a comparison of the absolute values of the trials to criterion measure of control groups in various reports are of special interest. Werboff and Kesner[1] reported that the control group required 9·5 trials to criterion as compared to Hoffeld and Webster's[7] 14·2 trials to criterion. These authors then refer to a previous report of Werboff, Havlena and Sikov[8] in which prenatal irradiation was administered with a combined control group requiring 13·1 trials to criterion. The fact that the data from the control group of this investigation[8] are more in keeping with the data of Hoffeld and Webster[7] is noteworthy. The pregnant animals in this work[8] had to be carted several blocks to be irradiated under a variety of weather conditions. Thus, they may have experienced the same kind of 'shipping' stress received by the Hoffeld and Webster[7] animals, which may explain the differences and similarities between the scores of the various control groups on the Lashley III maze.

In spite of the fact that it is difficult to replicate conditions exactly from one experiment to another, certain important methodological variables have been suggested that may explain the discrepancies noted and that require further experimental elaboration. These are the questions of dose-response characteristics of pregnant animals to various drugs, and the effects of compounding additional prenatal and postnatal stresses on subsequent behavioural development. If these discrepancies lead us to uncover new data and new relations between critical variables, then our efforts would be rewarded.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 3

PLASTICS INSTITUTE (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p m —Mr. J. E. Proctor: Young People's Lecture.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Sir Dudley Stamp, C.B.E.: "Philatelic Cartography".

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKING PARTY (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Human Co-ordination and Engineering Control".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. P. Prosser: "The Chemistry of Mineral Processing".

Tuesday, January 4

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Earthworks—Are Present Specifications and Construction Plant the Most Suitable for the Soil and Climate Conditions in the British Isles", introduced by Mr. S. Rodin.

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p m.—Mr. E. G. C. Burt. "Satellite Control".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. L. Croom: "Naturally Occurring Thermal Radiation in the Range 1–10 Gc/s"; Mr. E. Denison and Mr. G. L. Rogers: "Aerial Noise Temperature at 5,650 Mc/s".

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Accountancy as a Service to Engineering Management".

Wednesday, January 5

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Prof. D. V. Lindley: "Bayesian Robustness".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p m.—Discussion on "Operating Experience with Deck Machinery" opened by Capt. W. S. C. Jenks, R.N., and Capt. D. Campbell, R.N.

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Dr. John R. Baker, F.R.S.: "Experiments on the Function of the Eye in Light Microscopy" (Presidential Address).

Thursday, January 6

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S W.1), at 5.30 p.m.—Informal Discussion on "How Can Research Help Construction Methods", introduced by Mr. R. S. Taylor.

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Marine Reactor".

INSTITUTE OF METALS, LONDON LOCAL SECTION (at 17 Belgrave Square, London, S.W.1), at 7 p m.—Mr. R. S. Sharpe: "Current Research in Non-Destructive Testing".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 30 p.m.—Mr. C. Freeman: "The Economics of Research".

Friday, January 7

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p m.—Discussion on "Tracking of Insulation" opened by Dr. A. G Day, Mr. P. J. Jackson and Mr. A. W. Stannett.

Monday, January 10

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Taurus and Tibesti" (colour films of recent expeditions).

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (joint meeting with the London Section of the Oil and Colour Chemists' Association, at 14 Belgrave Square, London, S.W 1), at 6.30 p.m.—Mrs. S. M. Rybicka and Mr. A. F. Sherwood. "Surface Properties of Titanium Dioxide Pigments".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TECHNICIAN or SENIOR TECHNICIAN IN THE DEPARTMENT OF MICROBIOLOGY for duties which will include assisting with research and taking charge of the preparation of class material—Prof. D. E Hughes, Department of Microbiology, University College of South Wales and Monmouthshire, Cathay's Park, Cardiff (January 7).

SENIOR LECTURER (with an honours degree and prepared to teach the subject to final B.Sc. (Applied Chemistry) and A P.I standard, and preferably appropriate industrial and/or research experience) IN THE CHEMISTRY AND TECHNOLOGY OF HIGH POLYMERS—The Principal, Rutherford College of Technology, Ellison Place, Newcastle upon Tyne, 1 (January 8).

UNIVERSITY LECTURER IN ANCIENT PHILOSOPHY—The Secretary of Faculties, University Registry, Oxford (January 8).

GRADUATE (with at least an upper second-class honours degree in botany, agricultural botany or plant physiology) to carry out individual research on the biology of perennial grass weeds in arable land—The Secretary, A.R.C. Weed Research Organization, Begbroke Hill, Kidlington, Oxford (January 10).

LECTURER or ASSISTANT LECTURER (with a special interest in one or more of the following: (*a*) economic geography; (*b*) planning; (*c*) a region other than Western Europe or the Americas) IN THE DEPARTMENT OF GEOGRAPHY—The Deputy Secretary, The University, Southampton (January 10).

ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (January 13).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with an honours degree (or a diploma in technology) in entomology, engineering, botany or chemistry, and preferably some tropical experience) to spend periods overseas undertaking original investigations into problems associated with the handling and storage of foodstuffs in tropical climates, to evolve methods of improving quality and reducing losses, and to assist in the training of local personnel—The Director, Ministry of Overseas Development, Tropical Stored Products Centre, London Road, Slough, Bucks, quoting Ref. V/I (January 14).

ASSISTANT LECTURER (preferably with special interests in the scientific aspects of geography, for example, biogeography or climatology) IN GEOGRAPHY—The Registrar, The University, Manchester, 13, quoting Ref. 226/65 (January 15).

ASSISTANT LECTURERS, LECTURERS OR SENIOR LECTURERS IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Sheffield (January 15).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE SCHOOL OF PHYSICS—The Registrar, The University, Newcastle upon Tyne 2 (January 15).

LECTURERS or ASSISTANT LECTURERS (with special interests and qualifications in one of the following fields: experimental psychology; social psychology; personality and individual differences; psychometrics) in the Department of Psychology—The Deputy Secretary, The University, Southampton (January 15)

RESEARCH FELLOW or SENIOR RESEARCH FELLOW IN APPLIED MATHEMATICS—The Registrar, University of Queensland, Brisbane, Australia (January 15).

DEPUTY LIBRARIAN (honours graduate)—The Librarian, The University, Keele, Staffs (January 17).

CHAIR OF PHYSICAL CHEMISTRY—The Registrar, The University, Sheffield (January 22).

SENIOR LECTURER IN THE DEPARTMENT OF PATHOLOGY—The Registrar, The University, Sheffield (January 22).

LECTURER/SENIOR LECTURER IN VETERINARY VIROLOGY IN THE DEPARTMENT OF VETERINARY PATHOLOGY AND BACTERIOLOGY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 28).

LECTURER or SENIOR LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS—The Registrar, University College of South Wales and Monmouthshire, Cathay's Park, Cardiff (January 29)

RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST (with a Ph.D. degree in an appropriate field or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability) TO THE LEATHER RESEARCH SECTION OF THE DIVISION OF PROTEIN CHEMISTRY, Commonwealth Scientific and Industrial Research Organization, Parkville, Melbourne, Australia—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2 quoting Appointment No. 462/244 (January 29).

ASSISTANT or ASSOCIATE PROFESSORS (with research experience in biochemistry, plant physiology or experimental biology) IN BIOCHEMISTRY AND BIOLOGY—Head of the Bioscience Department, Brock University, St. Catharines, Ontario, Canada (January 31).

CHAIR OF STATISTICS—The Secretary, The University, Aberdeen (January 31)

LECTURER IN THE SCHOOL OF SOCIOLOGY (Seconded to the Faculty of Medicine), University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31)

LECTURER (with a good honours degree in engineering or an equivalent qualification, and preferably some industrial experience) IN THE STRENGTH OF MATERIALS SECTION OF THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C 1 (January 31).

READER IN EXPERIMENTAL PHYSICS AT QUEEN MARY COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 31).

SENIOR LECTURER/LECTURER IN BIOCHEMISTRY at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, January 31)

TECHNICAL ASSISTANT IN THE DEPARTMENT OF PSYCHOLOGY, Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W.1 (South Africa and London, January 31).

LECTURER (preferably with experience in experimental fields of zoology) IN THE DEPARTMENT OF ZOOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 1)

SENIOR LECTURER/LECTURER IN DAIRY HUSBANDRY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or the Registrar of the University (New Zealand and London, February 3)

LECTURER (preferably theoretical physicist with experience in crystal or solid state physics) IN PHYSICS at Queen's College, Dundee—The Secretary, University of St Andrews, Queen's College, Dundee, Scotland (February 12).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY AND PHARMACOLOGY, OR ANY RELATED SUBJECTS—The Registrar, The University, Liverpool, quoting Ref. CV/359 (February 14).

LECTURER/SENIOR LECTURER (with a degree in civil or agricultural engineering with experience and interests in the field of soil and water engineering) IN AGRICULTURAL ENGINEERING (SOIL AND WATER) at Lincoln College, University of Canterbury, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 14).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with a Ph.D. degree or equivalent research experience, and below the age of 30) IN APPLIED MATHEMATICS, BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY, OR PHYSICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (February 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (with a Ph.D. degree or equivalent research experience, and preferably below the age of 30. IN CHEMISTRY, CHEMICAL ENGINEERING, ENGINEERING SCIENCE AND PHYSICS—The Academic Registrar, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (February 28).

ASSISTANT or ASSOCIATE PROFESSORS (with a Ph.D. degree; special consideration for one position will be given to candidates with research interests in theoretical physics) IN THE DEPARTMENT OF PHYSICS—The University of Victoria, Victoria, B.C., Canada.

BACTERIOLOGIST (with at least three years' experience in bacteriology) to work as Assistant to Dr. E. S. Anderson, Director of the Enteric Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W.9—The Secretary, Public Health Laboratory Service Board, 24 Park Crescent, London, W.1.

BIOCHEMIST (preferably with experience with chromatographic and electrophoretic techniques) to join a team studying aspects of copper and magnesium metabolism in animals—The Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh, 9, Scotland.

BOTANIST (morphology, anatomy, morphogenesis) IN THE BIOLOGY DEPARTMENT—Prof. E. Gorham, University of Alberta, Edmonton, Alberta, Canada.

CHAIR OF THEORETICAL ASTRONOMY—The Registrar and Secretary, The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential".

ELECTRONIC TECHNICIAN (Senior or Technician Grade according to experience) FOR THE DEVELOPMENT, CONSTRUCTION AND MAINTENANCE WORK IN THE RADIOTHERAPY, PHYSICS AND MEDICAL ELECTRONICS DEPARTMENTS—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. No PT/999.

GEOLOGIST (male, national of the United Kingdom or the Republic of Ireland, with a first- or good second-class honours degree in geology, preferably with experience of geological mapping of metamorphic terrain) IN MALAWI, to undertake geological mapping, geochemical surveys, investigation of mineral deposits and possible water supply investigations—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 218/134/02.

GRADUATE RESEARCH ASSISTANT for work on serology of tuberculosis including fluorescent antibody techniques—The Secretary, Harefield Hospital, Harefield, Middlesex.

GRADUATE RESEARCH ASSISTANT (preferably with an honours degree) for biochemical studies in muscle disease—Dr. B. P. Hughes, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1.

HISTOPATHOLOGIST (with either a veterinary or medical qualification); and a TOXICOLOGIST or PHARMACOLOGIST (recent graduate in pharmacology, pharmacy or zoology) IN THE APPLIED PHARMACOLOGY DIVISION, for work concerned primarily with the pathology and toxicology of drug-induced lesions in experimental animals—The Personnel Manager, Arthur D. Little, Ltd., Inveresk Gate, Musselburgh, Midlothian, Scotland.

LECTURER and an ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Registrar, Hatfield College of Technology, Hatfield, Herts.

LECTURER IN BIOCHEMISTRY and a LECTURER IN MICROBIOLOGY—The Registrar, Hatfield College of Technology, Hatfield, Herts.

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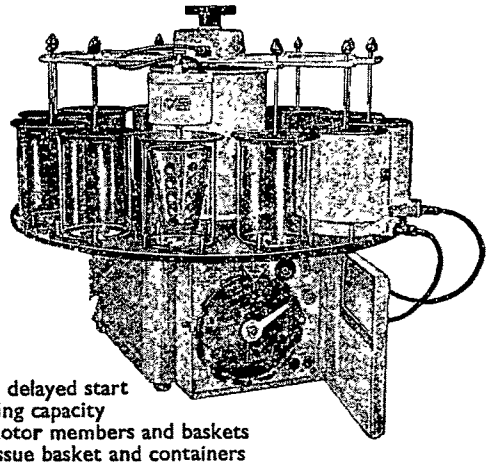
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DEPLOYMENT OF MANPOWER IN SCIENCE AND TECHNOLOGY

THE Committee on Manpower Resources for Science and Technology, appointed in February by the Secretary of State for Education and Science and the Minister of Technology, with Sir Willis Jackson as chairman, has issued an introductory review of the scope and problems of policy for scientific and technological manpower*. The Committee has interpreted its responsibility of advising on manpower resources for science and technology as including the effective use of Britain's entire scientific and technological manpower, as well as the output of newly qualified individuals, in relation to the opportunities for employment. It believes that the deployment within the economy of existing scientists and technologists, and their utilization within any organization, demand as careful and urgent consideration as the continuing supply from Britain's educational system. Moreover, it considers that these problems should be studied against the background of the deployment of all highly qualified manpower.

Thus this authoritative Committee, at the outset, puts its finger on a key aspect of the manpower situation—the efficient use of trained manpower—which, although frequently noted, has seldom received more than passing discussion. That in itself should help to ensure that in future more attention is given to the question of supporting staff and that the triennial surveys of scientific and technological manpower will cover technicians. It is worth noting in this connexion that Sir Willis Jackson is also chairman of a committee set up in 1962 to consider the parameters of an enquiry into the employment of technicians in industry, the universities and the scientific and professional Civil Service. As a result of this enquiry, a new section on technicians and other technical supporting staff is being incorporated in the 1965 Manpower Survey, on the findings of which the Committee on Manpower Resources proposes to base the complete programme of its work. It might be added that another member of the Committee, Prof. C. A. Moser, is at present engaged in a survey of correlations between academic qualifications and performance in industry and that the Council for Scientific Policy has invited another member, Prof. F. S. Dainton, to study the flow from schools into places for science and technology offered in higher education.

The Committee is itself associated with this study and will be examining its results. To illustrate the representative character of the Committee, on which the Science Research Council, the University Grants Committee and the Ministry of Overseas Development, *inter alia*, have representatives, it should suffice to mention the names of Sir Leon Bagrit, Sir Peter Venables, Lord Brown of Machrihanish, and Prof. J. R. N. Stone. The Committee is basing its statistical surveys largely on the Census of Scientific and Technological Qualifications carried out in April 1961, and which is to be brought up to date by a recent enumeration in 1966, and on the triennial surveys, covering about 80 per cent of Britain's scientific manpower, which have been carried out by the Ministry of Labour since 1956. The latest survey, relating to January 1965, which is now being processed, has been designed to interlock with the more comprehensive enumerations of the 1961 and 1966 censuses of population. Data from this survey will enable the Committee to assess further the supply and demand for qualified manpower, both at present and in relation to employers' forecasts for 1968. In the light of this evidence and of other enquiries undertaken for purposes of general economic planning, the Committee proposes to attempt to determine how far present deficiencies within particular categories of scientific and technological manpower are due to overall quantitative shortages.

On the supply side, the Committee is looking to the statistics provided by the University Grants Committee, the Education Departments and the professional institutions. These statistics show the inflow of scientists and, when set against estimates of wastage of all kinds, will allow more accurate forward estimates of the stock of scientific manpower in Britain. The Committee points out, however, that an accurate assessment requires a comprehensive understanding of the relation between scientific and technological education on one hand, and employment on the other. This has profound implications for professional education and the number of places universities offer in particular disciplines, as well as in respect of the broad basis of scientific and professional education and the transfer between subjects, if not between disciplines. Employers, for example, in replying to questionnaires about future demand, often start with their own needs for specialized technologists and estimate the number of individuals who are likely to fill these posts from the disciplines listed.

The material the Committee is seeking to acquire has, therefore, implications for estimates of material needs in terms of disciplines and may call for some further thinking about the expansion of higher education. The Robbins Report based its estimates of the scale, and its concept of the pattern, of the future supply of qualified manpower from the educational system on a forecast of the numbers of boys and girls from the schools who would justify education at university level in subjects of their own choosing. Apart from the question (only superficially examined in the Report) of where that higher education would most appropriately be given, this suggests that, as counterpart to employers' forecasts of needs, an examination is desirable of factors affecting or determining the choice of subjects of study at school and in higher education. This also the Committee proposes to examine, together with the motives, character and abilities relevant to science and technology, and the extent to which they are encouraged or inhibited at each stage of education and training. The part played by imaginative teaching and up-to-date curricula in influencing choice for or against science and technology will also be examined.

These problems are already being investigated by the Schools Council in relation to England and Wales, and the Scottish Council for Research in Education is studying the validity of aptitude tests in selection for university places in relation to the subsequent performance of school

* Committee on Manpower Resources for Science and Technology. *A Review of the Scope and Problems of Scientific and Technological Manpower Policy*. Pp ii+17. (Cmnd. 2800.) (London: H.M.S.O., 1965.) 1*s* 6*d*. net.

leavers in higher education. The Committee also emphasizes its concern that sixth-form pupils taking science and mathematics should, in the course of their studies, come into contact with some teachers who have up-to-date technological backgrounds, and who have had experience and an interest in the application of science to practical ends. Few graduates in science and technology, however, enter the schools after industrial experience, and means should be devised to tap the knowledge and energies of those with recent and up-to-date experience in research and in industry. The problem is being examined by many bodies, but no proposals have so far been advanced which can make a measurable contribution in the schools themselves and, for the present, the Committee on Manpower Resources is content to emphasize the importance of this problem.

The Committee is not content, however, to accept the classification of scientific and technological manpower based on the disciplines in science and technology in which degrees are normally taken. It is sometimes necessary to supplement this education by a period of organized training in employment. This is not necessarily an unfavourable reflexion on the teaching given in institutions of higher education, particularly if the period of adaptation proves to be short. It is to the national advantage rather than otherwise that universities and other institutions should prepare individuals in the first instance broadly for their future responsibilities, so that they are adaptable to a wider variety of circumstances throughout their careers.

That a high proportion of vacancies available in industry are for types of technologists not produced solely by the educational system is not necessarily disturbing, provided it is remembered that, as the Manpower Committee points out, proportionately fewer young people capable of qualifying as scientists and technologists will start from the factory floor—this is especially so now that proportionately more young people go on from school to higher education. Nor does this trend merely affect the provision of part-time or full-time census for technicians and others in technical colleges; it accentuates the danger of too many students in institutions of higher education being orientated towards theory and research and away from design and production.

For this reason, the Committee on Manpower proposes to consider whether, and at what stage, the emphasis in courses in university or college of technology should be changed to match industrial needs more closely. This is partly a matter of communication between industry and higher education and, as a first stage, a working group has been appointed under Mr. G. S. Bosworth to study the problems involved in stating the requirements of industry in terms relevant to education. A member of the Committee, Mr. S. L. Bragg, is examining how far individuals from different academic disciplines are in practice interchanged or interchangeable. This could help to establish what kind of basic scientific or technological education would give the breadth of view needed to cope with new developments.

In this context the Committee welcomes the steps which are being taken, as the colleges of advanced technology become universities, to preserve and to develop those elements which have encouraged close contacts between educational courses and the practical requirements of employers. It particularly emphasizes the importance of preserving both the value of the sandwich course (above all, that which ensures an intimate contact between the educational course and industrial practice) and the opportunity for a candidate with an Ordinary National Certificate type of qualification to continue his education through a full-time sandwich course at university-level. It questions the validity of basing entry to certain advanced courses solely on academic criteria and proposes to examine this difficult problem further. There is some evidence that industry in general is not sufficiently aware of the role of the universities in providing postgraduate courses and that universities are not fully aware of the needs of employers. The Committee has accordingly recommended publication of a report by Mr. H. Arthur on an enquiry undertaken at the request of the Committee on Scientific Manpower in December 1963†.

This report has now been published and has been examined by the University Grants Committee, by the Postgraduate Training Awards Committee of the Science Research Council and by professional institutions concerned with technological education. Discussion of Mr. Arthur's findings has increased the awareness that industry must formulate better its needs for postgraduate courses if educational institutions are to cater for them effectively. However, this question of postgraduate courses may here be left with the note that the Committee on Manpower Resources supports the steps being taken by the Postgraduate Training Awards Committee of the Science Research Council to encourage the development of suitable courses at postgraduate level in technology. Advanced course studentships are being allocated more generously in relation to the demand for technology than for pure science subjects and the award system is being comprehensively reassessed so as to ensure that its resources are deployed as effectively as possible.

Apart from the question of technicians and other supporting staff and Prof. Moser's enquiry, other matters bearing on the more effective utilization of qualified manpower, which the Committee proposes to examine, include the optimum distribution of highly qualified people among private industry, the public sector and higher education; mobility between employments (including the need for adequate numbers of scientists and technologists in management); and courses in new technologies. The Committee proposes to keep in touch with the newly established Industrial Training Boards which have a major part to play in training technological manpower. It is also seeking to chart more fully the wastage of scientists and technologists whether by emigration or in other ways.

All this amounts to a considerable programme even when set against the research projects known to the Committee which are listed in an appendix. The Committee itself proposes to pay particular attention to the implications of long-term economic planning for policy for scientific and technological manpower; to identify motives and abilities relevant to science and technology and the factors governing the choice of a career in science or technology; to identify the main categories of scientists and technologists needed by industry and the relation of industry's needs to educational courses; to study the extent to which present resources of ability are fully mobilized, and problems of effective deployment and utilization of scientists at all stages in their career; to relate the interactions between scientists and technologists and their supporting staff; to investigate the migration of scientists and technologists internationally

† Department of Education and Science. *Enquiry into Longer-term Postgraduate Courses for Engineers and Technologists, 1964–65.* By H. Arthur. Pp. x+54. (London H.M.S.O., 1965.) 4*s.* 6*d.* net.

and within the economy. The present Manpower Report sets forth the broad approach of the Committee and the lines on which it is now thinking, and will doubtless provide in due course a yardstick against which its achievements will be measured.

Nevertheless, even at this early stage the Committee has identified some matters on which immediate action could, and should, be taken, and it concludes its report with five specific recommendations. First, universities should continue their efforts to provide more facilities for the increasing number of candidates in science and technology. Secondly, ways should be found to use imaginative and resourceful individuals with practical experience in research and development establishments and industry, to widen the scope of science and mathematics teaching in the schools and to demonstrate the challenge of careers in applied science and technology. (The Committee does not indicate where the responsibility for instigating this second recommendation should lie, but it clearly lies in part on itself, though more especially on the Department of Education and Science, and also on the professional institutions.)

Thirdly, the former colleges of advanced technology and the leading colleges of technology should be encouraged to maintain the resources devoted to sandwich courses, and to keep open opportunities for holders of national certificates to enter higher education in technology. Here, the major responsibility lies with the Department of Education and Science, as it does also for the fourth recommendation: that recommendation is for a closer partnership between the universities, colleges of technology, Government establishments and industry in promoting and conducting postgraduate courses in technology. At the same time, the Government should strengthen the preference given to supporting courses to which industry would send candidates with industrial training and experience, and which are well planned to prepare science graduates for technological employment. Industry should increasingly provide lecturers on particular technological subjects.

On the difficulties with regard to this last-mentioned point, some comments in Mr. Arthur's report are pertinent. To be really effective, he observes, staff of good calibre and experience are required, and this means they will be in senior positions and will be men that industry can least spare on full-time secondments. Firms and organizations are unwilling to contemplate full-time secondments for as long as a year, nor do the staff themselves wish to be absent for so long a period. Full-time secondment for a period from 3–6 months is more favourably regarded; it offers sufficient time to broaden the outlook of the staff seconded and will give useful experience to their deputies. The universities, however, seem only lukewarm to such full-time secondments: they feel that it takes an appreciable time for a man to settle down and become an effective member of the academic staff.

The universities would accordingly prefer the services of an industrialist on a part-time basis regularly over a period of several years. This arrangement involves considerable strain on the individuals concerned, but should be possible if the academic load is not too heavy and if the individual has the support of competent staff on the industrial side. A few universities have already made arrangements with large industrial organizations for the appointment of honorary readers or part-time or visiting professors and lecturers, and possibly the Committee on Manpower Resources could encourage the spread of this practice. It could also be helped by the growth of secondment of academic staff for periods of experience in industry. Here, however, the difficulties are more considerable and much may depend on how far the Department of Education and Science is able to assist universities and colleges of technology with the cost of industrial fellowships of this type, and also on the development of liaison generally between industry and the universities and colleges of technology.

The Committee's fifth, and final, recommendation, namely, that Industrial Training Boards should give particular attention to more graduate facilities within industry, is essentially a matter for the Minister of Labour. Here also, however, the Committee itself could act as a general co-ordinating authority as well as a central forum for considering the problems which arise. Its vision and energy will call for the firm support of other Ministers besides those to whom this report is presented to secure the necessary action based on the fuller understanding which the investigations it proposes should provide.

THE SCIENTIFIC ESTATE

The Scientific Estate

By Don K. Price. Pp. xi+323. (Cambridge, Mass.: The Belknap Press of Harvard University Press, 1965.) n.p.

THE SCIENTIFIC ESTATE, which is likely to become a major authority in the field of science and administration, represents an appreciation of the new and important part science is playing in government. It might be taken as another example of the development of the 'science of science', except for the fact that it is not written by a physical scientist but by a distinguished social scientist who has had a long experience in government and industry and is at present Dean of the Graduate School of Public Administration of Harvard University. It represents an attempt to fit scientific research into the framework of government and administration and, in a way, it has succeeded in doing this. But reading the book yields a very different picture; from what we know from other sources in the United States and in Britain, the picture presented of the relations of science to politics is an idealized one and still far removed from reality.

Prof. Price has chosen to treat science in the United States as one of the estates of the realm: "The four broad functions in government and public affairs—the scientific, the professional, the administrative and the political—are by no means sharply distinguished from one another even in theory, but fall along a gradation or spectrum within our political system. At one end of the spectrum, pure science is concerned with knowledge and truth; at the other end pure politics is concerned with power and action. But neither ever exists in its pure form. Every person, in his actual work, is concerned to some extent with all four functions. The laboratory scientist is probably interested in some professional association with his colleagues, in the administration of his laboratory, and in the support for his work that comes only from money or influence. At the other extreme, the Congressman or the President must be interested in the ways in which new knowledge affects his status and his purposes, and in which professional skills and administrative competence can support them" (p. 135). He is motivated not by any hostility to science but by the fear that scientists might use their specialized knowledge to determine policy and to pervert the proper functions of democratic control. To make his point, Prof. Price has to enter into a long historical discussion of the relations between science and government, particularly in the United States, but including much of the history of science in Britain with which he shows himself to be exceptionally well acquainted.

(Prof. Price, as might be expected, has been a Rhodes scholar at the University of Oxford.)

Ostensibly, the control of science in the United States is not so much the concern of the President or of the executive organs of state as of various congressional committees which are associated with different scientific programmes. The reference to congressional committees is assumed to guarantee control of scientific investigations and the policy arising from them, but that the control is really democratic cannot be uncritically accepted by anyone with more than superficial knowledge of how science is run in the United States; even an attentive reader of the book can see that the picture is unintentionally scarcely more than a 'cover story' for what actually goes on.

The picture presented is, in fact, one of very extensive administrative chaos because major considerations are necessarily left out. The enormous sums of money spent on science in the United States, for example, easily more than in the whole of the rest of the world put together, are not distributed according to any rational system but are due to the interplay, less of democratic than of military and economic considerations.

The underlying aim of the presentation here given of the scientific estate is to ensure that the scientist is 'on tap' but not 'on top'. But the scientist is at the same time a man and is recognized to have motives and interests of his own: "If we understand that the scientist and the engineer cannot be guided in their political actions entirely by what science teaches them, and that even when they think they are doing so they may be deceiving themselves and others, we are led to a realization that the fruit of the tree of knowledge is not always peace" (p. 142). The fundamental difficulty which Prof. Price never resolves is to work out a way in which people essentially ignorant of the facts of science can direct policy which more and more involves science. This applies equally to the United States and British systems.

The book will make difficult reading for a British reader unless he knows the United States very well, but the realities are easier to understand than the official theory which covers them. In various places Prof. Price realistically likens the administration of science to the feudal system where a number of rival barons are occupied in fighting against each other for the appropriations which would enlarge their prestige. They are kept in control, with varying success, by powerful agents of the President. The enormous sums spent on the policies on which they are based really speak for themselves. The underlying theme is war, of an objective or mechanical nature, or its equivalent in popular prestige, which is really war in another form, as applied to the Kennedy objective of landing men on the Moon by 1970. This is the objective of the aerospace agencies, for which a great effort is undertaken, equivalent to more than all that is spent on fundamental science in other fields, regardless of scientific opinion, but having the necessary public appeal and at the same time being extremely profitable to the firms involved, even to whole areas in which they operate for the time being.

In Chapter 4, "The Established Dissenters", Prof. Price shows how scientists themselves have used the prestige they have gained by their contributions to military success to attempt to influence policy directly, often in opposition to the policy laid down officially. Any logical plan for scientific expenditure can always be overridden in the military field because where victory or survival is at stake it is pointless to count the cost. Evidently this puts the direction of science as a by-product of the 'military-industrial complex' of President Eisenhower. But this is not developed in detail. His references to Oppenheimer are scanty and, among the established dissenters, the name of Linus Pauling does not appear in the index between 'Patronage' and 'Pearl Harbour'.

The book contains a fascinating account of the origins of successive administrative scientific bodies which have been set up to organize science in the United States since the time of Jefferson. How the United States ever came to be such a scientifically great power is not apparent. Evidently it was just as much an accident as the acquisition of the British Empire—in a fit of absence of mind. This is, however, very far from the whole truth. The principal policy has been to relegate basic science to the universities—all definite project research is paid for by the government and outside the ordinary funds of the university—and to entrust development contracts to leading industrial firms.

There have been from time to time various efforts at retrenchment but these have been quickly suppressed on account of the needs of recent wars, such as those of Korea and Vietnam. The basic principle is that universities cannot afford not to develop science to the utmost, but they do not wish to do this, mainly on the advice of the scientists themselves who are apt not to take the policies of their government as automatically perfect. In the process, the legislative forces are generally easily carried along on the basis of national security, containing communism, or showing that the United States has the biggest and most destructive weapons.

Prof. Price has chosen as an example the great post-War development of oceanography research: "Oceanography, for example, was to each of more than a dozen agencies an important means towards its particular purpose. But each agency, if left to itself, would have planned its research and development work in the way that would contribute to its own purposes, regardless of the interests of others. To make the programme into an effective whole, the Office of Science and Technology had to work with the agencies concerned. If possible it had to persuade each of them to include the appropriate share of the total programme in its budget, even though this meant that each had to undertake some work not directly relevant to its own purposes as it saw them and consequently that, in order to stay within its budget, it would have to cut out something that it would prefer not to do" (p. 246). But the central *raison d'être* of the oceanographic programme, though admittedly furthering the activities of the *Polaris* submarines, is hardly touched on.

The book is eminently readable but essentially of more interest to the administrator than to the scientist and carries valuable lessons for the comparatively cautious and unenterprising science in Britain, but, as can be seen from these comments, it leaves out as many important factors as it brings in.

J. D. Bernal

COMPENDIUM OF SPACE LORE

Sourcebook on the Space Sciences

By Samuel Glasstone. Pp. xviii+937. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1965.) 62*s*.

When the United States Government founded the National Aeronautics and Space Administration (N.A.S.A.) in 1958, the new organization was instructed not only to explore space but also to "provide for the widest practicable and appropriate dissemination of information concerning its activities". N.A.S.A. has faithfully performed this task by giving advance details of all projects and reporting the results of the experiments. To supplement these frequent Press releases and reports of current events, the Information Division of N.A.S.A. commissioned Dr. Samuel Glasstone to prepare a compendium of basic knowledge in the space sciences: this is the origin of the 937-page volume under review. N.A.S.A. holds the copyright, and the list of acknowledgments shows that no fewer than eighty-two individuals, most of them connected with N.A.S.A., gave their opinions of particular chapters in the book. Thus, although Dr. Glasstone is the author, *Sourcebook on the Space Sciences* must be regarded as bordering on an official N.A.S.A. publication:

the text and illustrations are both strongly orientated towards United States achievements. When we have made allowance for this bias, however, we are left with a book which it would be difficult to improve upon, except in minor details. The topics are well chosen, the treatment is accurate and assured, and the author is never guilty of introducing a needless fog of technical detail. The most pleasing features of the book are the clear and direct style of writing, the historical touches which so often illuminate descriptions of the present situation, and the author's courtesy in pausing to elucidate the origins of technical terms which in most books are flung at the reader in a daunting barrage.

The book begins with a historical review of astronautics and rocketry, from science fiction and twelfth-century Chinese rockets to the first actual launchings in 1957. Chapter 2 describes the basic properties of Earth-satellite orbits and space trajectories, while keeping clear of heavy mathematics. Chapter 3 is a thorough treatment of propulsion and power supplies, including an outline of the main United States launch vehicles. In Chapter 4, on guidance, tracking and telemetry, the section on tracking is deficient because it describes only those facilities supported by N.A.S.A. Chapter 5 is an account of the peaceful applications of satellites in meteorology, communications and navigation.

Chapters 6–12, which make up nearly two-thirds of the book, bring a change of emphasis, and are concerned with pure space science and astronomy. In Chapters 6 and 7, entitled "The Sun" and "The Solar System", respectively, the author steers a judicious course among the various controversial rocks on which the narrative might have been wrecked (for example, the origin of tektites—or of the solar system). Chapter 8 is a long and well-balanced survey of the Earth and its environment. Chapter 9 is devoted to the Moon, and again the controversial topics, such as the surface, figure and origin of the Moon, are discussed with such willingness to see both sides of the question that criticism is quite disarmed. In Chapters 10 and 11 we travel to the inner and outer planets, respectively, while Chapter 12 enjoys the comprehensive title "The Universe", and ranges from the minutiae of the Hertzsprung–Russell diagram to the starry-eyed speculations of cosmological theory. After these philosophical flights, there is a touch of bathos about the final chapter, "Man in Space", which might have had as its motto:

> The time has come, the Walrus said,
> To talk of many things,
> Of pressure suits and calcium loss,
> Of moons and Saturn's rings,
> Of waste disposal up aloft
> And whether LEMs* have wings.

This last chapter is certainly a useful reminder that although man may have his head among the stars, his feet are still in the gutter (if a misquotation from Oscar Wilde may be allowed to balance that from Lewis Carroll).

Besides being well written, the book is up to date and excellently printed, with very few misprints or minor Mistakes. In a book of such length, however, some errors have inevitably crept in. For example, in the map of Minitrack stations on p. 218, Winkfield has been translated from English soil to Dutch, and on p. 224 Canberra has slipped into Western Australia. The Sun seems to be rotating the wrong way on four diagrams (pp. 532–542), and, more serious, the derivation of the basic rocket equation on p. 171 is fallacious, even though it leads to the right answer.

The book carries good indexes of subjects and names, but there are no references or bibliography: a full bibliography would have been excessively lengthy, but at least there might have been a list of journals which normally publish papers on space science. Indeed, the title 'Sourcebook' is slightly misleading, since the book is not a collection of original papers. These are but minor flaws, however, in a masterly book which every student of space science will wish to acquire. Reviewers cannot even voice their standard complaint that the book is too expensive: for its price is less than a penny a page.

D. G. King-Hele

* LEMs, Lunar Excursion Modules.

NATO AND THE SOLAR SYSTEM

Solar System Radio Astronomy

Edited by Jules Aarons. (Lectures presented at the NATO Advanced Study Institute of the National Observatory of Athens, Cape Sounion, August 2–15, 1964.) Pp. xi+416. (New York: Plenum Press, 1965.) 17.50 dollars.

THIS expensive book is a series of lectures given at a summer school on the radio- and radar-astronomy of the solar system. Forty-three such schools were sponsored by NATO during 1964 at a cost of 650,000 dollars. No restrictions are placed on the form or organization of these schools, except that they take place in a country of the alliance; participation and financial support are not, however, limited to citizens of the NATO nations. In this case the country was Greece, and the Director of the Summer School was Prof. Michail Anastassiades of the University of Athens, who collaborated with Dr. Jules Aarons of the Air Force Cambridge Research Laboratories (U.S.A.) in organizing the programme. Their success is evident in *Solar System Radio Astronomy*: in many cases the lecturer was the acknowledged international authority on his subject. The students at the school were young scientists at the beginning of their research careers, and much opportunity for informal discussion was provided in the pleasant surroundings of Cape Sounion.

More than half the book is devoted to the radio emission from the Sun, both quiet and disturbed, and forms a useful summary of this immensely complicated subject, mainly from the point of view of the observer. Then follows a discussion by Hewish of the investigation of the far-out corona by the now-classical method of radio source occultation. This ends with a mention of the important subject of interplanetary scintillations, by means of which Hewish has detected many quasars.

The investigation of gaseous regions in the Solar System by radio and radar techniques, including sun radar, is discussed by von Eshleman. Since this chapter was written these methods have scored a major success in the measurement of the atmosphere and ionosphere of Mars by observing the occultation of the probe *Mariner* 4. This work was planned by a Jet Propulsion Laboratory–Stanford group. Propagation studies within the Solar System, either by ground-based radar or using probes as transmitters or transponders, will clearly continue to make important contributions; this lecture also includes a discussion of the Stanford electron density measurements of the *cis*-lunar region by harmonically related multi-frequency radar.

The rest of the book is devoted to the rapidly growing field of the radio- and radar-astronomy of the Moon and planets. Confronted with the volume and complication of the information now available, it is hard to recall that the first Venus echoes were observed as recently as 1961, and that the effective study of the planets by passive radio-astronomy is scarcely an older subject. A measure of the speed of advance in these fields is provided by the discoveries made since the summer of 1964 when the lectures were given, for example, the non-synchronous rotation of Mercury as detected by radar.

This is altogether an exciting book, reflecting the renewal of interest in the solar system, stimulated in part by the space age, but probably more by the new techniques of radio and radar. It will date less than do most conference proceedings, being the lectures of a summer school designed to be tutorial as well as topical.

J. H. Thomson

MAST CELLS

The Mast Cells

By Dr. Hans Selye. Pp. xxix + 498. (Washington, D.C.: Butterworth, Inc.; London: Butterworth and Co. (Publishers), Ltd., 1965.) 135*s*.

SIR ERNEST KENNAWAY once observed that "cancer research is tending to become buried under its own literature". Similar thoughts about the mast cell were evidently in the mind of Prof. Selye of Montreal when he set out to review, in *The Mast Cells*, an expanding literature already comprising some 2,500 titles. The treatment is novel—'a book within a book'. Each chapter and major sub-division opens in large type: collectively, these portions of the book form a running narrative, unencumbered by references, though legitimately coloured by the views and experience of the author. The remainder of the text, in smaller type, is made up of brisk, telegraphic abstracts of the pertinent literature. A numbered code guides the reader thence to a full bibliography. The final index is printed on tinted paper. Every aspect of the mast cell is dealt with. The result is superb.

There are two types of mast cell, both discovered by Paul Ehrlich early in his career. The first is the tissue mast cell which develops, dies and, as many believe, has its function, in the connective tissue. The second is the basophil (blood mast cell or mast leucocyte) the origin of which is in the bone marrow and the habitat of which is the peripheral blood. Both types of mast cell elaborate cytoplasmic granules which stain metachromatically with various basic dyes. They were named *Mastzellen* in the belief that their granules form under conditions of hypernutrition (*masten*, to fatten).

Sixty years after Ehrlich's discovery, Scandinavian workers gave to the mast cell its first plausible function. They were seeking a cellular location for the powerful anticoagulant, heparin, and found it in the metachromatic granules of the tissue mast cell. Fifteen years later it was shown, by us, in Scotland, that the mast cell is as rich in histamine as it is in heparin. This stimulated intensive investigation on the mode of action of the newly discovered histamine-liberators. There was more to come. American workers then demonstrated 5-hydroxytryptamine in the mast cells of mouse and rat. All these pharmacologically active agents are synthesized and stored within the mast cell. The mast granules are also curiously rich in protease and contain smaller amounts of other enzymes. Recent observations suggest that catecholamines may be present in certain types of mast cell. For long the mast cell lacked a function. Now it has too many. This is a baffling situation for the investigator: the newcomer, especially, looks for guidance.

Prof. Selye's book supplies his needs. There have been reviews in the past, of which, perhaps, Michels's brilliant chapter in Downey's *Haematology* (1938, and reprinted as part of a mast-cell symposium, *Ann. N.Y. Acad. Sci.*, 103, 491; 1963) remains outstanding. Specialized monographs are available on heparin, histamine, hyaluronic acid, histochemistry, mast cells and lymphocytes, mast cells and anaphylaxis, the basophil—there are many excellent shorter surveys. Selye has out-reviewed us all. For the moment the mast cell has been brought within the compass of the individual.

Armed with this lavish compendium the reader can see at a glance where knowledge is well advanced and where gaps remain. Short chapters on history, definition and terminology are followed by a long chapter on histology, including histochemistry. Embryology, a mere two pages, could already be profitably expanded by the inclusion of recent work by Burton (*Anat. Rec.*, 150, 265; 1964) and Combs, Lagunoff and Benditt (*J. Cell Biol.*, 25, 577; 1965). Likewise, the next section on comparative anatomy could usefully draw on the comprehensive survey by Grünberg and Kaiser (*Zbl. f. Veterinarm.*, 11, 729; 1964; 12, 18; 1965). The longest chapter, "Agents Affecting Mast Cells", includes a detailed account of Prof. Selye's own contribution to the mast-cell story: 'calciphylaxis' implies the deposition of calcium, often massive, around mast cells or their products in hypercalcaemic rats; 'calcergy' denotes a somewhat similar calcification in normal rats previously injected with certain metals. Calciphylaxis was itself the subject of a considerable monograph by Prof. Selye some three years ago. Excellent chapters then deal with the diseases of the mast-cell system, the mastocytoses and mast-cell dermatoses in man, and the mastocytomas in animals. The same thorough treatment leads through biochemistry and the blood basophil to theories. Here, though many points of view are considered, the reader will seek in vain for a hypothesis to cover all mast cells in all species.

Heparin can certainly be extracted chemically from mast cells, yet there is little to suggest a physiological release of heparin into the blood: in some quarters the mucopolysaccharide is now regarded more as a structural component of the granule than a potential secretion. Histamine is undoubtedly synthesized and stored in mast cells, and is easily released. However, one would question the statement (p. 366) that "virtually the whole histidine-decarboxylase activity of the body is concentrated in mast cells": even the specific enzyme (*p*H 6·5 and sole substrate, histidine) has a wider distribution. 5-hydroxytryptamine (serotonin) is restricted to the mast cells of mouse and rat. Calciphylaxis and calcergy have likewise as yet been demonstrated only in the rat: one has seen nothing like them in mice or men.

In the end, Prof. Selye ("Overview", p. 403) is driven to conclude that "the mast cell is a pluripotent cell, a kind of 'emergency kit' which contains a number of compounds that tissues may need, especially in regions exposed to local stress". This would certainly find more favour with me, as I have myself been advocating a local 'mast-cell cycle', intermittent in action and motivated by trauma, for the past dozen years (*Lancet*, i, 841; 1954). Perhaps the real 'emergency kit' is the book itself. No future explorer on the mast-cell trail can afford to travel without it. Author and publisher alike are to be congratulated on a splendid achievement. JAMES F. RILEY

BONE

Proceedings of the Second European Symposium on Calcified Tissues

Held at the Domaine Provincial de Wegimont, March 31st–April 4th, 1964. Edited by L. J. Richelle and M. J. Dallemagne. Pp. xv + 471. (Liège: Université de Liège, 1965.) n.p.

CALCIFIED Tissues contains forty-six contributions on cell differentiation, fluorine, bone remodelling, collagen and kinetics of bone metabolism. It opens with a paper by Prof. F. C. McLean, the recognized doyen of osteology, summarizing some of the progress made in recent years towards the understanding of the mechanism of internal bone remodelling. The picture, while a hopeful one, is far from being rosy, for, as the speaker recognizes, the resorption cavities originally described by Tomes and De Morgan more than a century ago are still not well understood to-day.

Parathyroid hormone seems now to play little or no part in the remodelling of compact bone, contrary to the belief upheld until recently by most people. The concept of 'calcifiability' of the organic matrix is gaining support together with the belief that local acidity, probably caused by the metabolic activity of the osteocytes under the partial control of vitamin D and the parathyroid glands, is responsible for the solubilization of bone mineral.

It is beyond my powers to comment on most of the contributions but attention will be paid to a few of them; beginning with the work on cell differentiation by the use of tritiated thymidine and tritiated glycine by Maureen Owen, who found evidence in the periosteum of the femur of young rabbits of the negligible role that the fibroblasts take in increasing cell population in the active bone-forming layer of the periosteum. The pre-osteoblasts, on the other hand, increase at a rate of about 33 per cent per day while the osteoblasts take an average of 3 days to lay down the matrix and become osteocytes. This work contributes substantial evidence that the specialized cells of bone are all different functional states of the same cell and that they can revert easily from one state to another. No conclusion is reached as to the origin of the progenitor cell but the suspicion is advanced that it may be a fibroblast, while no mention is made of the possibility that the source is the vascular endothelium which in perfused specimens is seen to occupy exactly the mid-position between the fibroblast layer and the pre-osteoblast.

Vaes contributes with a good paper in support of the view that the production of acid hydrolase of bone is segregated within special particles surrounded by an impermeable membrane analogous to the lyosomes found in liver.

Danis and Donizel in a paper on heterotopic ossification advance the view that the pre-osteoblast originates from migrating white blood cells. In this work they do not use vascular perfusion to determine the relative position of the vessel wall and the line of ossification.

The field of osteoporosis is well covered by several papers; in one of them Pratt and McCance add further data on the atrophic changes induced in the pig by under-nourishment, contributing with the finding that bone in this condition was always hypercalcified, reaffirming the previous observation that bone from an undernourished animal was as heavy as a bone of the same length from a well-nourished animal. Chalmers brings data on the different survival rate of the growth and articular cartilages in grafted limb rudiments, and Rutishauser and Tschantz investigate the early effects of pressure on bone. These are the only contributors who refer to the vasculature of bone and conclude that remodelling in the cortex under compression depends on the proximity to or separation from the vessels.

A detailed and lengthy work on collagen by Tristram and the discussion of his paper by Horman make a section by themselves, complemented by two or three other works on the subject.

The relation of bone matrix lipids to calcification is examined by Irving, while Ascenzi and Bonucci investigate the changes in the tensile strength of isolated osteons with advancing age and conclude that calcified and decalcified old osteons have tensile strengths of similar magnitude. This brings further evidence that the collagen fibres of the matrix mainly determine the mechanical properties of bone tissue.

Foster and his collaborators present the result of their investigations on the origin of calcitonin or hypocalcaemic factor, favouring the view that the thyroid is the source of this hormone and not the parathyroids. Haas and collaborators add evidence that calcitonin, so far demonstrated to be present in the dog, is also likely to operate in man.

It is sad that an excellent paper on autoradiography in the study of phosphates in bone is the posthumous work of Prof. J. Vincent.

Summarizing, this volume contains material by many of the experts on calcified tissues and thus makes available works appearing in journals scattered around the world. To me, a limitation is still evident which may explain the lack of progress in some respects pointed out by McLean in his introductory remarks; this refers to the lack of interest in studying the part that the circulatory system, both in its container—the vessels—as well as in its contents—the blood—plays in the regulation of the unending process of bone deposition and bone removal. Apart from a couple of paragraphs by Rutishauser and his group, no reference is made to this important factor in osteogenesis.

J. Trueta

A SUPERFICIAL VIEW OF MENTAL HEALTH

Mental Health in a Changing World

Edited by Kenneth Soddy and Robert H. Ahrenfeldt, with the assistance of Mary C. Kidson. (Vol. 1 of a Report of an International and Interprofessional Study Group convened by the World Federation for Mental Health.) Pp. xxiv+192. (London: Tavistock Publications, Ltd., 1965.) 35s. net.

THIS is a disappointing book. It is a report of the work of sixteen psychiatrists of various nationalities, two psychologists, two child psychologists, a biologist, an anthropologist, an educationalist, a scientific journalist, and a priest.

They formed a study group, first convened in 1961. Unfortunately, the journalist died and another member had to leave for emergency surgery. The rest were in continuous consultation from June 12 until June 24, 1961; but it is difficult to discover from the book how many times, if at all, they had further discussions.

The material for discussion was arranged into eight sections, which were further sub-divided. They consisted of clinical, research, prophylaxis, professional training, public education in mental health, mental health implications in other fields of science and professional practice, mental health implications in social and community life, and mental health and cultural attitudes.

One might have expected with such a distinguished study group, covering such a wealth of material, that something akin to the symposia of the Ciba Foundation Colloquia might have resulted. This has not occurred.

Thirty pages, out of 120, are devoted to the introduction, the background of the study group, and its work. The other 90 describe its conclusions. The rest of the book is composed of the bibliography. The conclusions are platitudinous and banal: the style is dull, pompous and repetitive. No doubt this is partly due to the death of the scientific journalist, but it does not add interest to the book.

Nothing fresh seems to have emerged, but some of the statements are dubious and do not bear examination. For example, it is stated that: "The very fact that some form of compulsion is necessary to get boys to fight in any national war is suggestive evidence against the assumption that war represents an outpouring of a national aggression which must inevitably find expression in that particular manner". Such a statement is not true; some young men do not need compulsion, some even welcome a chance to fight. Only in those who need compulsion is there lack of aggression. Most of us have seen films of young Indians shouting to have a chance to fight Pakistan in the recent conflict.

Again, surely every psychiatrist appreciates that people are often suspicious of him because they feel that psychiatry can be used harmfully as well as beneficially. To retail anecdotes of a military psychiatrist being greeted by a general at the start of official conversations with "Don't try to psychoanalyse me" seems futile.

These are but two examples of the sort of material described. The complaint which can be made against the book is that it is an attempt to cover too wide a field, with resulting superficiality. Indeed, if, as seems to have been the case, there was only one period of twelve days to discuss everything there was obviously not enough time to do so adequately.

The bibliography is a valuable one, so far as it goes. It is composed of books published on the subjects discussed, between 1948 and 1962. There are 675 references with the addition of some 66 supplementary titles. It does not go beyond the summer of 1962 because, as the authors say: "We lacked facilities to ensure that the list of recent additions is complete". No explanation for this is given.

The index is adequate and the book is well printed and nicely bound in black cloth with gold lettering.

Two further volumes are promised from the International Study Group, but it is to be hoped that they are more profound, and more useful than the present one.

Clifford Allen

CHILD PSYCHIATRY

Modern Perspectives in Child Psychiatry

Edited by J. G. Howells. Pp. xvi+595. (Edinburgh and London: Oliver and Boyd, 1965.) 105*s*.

THIS omnibus volume is a 'double decker'. The lower deck is designed to provide the scientific basis of child psychiatry and the upper the clinical superstructure. Unfortunately, its designer has omitted to provide a connecting stairway. By and large, there is very little contact between those who write about the development of normal children and the experts in child psychiatry. Nor, it would seem, is a great deal being done in child psychology at present that looks like being relevant, in any direct way at least, to psychiatric issues.

The lower deck is occupied almost exclusively by academic psychologists, who write from a variety of points of view and with varying degrees of understanding of science and children. Indeed the most useful are probably those in which neither science nor children are particularly obtrusive. Among the topics dealt with are research methodology, genetical issues, the development of perception, thinking, remembering and imagining, and the application of learning theory to child psychiatry. There are also contributions written from the standpoint of ethology and (inevitably) that of Prof. Piaget. The child psychiatrist in search of a 'scientific basis' for his work must pay his money and take his choice—or, as is more probable, do neither.

The psychiatrists upstairs provide a more coherent picture, in so far as their work reflects the investigation of relatively well-circumscribed groups of disabilities, for example, speech disorders, mental subnormality, childhood psychoses and epilepsy. There are also chapters with a more distinctively social slant, such as those on delinquency and on the psychiatric aspects of adoption. For the most part, these contributions are well organized, up to date and surprisingly free from jargon. Only in one or two cases do preoccupations of a somewhat fanciful character obtrude.

Modern Perspectives in Child Psychiatry is neither sufficiently authoritative to be accepted as a handbook nor sufficiently original to be taken as a contribution to research. It does, however, bring together recent work relevant to a considerable number of important topics in its field, and the editor, Dr. J. G. Howells, merits warm commendation on the judgment he has displayed in his choice of contributors. Nearly all are good and there is surprisingly little overlap. His own review of the child psychiatric services in Britain is also extremely capable.

While omnibuses have their uses, they are undeniably cumbrous, and in some circumstances pretentious. The way to establish a more adequate scientific basis for any part of psychiatry is simply to undertake more and better research, which may or may not involve the systematic investigation of normal behaviour. What is wanted, it might be suggested, is an amphibious vehicle, equally at home in the disparate media of psychology and psychiatry, and able to carry out pilot raids on any part of the psychiatric coast line that might seem vulnerable to scientific attack.

O. L. Zangwill

BELIEF SYSTEM OF A CARGO CULT

Road Belong Cargo

A Study of the Cargo Movement in the Southern Madang District, New Guinea. By Peter Lawrence. Pp. xvi+291 +6 plates. (Manchester: The University Press, 1964.) 35*s*. net.

SINCE the late nineteenth century, many native-led movements, of varying intensity and duration, with the goal of quick acquisition of European goods, have appeared in Melanesia. Now Peter Lawrence's book, *Road Belong Cargo*, can join a small group of anthropological studies which describe and reconstruct particular cargo cults.

Lawrence's study is historically meticulous: he traces the impact of Europeans in the Madang District of New Guinea from Miklouho-Maclay in 1871 to the time of his own field work between 1949 and 1958. His account includes the economic changes, influence of missions, and reactions to administration. He pays particular attention to the relations between the races at different times, and he presents a full investigation of the life of Yali, the principal post-War leader in the area. Perhaps his most important contribution to studies of cargo cults is his analysis of the traditional belief system and historical account of the interaction of traditional belief and myth with mission teaching in the actual doctrines of the cults.

The five distinct cargo beliefs and other movements in the Madang area are described. They vary: pagan in the early days, Christian, syncretic, and later a pagan revival. In the course of these variations there were also shifts to anti-European hostility, and in the explanation of why the natives do not have the cargo they so greatly want. The bitter experiences with Europeans, and continued low economic status, brought, in later cults, militant nationalism. After the phase in which the natives realized that the Europeans are not deities, and believed that they forfeited rights to cargo because of their stupidity, there was in the 1930's a conversion to a kind of materialistic and anthropocentric Christianity. But as race relations declined, the following cult believed that the missionaries were holding back information; native deities were identified with God and Christ.

In the careful examination of the career of Yali as policeman, army recruit, leader of a rehabilitation scheme, and cult leader, we can see a combination of reality and fantasy which probably exists in many parts of New Guinea; it is clearly evident in some other accounts of native movements and their leaders—Tommy Kabu and Paliau. These movements are for local improvement: villages are rebuilt, clean living and hard work are urged for the common good, and some revision of custom is proposed. But periodically, under the influence of cult prophets, this road to cargo is abandoned in favour of ritual means. Lawrence gives us precise turning points: Yali's humiliation and disappointment in Port Moresby in 1947, and his acceptance of a new cult prophet a few months later.

Discussions of cargo cults often stress the abnormal thinking and hysterical behaviour of cultists, and present an explanation in terms of the suspension of the normal. From this investigation and a few others we begin to see that traditional beliefs in Melanesia are entirely compatible with the expectation of a divine gift of cargo.

Paula Brown

The Theoretical Significance of Experimental Relativity

By R. H. Dicke. (Documents on Modern Physics.) Pp. xii+153. (London and Glasgow: Blackie and Son, Ltd., 1964.) Cloth 32*s*. 6*d*. net; Paperback 17*s*. 6*d*. net.

THIS book is a reprint, without change, of the contribution by R. H. Dicke to the Les Houches Summer School in 1963, which originally appeared in the larger volume *Relativity, Groups and Topology*, published by Gordon and Breach (1964). Possibly because of a difference in paper, the overall appearance of the book is slightly less clear than in the original form. The author takes the position that the experimentalists should not be working on the complicated applications of general relativity but rather on its foundations. He begins by discussing null experiments like that of Eotvos, and then considers in some detail the three so-called 'crucial' tests. There are fairly complete references to experimental results, and the greater part of the book consists of appendixes—mostly on the author's characteristic theories of Mach's principle. The contents of these is best summarized by their titles: "Experimental Tests of Mach's Principle"; "Mach's Principle and Invariance under Transformation of Units"; "Long-range Scalar Interaction"; "Field Theories of Gravitation"; "Cosmology, Mach's Principle and Relativity"; "Significance of Spatial Isotropy"; "Mach's Principle and a Relativistic Theory of Gravitation"; "Lee–Yang Vector Field and Isotropy of the Universe"; "The Earth and Cosmology"; "Implications for Cosmology of Stellar and Galactic Evolution Rates"; "Dating the Galaxy by Uranium Decay"; "Dirac's Cosmology and the Dating of Meteorites".

The book fulfils a useful purpose in collecting together published work on experimental checks of the theory, although from a rather idiosyncratic point of view.

C. W. KILMISTER

Quantum Electrodynamics

By A. I. Akhiezer and V. B. Berestetskii. Translated from the Second Russian edition by G. M. Volkoff. (Interscience Monographs and Texts in Physics and Astronomy, Vol. 11.) Pp. xix+868. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 170*s*.

QUANTUM Electrodynamics gives a detailed and systematic presentation of quantum electromagnetic theory. Like all good books on the subject, it does not conceal the defects of the theory, and emphasizes that a complete removal of the unsatisfactory features of quantum theory will probably not be achieved without new and basic physical concepts. The authors have therefore given much care to expressing the existing theory in its most logical and consistent form; for example, in preparing this second edition of the book they have extensively revised the section on renormalization, so as to present the concept from a simple physical point of view, avoiding purely prescription-like methods of eliminating divergences.

The book is divided into nine chapters. The first chapter, which starts from Maxwell's equations and is devoted to deriving the quantum mechanics of the photon, is followed by a chapter on the relativistic quantum mechanics of the electron. In the next two chapters the Lagrangian, the method of second quantization, and the scattering matrix are introduced and explained in detail, leading to a chapter on the interaction of photons and electrons, and the retarded interaction between two charges. In Chapters 7 and 8 the investigation of the scattering matrix is extended to higher orders (radiation corrections). Finally, there is a chapter on particles of spin zero.

The great range of the book, and the mathematical detail of the applications, will make it a valuable reference book for workers in the subject; but it will appeal equally to non-specialist readers, since it assumes only a very elementary knowledge of quantum mechanics. Even when the mathematical development becomes very elaborate, the lucid accompanying text would enable the reader to obtain a clear idea of the procedure without following the mathematics in detail.

P. M. DAVIDSON

Dictionary of π-Electron Calculations

By Prof. C. A. Coulson and A. Streitwieser, jun. Pp. xxx+358. (London and New York: Pergamon Press, 1965.) 100*s*. net.

DICTIONARY of π-Electron Calculations is a compilation of the results of simple molecular orbital calculations on planar conjugated hydrocarbons such as polyenes, polyacenes, various conjugated radicals and hydrocarbons and some heteromolecules. It will be very useful to people who are interested in the electronic structures of conjugated systems; I have already used it twice in connexion with a series of lectures on electronic theory.

The essential thing about a compilation of this sort is that it should be accurate. The risk of error has been minimized by photographing directly the printed output of the Mercury Computer at Oxford. This is about as fool-proof a method of ensuring accuracy as one could reasonably demand.

The book also contains an introduction which outlines the essential features of the Hückel method. The introduction is an excellent little monograph which could be recommended to people who want to find out the bare bones of the theory. There is also a section entitled "How to Use the Tables", which will teach the reader something about elementary group theory. Altogether, a most business-like and attractively produced volume and one which should be on the shelves of every chemistry library.

H. C. LONGUET-HIGGINS

Technique of Inorganic Chemistry

Vol. 4. Edited by Prof. Hans B. Jonassen and Arnold Weissberger. Pp. ix+401. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 115*s*.

TECHNIQUE of Inorganic Chemistry is the fourth volume of a series which sets out to give an account of the techniques used in inorganic chemistry and contains, like its predecessors, a rather mixed selection of topics, of which the chapters on "Magnetochemistry" by B. N. Figgis and J. Lewis and "Optical Rotatory Dispersion and Circular Dichroism" by F. Woldbye are of the most immediate interest and value; both of these are very comprehensive and pleasingly written, and the book is well worth having for them alone.

Of the remaining chapters, those on "The Growth of Oxide Single Crystals from the Fluxed Melt" by E. A. D. White and "High Temperature Technique" by R. G. Bautista and J. L. Margrave are of less general application, but are likewise extremely interesting, well-written and documented accounts, and may well suggest potential applications of the techniques to the reader who has hitherto had no occasion to use them; the chapter on "Ion-Exchange" by R. Kunin is, unfortunately, far too brief an account and suffers, in comparison with the other chapters, because of its brevity and an extremely short bibliography, although it does give a fair picture of the uses and techniques involved.

This volume is as well produced as the earlier volumes, and there are very few errors of any kind; those observed were innocuous, such as the extra metal *d* electron in Cs_2CoCl_4 (Fig. 8, page 169), and actinides are not all transuranics and vice versa, as is implied on page 144. The value of the book, as of the rest of the series, lies in the good coverage of most of the topics and in the bibliographies; it is these which make it well worth having.

K. W. BAGNALL

IMPORTANCE OF TECHNOLOGY

By Prof. JAMES K. FEIBLEMAN
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PURE science—basic research—has been highly praised; but this is chiefly due to the products of applied science, in which the average citizen is able to see at first hand the brilliant results of science itself. The poor reputation of technology stands in sharp contrast to these successes. Although technology is very much older than the sciences, it is stronger than ever to-day. Yet it has never enjoyed great prestige. Such neglect is undeserved, for in technology the highest of human values are involved.

The term 'technology' comes from the Greek *téchnē*, a word for art or skill, a regular method of making something. But the meaning has shifted. Technology once meant very much what the Greeks meant by *èmpeiría*: a rule of thumb applied without any theoretical understanding of what is involved, to get something done which needs doing. To have a technology at one's command was *emperírōs tinòs échein*, to know a thing by experience. It is the skill of the practical man long accustomed to the doing. In the modern meaning of technology, however, a material tool is usually part of a 'short cut' method of accomplishment. Science is theoretical and technology practical. Science satisfies the need to know and technology the need to do. But since the seventeenth century we have learned that by doing it is also possible to satisfy knowing. We learn by doing but only if we have an adequate technology.

By technology now is meant the knowledge and use of the techniques and devices by which man exploits his environment to meet his needs. A given stage of technology is measured by its efficiency. The essential point seems to consist in getting a piece of matter or energy to move against another piece. An artefact—that is to say, a material object altered through human agency—is to be produced out of raw materials by tools in accordance with some process. This may mean one thing operating on or affecting another thing in some way or other. The operation is technological so long as it is physical and employs a physical instrument or technique.

Whenever in a field of endeavour the material objects designed or the practices used become routinized or modified as a result of experience, then we are in the area of technology. Every particular endeavour has its own technology, whether farming or inscribing cameos. Having a technology is a minimum requirement for any discipline. Technology is always at the physical level and always uses physical means, and this remains true whatever the end. Technology thus includes the large theoretical areas concerned with instrumentation. Getting things done with the kind of advanced technology rapidly becoming available does not mean doing them; it means designing instruments to do them. In this way applied science and technology are rapidly coalescing under the general heading of technology, with pure and applied science furnishing the theory, and technology the devices.

We still tend to think of technology only as the effort to solve a practical problem by trial and error with tools. The technologist is known to be one who tries to discover a new method of meeting an urgent need by improvising wherever the work has taken him and by using whatever materials are ready to hand. This is one of the meanings subsumed under the definition we have adopted. But this is a highly significant one. Indeed we seldom comprehend the sweeping implications of such a conception. For in this sense, all civilization has its origins in technology. The first ape-like man who stood erect in order to have his hands free to throw a stone at an animal too swift to catch was also the first technologist, for his action fits the definition already given here. This happened according to the most recent findings some million and a quarter years ago.

It is interesting that civilization can be dated from the beginnings of bipedalism, and that technology was responsible for bipedalism and not the reverse[1]. Men stood erect in order to throw stones, they did not throw stones because they were standing erect. Thus it is technology which is responsible for there being men at all. In technology we are turning the environment in a certain way against itself, getting it to take shapes by using parts of Nature against other parts in order to reach goals we could not otherwise reach. We have found that through the use of tools accomplishments can be on a grander scale. There are no records of human beings without tools. Even the most primitive people alive to-day, the Australian aborigines, the Bushmen of South-west Africa, and the Guaharibos of the Brazilian jungles, have tools of sorts: digging sticks, throwing spears and cooking pots.

Of course, man-made tools are not the only ones. There are bird nests, beaver dams, beehives and anthills, and many more. However, human toolmaking is advanced in several important respects over the others. There is a continuity to the human effort made possible by social co-operation. Furthermore, human tool-making is extended into the production of quite another sort of tool: the sign. Signalling systems of a complex character which added to the range of the human capacity rely on technologies. Writing and printing, the manufacture of paper and books, are technologies which make possible the storing of information, and thus provide for a continuity of effort. Since there were so far as we know no theories of communication antedating efforts at communication, it may be supposed that the earliest means of communication were products of technology.

Tools are material objects which have been altered through human agency. But the tools that man makes are responsible for marked changes in his behaviour. Tools in fact force skills. With each new technological device, a skill must be developed in order to use it. Technicians who excel in the operation of instruments have existed at all times. Some can throw a spear farther, others can write better, still others have a native capacity for designing and executing better utensils. More often than not the use of tools requires prolonged effort. Not everyone can bring a piano to its greatest proficiency, and evidently the same statement applies to the operation of spacecraft.

Life of the human variety is mainly due to the device of an interchange between animal and tool. Man makes tools, but then he is affected by them, and this can be observed in many of his adjustments and ailments, from

habituation to occupational diseases. What man makes in a certain sense also makes him, and as he continues to improve his tools he also continues to be altered by them. It is a rare thing now for anyone to encounter in his ordinary experience much of the environment that his fellows have not had a hand in re-making. The clothes he wears, the shelter under which he lives, the food he eats, the ground on which he walks, and almost the very air he breathes, is not what it would have been without the performance of some human operation. We have produced our own environment and we are continually being affected by it. Primitive man had a few very simple tools. Civilized man in a modern industrial society is surrounded by highly complex machines. The degrees of civilization between these extremes is determined to a large extent by the current stage of development in technology.

From the point of view of human behaviour, it is necessary to remember that although technology is ordinarily considered a means and not an end, the situation is not actually that simple. For means often influence the ends they serve, while ends do not altogether determine the means which shall be employed. A technical device or skill is a preparatory response undertaken in view of operant behaviour. But if technology is always the means for getting things done it still has to be decided what has to be done, and this is a question of preferences. The first use of tools by primitive man was to reduce his hunger. The human needs do not change, but there are changes in the decision of which needs are to be emphasized. And in the making of such decisions the availability of technological tools is extremely influential. To-day we are devoted to transportation and communication on which we are committed to the spending of a disproportionate amount of our income. But there have been cultures in which the individuals were far less concerned with such matters. Certainly the problems which occupied everyone in Tibet were quite different. The emphasis of the culture will determine what the problems are to be, and the technologists will then address themselves to solving them. The Chinese had steam engines millennia ago, but used them only to open temple doors. The Hellenistic Greeks understood and to some extent used mechanical devices, but they were not very interested in this approach. With them as with the ancient Chinese the technology of ceramics was pursued with far greater avidity.

And what is science after all if not a more deliberate kind of technology? It was by means of tools that man enabled himself to found institutions and advance to a higher stage of culture. We can see this if we examine the procedures a little more closely. Once man had gained a little headway in providing himself with the articles necessary for immediate survival, once he had stored a little food and clothing and taken measures to protect himself in advance against the encroachment of enemies, he found himself in possession of that rare commodity, leisure time. By utilizing it properly, which is to say, by thinking about the nature of things without regard to any particular need but only for the acquisition of abstract knowledge (which might conceivably have included the problem of the satisfaction of the needs in general), he could set about devising new and more efficient methods for achieving particular need-reductions in the future.

One of his needs was, of course, to satisfy his innate and powerful curiosity, and for this purpose there were things he could do as well as things he could merely think about. Scientific method so far as it is the method of pure science is directed toward theoretical ends. But then almost by chance he found that such theoretical scientific discoveries could have immense practical consequences. Applied science, so beneficial to man in a practical way, was found to be the application to human problems and needs of knowledge obtained in quite another way. By endeavouring to reduce his need to know, man has come on principles the application of which in practice was quite capable of helping to reduce his other needs: his hunger, for example, his need of accomplishment, his need for shelter and for medical attention. If these principles could only be implemented, the gain would be enormous.

Thus the line between technology and applied science became thin. The only difference is that technology is closer to the practical problem. What I am referring to when discussing science, applied science and technology are three grades of increasingly particular concrete approaches to the concrete situation. Through pure science the principles are discovered, through applied science the method of application is found, and through technology the actual practice is worked out. Usually three sets of men possessing three different sorts of temperament and having three different but related kinds of interest are involved. The first group consists in men of a theoretical turn—mathematicians and mathematically minded experimental scientists. Men of the second group uniquely combine theory with practice, but not with an equivalent interest: they must be familiar with the theory but only because they wish to put it into practice. The third group contains the men on the scene, practical operators who must solve immediate problems with a minimum of equipment in order to get things done.

Every use of an abstract scientific principle must always be an application to some particular concrete situation. The actual world is a rich one. It always contains more than is indicated by our limited schemes. Thus unexpected developments are bound to occur in the course of any human endeavour. These often tend to upset or defeat the plans of the applied scientist. New calculations must be made or new adjustments devised: and so it becomes the task of the technologist to meet such challenges to successful practice.

The development of technology has brought with it a whole host of attendant theoretical problems. The theory of general propositions required for scientific laws has proved difficult enough to formulate. From the classic Greek period to our own day, discussions on this topic have been conducted without reaching any satisfactory solution. This is not due to the peculiar character of the discussion but to the extreme complexity of the issues. But if a suitable theory of general propositions has proved difficult to construct, this is as nothing compared with the problems presented by the theory of the concrete individual. A material object or event is always involved in the labours of the technologist. Its analysis is a prerequisite of the understanding of his work. Such an analysis has scarcely been begun. Its successful outcome would be of great interest to the theoretical explanation of technology.

All abstract formulations are of necessity ideally and universally stated. The theoretical scientist is concerned with them. It is he who discovers a hypothesis but the experimental scientist who verifies or falsifies it in the laboratory, usually by testing the deductions from it. The speculative area of concern to the applied scientist is some particular sub-class under the indefinitely large class of the ideal formulation. It is the applied scientist who finds the special class of occasion on which a law can be effectively applied in practice. But the area of the technologist is narrower; it is some individual and particular occasion. The analysis of material is the work of the pure scientist. The way in which such materials could be combined or used in industry is the work of the applied scientist. But the way in which materials could be brought to bear to solve a particular task is usually the work of the technologist. He is the one who learns when he actually applies the law to a particular occasion that its application can be improved. He is the man on the site who cannot avoid the fact that an insufficient number

of variables were taken into account in the original formulation when this particular job was being planned.

In order to see how the technologist works, let us, for example, consider a problem in structural engineering. Suppose a river bridge is to be built. So far as this task is concerned, the work of the theoretical scientist was done long ago when he discovered the engineering principles which will of necessity be involved. His field is mechanics, the mechanics of shears and moments, of stresses and strains. The applied scientist is he who will make the calculations from mechanics which are necessary to solve the particular problems. Once he knows the maximum weight the bridge will have to bear, the width and speed of the river at the site, the character of the soil on the banks, and other details of this sort, he can design the structure and specify the materials.

But there are other problems more particular still which cannot be worked out in advance. For no matter how thorough and precise the calculations, difficulties which had not been anticipated are bound to present themselves as the work progresses, and these require the presence of an engineer. For example, test borings at the site may have indicated granite—but not broken granite: and so now the engineer must decide what to do. Should he go down into the stone another ten feet to see what the conditions are, or should he support the structure on the broken stone by increasing the footings sufficiently to take care of it? Again, somehow in designing the bridge, signal lights on the abutments had been forgotten, and he will have to make last-minute arrangements to install the necessary conduits. Finally, the expected stone copings for the abutments were not delivered. Should he try to produce a mixture of fifty yards of exceptionally strong and durable concrete to replace the stone?

Science, then, is not the same as technology; but the relationship becomes increasingly clear. Science is technology looking to its own future. A good illustration is to be found in present-day developments of projects intended to further the exploration of outer space. In the design of propulsion systems, in the techniques of launch, separation, re-entry, and recovery of spacecraft, to say nothing of the design of the booster rockets themselves, technology furnishes the means to applied science. The search for reliable knowledge by means of the scientific method is only a variant and a late development of the search for practical knowledge, and science itself a further stage of technology.

If technology and applied science are intimately related, so are technology and pure science. Pure science and technology are so inextricably interwoven that one has often led to the other. In the case of the steam engine the technology came first and the science afterwards. Carnot developed the physical science of thermodynamics after the investigations of the implications of the steam engine. In the case of atomic energy, the science of quantum mechanics came first and the technology second. Planck's work and that of many other theorists and laboratory experimentalists came before the atomic bomb and the plant in Shippingport, Pennsylvania. But in both cases, whichever came first, there is a technology and there is a science.

Finally, there is a certain sense in which it can be said that the scientific method itself depends on technology. Observing by means of instruments, setting up a hypothesis to account for the observations (and others of a like class not at present observable), testing for verification or falsification by means of experiments with still other instruments, and then making calculations from the established results, which we call the scientific method of discovery, is only after all a kind of advanced technology. It is a deliberate method built to replace the trial-and-error method, but one providing for surprises and containing the trial-and-error elements. It is, one might say, a planned technology rather than the classical sort of technology conducted under the pressure of exigencies, a way of anticipating the requirements rather than of meeting them at the last hour. Technology was once the way to improvise a practice, then it became the way to get from science to practice, now it is the step which facilitates both pure and applied science by means of invention.

For science would not be science without laboratory instruments, and applied science could not be applied without technical devices. In the former case, consider the enormous importance of the cyclotron, which has furthered nuclear research; and in the latter case, consider the aeroplane. In the case of Leonardo da Vinci, who speculated on the possibilities of flying a heavier-than-air machine, not only was the theory inadequate but the requisite technology was also lacking. With the proper theory but without the proper technology he still would not have been able to fly.

According to the meanings which have become established through usage, when an instrument is used to get a theoretical task done, we call it scientific; and when an instrument is used to get a practical task done we call it technological, even though the procedures used may be very much the same. Scientific advance depends on the development of laboratory instruments, and technological improvements make possible the progress of experimental science. Scientific advance rolls down in waves of concentration, from observations aimed at discovering regularities and through them the formulations of abstract laws, to the invention of new techniques and devices necessary if the laws are to be applied. The centre of interest shifts from the problem of how to discover new principles to the problem of how to provide new equipment.

Within the scientific method itself, in the step-by-step process by which it is conducted in the laboratory, one finds a host of particular technologies. In chemistry, for example, in selecting one at a time the techniques for analysing materials in order to discover their constituents and structure, one has at one's command if one is a professionally trained biochemist a number of alternative approaches, such as freezing, crushing, drying, grinding, solubilizing, heating in strong acid, suspending in fluids of various densities, centrifuging, evaporating, and many others. There is usually a 'working hypothesis' somewhere in the background, a proposition accepted as though it had been verified in order not to hold up the proceedings, and its consequences put to use when no experimental test that could result in verification has yet been devised.

Pure science cannot be tested in advance for utility. Fortunately, this is the case, for there are too many discoveries which are the result of chance, which is to say, of accident or the trial-and-error method. Technology, however, is not entirely hit or miss. It contains that kind of procedure too, but relies also on the intuitions of the technologist, his 'feeling for the job', based on years of experience of living with the tools and operations. Rubber was a discovery made by chance and improved by science. Often the relations of pure science, applied science, and technology are turned around. The discovery of free radicals, for example, was made by chance in the course of a routine scientific procedure and later adapted by technology to many practical purposes.

Science is certainly the best method which has thus far been devised for obtaining a certain kind of result. On the other hand, not everything is readily amenable to scientific treatment. The reliability of the method is all the more remarkable when we remember that it is not expected to yield certainty. For even the most accurate method known for deriving reliable knowledge is still only a way of probing in the dark. We do not know very much about what we shall find, and afterwards we can never understand altogether what we have found. The infinite abundance, the extent and complexity, of nature remain, however much we examine it, and always manage to escape our full comprehension. Hence the necessity

of having a last-ditch method of attack. Science will never accomplish so much that the more elementary approach to an unaided technology as a separate and independent becomes dispensable.

Science has reinforced technology—not eliminated it. Behind the technologist there is the theoretical scientist and his applied brother. They are able to carry the solution of the problem an immense distance—in fact, all the way to the frontier where work must at last be done. But at the frontier there always arise additional problems, and for these the skills of the technologist are necessary. The theorist was the one who estimated the amount of energy that would be released if the atom were split, and the applied scientist suggested how to split the atom and even devised a method for doing it. But it was the technologist who had to make the decisions when to pull the rods in order to slow down the reaction to a controllable rate.

In what is to-day called 'big science', the science of the linear accelerator and the orbiting astronomical laboratory, for example, exploration is a form of scientific experimentation and technology is the frontier. The bigger the science the more the problems solved on the spot call out the knowledge and skill of the experimenter. At this point technology becomes not only indistinguishable from applied science but also from basic research.

We tend to think of technology as physical, and so relate it to the physical sciences, and in particular the science of mechanics. It is not so limited. Every science has its technology, and there are many areas where no science ever existed which have their technologies as well. Every variety of human activity of the disciplined and formal sort has its own technology, the material means for getting things done. We think of technology as the practical side of physics simply because in it the two material aspects coincide, a physical means for doing physical things. But there are physical means for doing biological things, psychological things, and even anthropological things.

The technological frontier in the biological sciences is to be found in the adaptation of physical and chemical tools and techniques to the biological subject-matter. The applied branches of the biological sciences lie, of course, chiefly in the practice of medicine, and medicine was until recently almost entirely given over to the experience and intuitions of the family physician. The techniques used in the practice of medicine had arisen largely as a result of treating patients. Thanks to physics and chemistry, however, the scene has changed considerably. No one will deny that the progress made in medical practice in the past century equals that made in all the previous millennia. The rapid improvement can be attributed in large part to technology. The X-ray, the spectrometer, the electron microscope, did not emerge from practice but were introduced from the outside; and neither did the antihistamines, the antibiotics, the tranquillizers and energizers, which have produced such enormous improvements in therapy: these were the work of the biochemists. Technology contributed its part by bridging the gap between scientific discoveries and manufactured products. The intuitive method, of course, has been reduced, but it still exists at the bedside. But technology has taken over in the laboratory where the applied biochemist has the problem of reducing his theoretical knowledge to utilizable form. His instruments have to be continually invented or improved, and new techniques discovered or devised.

The technological frontier in psychology lies in such activities as the designing of instruments such as the animal maze and the Skinner box, and the devising of techniques for conducting experiments, for example, the implanting of electrodes in the brains of animals in order to test reactions.

Psychology is such a relatively new science that the pioneering of experimentalists often enables the technologist to take the lead. Consider, for example, the role of technology in Pavlov's earliest conditioning experiments. The invention of the dog's stand and special harness were necessary components, and made it possible for Pavlov to verify the existence of the conditioned reflex. In applied psychology, there is the professional use of the psychoanalyst's couch, the administration of electroshock, the injection of sodium pentothal as part of the technique of narcosynthesis, and the specially equipped tubs employed in hydrotherapy.

The technological frontier in anthropology relies on such technological aids as the carbon-14 dating method and various solutions for cleaning and preserving archaeological findings. Many trial-and-error methods of on-the-site reconstructions are resorted to by practical archaeologists. We have come to recognize in the recovered artefacts of a dead culture part of the technology of that culture.

Technology is far-ranging and cannot be confined to the sciences. The arts have their technologies as well. The equipment of the painter, for example, offers many opportunities for the practice of technology. New paints of a more durable sort have to be discovered, new surfaces adapted to the painter's needs. In our own day many industrial products have been used for this purpose. Painting on prepared surfaces with plastic paints offers many advantages. In sculpture new material media are available such as aluminium and welding iron. In music new electronic instruments have been invented, instruments with a larger range of sounds and more easily modulated. The technologist was always very much in evidence in music, which requires many precisely made instruments, such as the violin or the piano.

In addition to the pure sciences and the fine arts, which have long been in the habit of considering technological assistance adjunctive, there are still a large number of areas where technology alone prevails. Cooking is neither an art nor a science, but only a technology. The same could be said for a great many other trades, such as tailoring, lumbering, or even teaching.

To-day we are witnessing the rapid speed of technology. But such a prevalence has its attendant ills, the danger, for example, of living for technology instead of by it. Usually what we do is conditioned by what we can do. Wars have often been fought because they had been prepared. The transmission of vast accumulations of data brings with it the temptation to transmit vast accumulations of data even when such an operation serves no reasonable purpose. If men climb mountains because the mountains exist, it is also true that data are processed because of the rapid improvement in the technology of data processing. The threat to technology consists in not knowing how to use it properly, and misuse can consist as much in excess as in defect. In the direction of excess there lies waiting the danger of recessive tendencies, of populations weary of expensive toys and discouraged because the outlay of vast expenditures of capital and of energy has produced so little in the way of solid satisfaction: a gain in knowledge, perhaps, but not in human happiness. How much education will be required before a sufficient number of people can be made to understand that dedication to inquiry is the highest of human purposes? In this way they might authorize the society to continue its support. If the significance of technology were to fail, the patience with its increasingly large demands might fail also. We would not want that to happen when we are so near to the successful servicing of all human needs. The role of technology as the mediator between science and the other cultural values has yet to be sufficiently recognized and acknowledged, and this involves a certain risk to the continued achievement of the values themselves.

[1] Sphuler, J. N., in *The Evolution of Man's Capacity for Culture* (Wayne University Press, 1959).

THE 1964–65 BELGIAN TRANS-SAHARAN EXPEDITION

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DURING the winter of 1964–65, under the leadership of Mr. Petiniot, the Belgian Trans-Saharan Expedition, sponsored jointly by the Minister for National Defence and the Minister for Foreign Affairs, accomplished the first crossing of the Sahara from the Atlantic seaboard to the Red Sea (Agadir to Safâga).

It was composed of a team of twelve, mostly military personnel (navigators, radiotechnicians, mechanics, a doctor, a nurse and others), and 3 heavy Unimog Mercedes lorries.

The most difficult travelling was between Faya-Largeau (north-east Chad) and the Red Sea, through some 1,500 miles of desert regions, very difficult of access, rarely explored and virtually unknown from the botanical point of view[1]. This fact and the impossibility for a scientist of visiting these regions on his own, unaided by the many facilities afforded by modern science, prompted the Minister for National Education to appoint a botanist to benefit from the exceptional opportunities offered by the expedition.

Route

Fig. 1 shows the route followed by the expedition across the Sahara. After having stayed several weeks at the Chad Research Section of the Office de la Recherche Scientifique et Technique Outre-Mer, Fort Lamy (Chad), in the northern Cameroon and on Lake Chad, the botanist joined the expedition at Faya-Largeau (Chad) and crossed the desert with it as far as Suez.

Collections

The materials brought back are:

(1) 564 *herbarium specimens* (J. Léonard 3340–3903), collected in the Chad (387), in the Cameroon (67), in Libya (40), in the Sudan (9) and in Egypt (53), among which 8 were algae collected by Dr. Onclin in Algeria (4), in Morocco (2), in the Rio de Oro (1) and the Niger (1). Many delicate flowers and fleshy fruits were preserved in liquids. All these collections have been deposited in the State Botanical Garden in Brussels, but duplicates will be shared with the Royal Botanic Gardens, Kew, and the Museum in Paris. The collections comprised 2 fungi, 4 ferns, many algae and 522 phanerogams belonging to 71 families, the most important being Gramineae, Cyperaceae, Mimosaceae, Papilionaceae, Caesalpiniaceae, Capparidaceae and Euphorbiaceae. Special attention was paid to collecting algae and plankton. Throughout the whole route, plankton was collected in each water-hole; 36 such samples and various algae have thus been collected. They make up a unique collection which has been found by Mr. Compère of the Brussels Botanic Garden to be, on a cursory preliminary examination, extraordinarily rich and varied.

(2) 195 *pollen collections* authenticated by herbarium specimens. They will be kept as a reference collection for the identification of fossil pollens when an effort is made to reconstitute the former vegetation which covered the Sahara.

(3) *Seed samples* of 117 species, which, sown in the Botanic Garden in Brussels, have already produced 62 seedlings and some specimens already in flower.

(4) *Samples of sand* collected in various localities on the route. These sands, watered, have already produced seedlings after a few days in the hot-houses, thus disclosing that they contained disseminated viable seeds. The determination of the plants will roughly outline the ephemeral vegetation that springs up after every heavy shower.

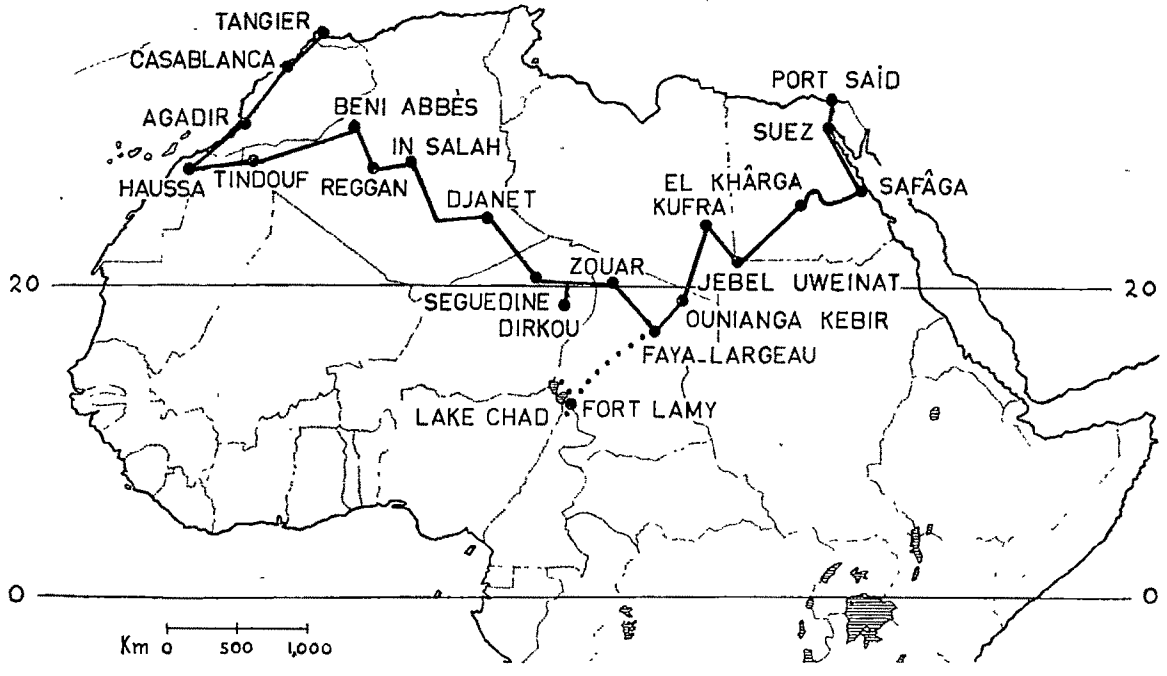


Fig. 1

(5) *Samples of various rocks.*

(6) *Fossils*, among which were silicified tree trunks, dating from the Cretaceous or the lower Eocene and half a lower mandible of a mammal living some 2–3 million years ago.

(7) *Many prehistoric tools* and more recent objects: carved stones dating back some 50,000 years, others 10,000 years, Neolithic polished axes, arrows, daggers, rings, necklaces, bracelets, etc. These collections have been deposited in the Ethnographic and Prehistoric Sections of the Museum for Central Africa in Tervuren, Belgium.

(8) *A colour film* illustrating the course of the expedition and several hundred colour slides illustrating landscape and vegetational types.

Botanical Researches carried out Prior to the Crossing of the Desert

Attention was specially concentrated on the aquatic vegetation in the region of Fort Lamy (Chad and Cameroon) as well as on the vegetation of the banks of the Rivers Chari and Logone: *Utricularia* and *Nymphaea* aquatic association, various aquatic grasslands (*Oryza*, *Vossia cuspidata*, *Echinochloa pyramidalis*, *E. stagnina*), riparian shrub vegetation periodically flooded (*Sesbania*, *Phyllanthus*, *Mimosa* and *Salix*).

Thanks to the kind invitation to join a foray organized by the Chad Research Section of the Office de la Recherche Scientifique et Technique Outre-Mer on Lake Chad, its little-known island and riparian vegetation were observed. The following plant communities were examined: aquatic vegetation (*Pistia* and *Lemna*; *Utricularia* and *Nymphaea*; *Potamogeton* and *Vallisneria*), semi-aquatic herbaceous vegetation (*Cyperus mundii*, *Vossia cuspidata*, *Echinochloa pyramidalis*, *Cyperus papyrus*, *Phragmites*, *Typha*) and also the wooded steppic vegetation of the sandy islands (*Acacia*, *Balanites* and *Leptadenia*).

The *Cyperus laevigatus* vegetation surrounding natron pools and ponds were examined in the course of a foray to the north of Lake Chad.

Crossing of the Saharan and Libyan Deserts to the Red Sea

The botanist joined the Trans-Saharan Expedition at Faya-Largeau (Chad), last important post at the edge of the desert. Ounianga Kebir (Chad) was reached after having covered 150 miles in a day and a half and collecting 26 plants. The expedition stayed there for ten days close to a lake situated in a paradisiacal landscape. The comparatively rich vegetation was intensively explored.

The oasis of Kufra, in southern Libya, was reached after having crossed the stark desolation of 460 miles, in two and a half days, of a stone and sand desert through which the track of the famous General Leclerc expedition of the Second World War was followed. On the last 400 miles, only five species of plants were seen and collected (*Capparis decidua*, *Cornulaca monacantha* and three different species of *Aristida*).

After studying the vegetation of the natron pools of Kufra, the expedition headed towards Jebel Uweinat, an important mountainous mass reaching 6,000 ft. and marking the boundary between Libya, Egypt and the Sudan. The 170 miles of country traversed were virtually devoid of vegetation. Only two species of plants were seen (*Cornulaca monacantha* and *Panicum turgidum*).

Many wadis radiate from the base of the Jebel. If they were dry at the passage of the expedition, they were nevertheless carpeted by a fairly dense and very interesting vegetation made up of about twenty-five species, nearly all in flower. Jebel Uweinat itself is a mountainous region barely known (certainly not with regard to its flora), the exploration of which should be of the greatest interest deserving of specialist investigation.

After having to endure a sand-storm, some 500 miles of most barren desert were covered in three days. What vegetation existed was strictly localized at the foot of the mountains lying on the route. Elsewhere, only rare tufts of *Aristida zittelii* were seen at long intervals. This region of very difficult access is most rarely visited although it reaches into the heart of Egypt close to the oasis of El Khârga. On reaching this place, the signature of the famous German botanist Schweinfurth was found engraved on the ruins of an old temple; he had visited this place a hundred years ago.

Having crossed the Nile, the expedition drove on once more into barren lands until the Red Sea was reached and later the Gulf of Suez and Suez itself. Thanks to recent rains, abundant collections were possible in the wadis and the ephemeral vegetation was examined.

Researches carried out during the Crossing of the Desert

Although time was very limited and insufficient to carry out any elaborate investigations, the following plant communities were nevertheless examined: aquatic vegetation of salt or natron waters with *Chara* and *Ruppia*; semi-aquatic herbaceous vegetation near freshwater springs with *Cyperus mundii* and *Paspalidium*; riparian herbaceous vegetation of the salt and natron lakes with *Cyperus laevigatus* and *Juncus*; herbaceous vegetation of the more or less moist sands with *Sporobolus spicatus*, *Imperata cylindrica*, *Desmostachya bipinnata*, *Panicum* and *Aristida*; vegetation of the sub-desert steppe with *Acacia*; vegetation of the grass steppes with *Chrozophora*, *Fagonia*, *Aristida*, *Aerva* and *Cornulaca*, and also the various vegetations of the wadis with *Aristida*, *Tribulus*, *Cleome*, *Aerva* and *Astragalus* (Jebel Uweinat), with *Aerva*, *Artemisia*, *Zilla* and *Zygophyllum* (between the Nile and the Red Sea), with *Haloxylon* and *Zygophyllum* (Red Sea) and with *Haloxylon*, *Panicum* and *Retama* (between Suez and Cairo).

Observations were also made on the adaptation of grasses to the wind-blowing of sand and their influence on their fixation. The indigenous food plants were also investigated.

Lastly, lists of vernacular names in the Gorane dialect and in Arabic with corresponding Latin names were drawn up for all plants encountered between Faya-Largeau and Jebel Uweinat.

An Edible Alga

In the market of Fort Lamy (Chad) one can purchase a greenish edible substance called *Dihe* which is sold as a flat cake. According to the information obtained in the market, it appears to be an alga collected on the bottoms of seasonally dried-up ponds and shallow waters in the north of Lake Chad and consumed by the local population. As the harvest period did not coincide with the visit of the botanist the inquiry was not carried further.

However, on arriving in the region of Ounianga Kebir, right in the middle of the Sahara, more than 750 miles to the north-east of Fort Lamy, the botanist was struck by the abundance of a microscopic alga in some lakes where it forms a real mash, easy to collect with a plankton net. Although the local population appears to be unaware that it might have a food value, the botanist suspected that it might be the same alga as that sold in cakes in the Fort Lamy market; he prepared some cakes according to the recipe obtained at the latter.

Both cakes, those bought in the market and those prepared at Ounianga Kebir, were examined by Mr. Compère (State Botanical Garden, Brussels). They are almost exclusively composed of a Cyanophycea: *Spirulina platensis*. Further, according to chemical analysis carried

out at the Laboratoire Intercommunal de Chimie et de Bactériologie in Brussels, it appears that it is a food-plant very rich in proteins and therefore very useful for consumption by the protein-deficient desert nomads.

Investigations are proceeding in this respect (chemical analysis, cultivation, ecology, etc.).

The Expedition has proved that, in spite of the many difficulties set by the terrain and above all those of petrol and water supply, it is possible to cross the Sahara from west to east provided proper equipment, specialists and technicians are available.

From the scientific point of view, the results of the expedition can be qualified as fruitful, not only from the abundance and quality of the material brought back, but also by the investigations which will result from it, and, perhaps later, the development of these regions, some perhaps barren, but others the wealth of which still is to be revealed.

[1] Léonard, J., and Brenan, J. P. M., *Webbia*, 19, 907 (1965).

PATTERN FORMATION IN AGGREGATIONS OF ENTITIES OF VARIED SIZE AND SHAPES AS SEEN IN MOSAICS

By RICHARD E. M. MOORE

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ANCIENT floor-mosaics provide material for investigating the formation of geometric patterns from the packing of irregular cubes in one plane. In contrast, modern mosaic stones are normally either uniform in size and shape or cut to suit.

More than 1,000 Roman and Greek mosaics were examined, largely from England, Italy, North Africa and the North East and East Mediterranean, and dating from *c.* 400 B.C. to about A.D. 530. As recently as 1955 the procedure of the mosaicist was regarded as unknown[1]. Apart from contributions[2,3] to the solution of this problem, the investigation has lent support to the hypothesis[3] that the packing of particles which vary randomly in sizes and shapes, within extremes, favours alignments of these particles at calculable intervals. This derived from investigations of bubble rafts, sand crusts, scums, the cells of leaves, and other particulate systems. It was also approached from a mathematical point of view (to be published elsewhere) considering random packing of cubes, irregular shapes and of spheres. Much attention has been directed to sphere packing[4], but the theory of cube packing has apparently not been investigated by others[5].

Materials

In simplest form a mosaic pattern is an area of stones of colour different from the others. The area is often circular or rectangular (Fig. 1*A*). A frequent pattern is a 'tracery' of stones of different colour from those forming the 'background' (Fig. 1*B*). A 'tracery' pattern often consists of several rows of stones, thus forming a thicker band (Fig. 1*C*). 'Tracery' patterns may be complicated by the band being itself patterned (Fig. 1*D*), or by a decorative rim (Fig. 1*E*).

Investigations of several thousand stones from floor-mosaics showed them to vary considerably in size and shapes within extremes, in each mosaic, in a largely random fashion. Trial also shows that 'random' stones result from the methods (to be published elsewhere) probably used in antiquity for stone manufacture.

In floor-mosaics, stones that disrupt patterns (but which would have been suitable for use elsewhere) were frequently found, indicating that they had not been carefully picked by the mosaicists, but used 'as they came'. In contrast, stones of wall-mosaics were apparently often specially cut to fit. Some wall-mosaics are more akin to marquetry.

That stones in floor-mosaics were not specially spaced out so as to conform more closely with the attempted patterns is apparent from the stones being tightly packed. Removal of the cement shows that even stones widely separated at the surface of the mosaic are often in contact deeper down.

The difference between the ancient floor- and wall-mosaic crafts was sufficient for the workers to be known by different names (for example, *tesselarii* and *musivarii*, respectively[6]). Wall-mosaic workers were largely concerned with pictorial scenes, often using materials unsuitable for flooring. Mosaic floors were normally ground smooth, whereas the stones in wall-mosaics were frequently of reflective materials.

Some floor-mosaics have, at the centre, a small, finely constructed mosaic picture. Because these 'centre-pieces' are so different from the surrounding mosaic (and from floor-mosaics generally), but exhibit the peculiarities of wall-mosaics, it is likely that they were made by wall-mosaicists. This is further indicated by these centre-pieces being set in trays (still remaining in the floor) and thus at one time would have been portable mosaic panels. These are known as *emblemata*.

An examination showed that stones for floor-mosaics were normally formed by breaking, but some of the earliest Greek ones are natural pebbles. Measurement of about

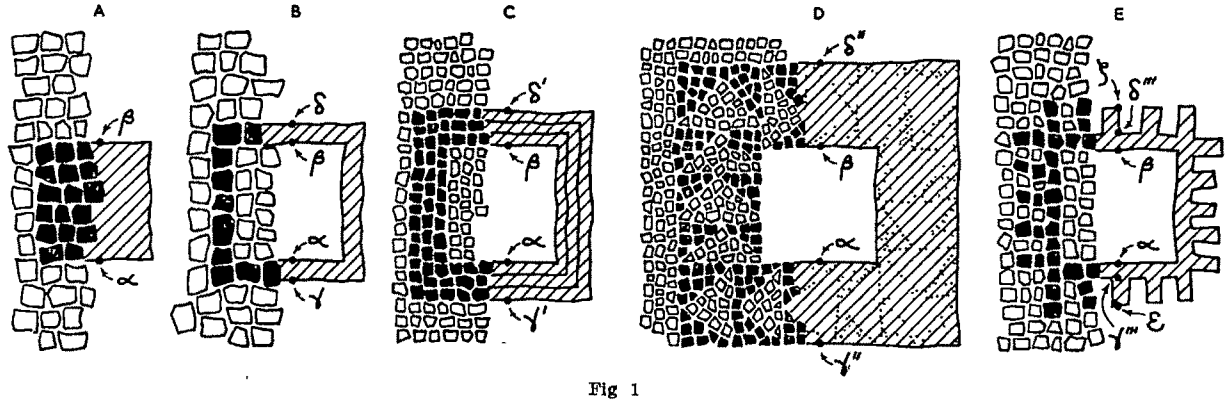


Fig 1

30,000 stones shows a typical modal size of their top faces (per mosaic) to be very nearly 1 cm × 1 cm. Some mosaics consist of stones about 0·2 cm, whereas others have a modal value greater than 4 cm. A typical size ranges between −48 per cent and + 34 per cent of the modal value, but is often greater (> ± 55 per cent). Ranges are normally smaller for small stones, and curiously for the larger stones also. The medium sizes exhibit the greatest ranges.

The stones generally lie in rows. Their dimensions measured tranversely to the row are normally more constant than their dimensions longitudinal to the row. From observation and experiment, the size of a stone appears to depend partly on the material used. It is often said that the average size of stones depends on date of construction, but the small number of measurements relied on, and the uncertainty of dates, do not allow a definite conclusion.

It is common to find two distinct sizes of stones in a floor. The bigger stones, of 3–4 times the side length of the others, exist solely as a peripheral region occupying the space between the perimeter of the true mosaic and the walls of the room. Mosaics are often set slightly askew with respect to the contemporary floor limits, and are sometimes considerably smaller than the area available. In England, these big stones are normally broken tiles.

Observation : Mosaic Growth

Stones which are unusual in size, shape or positioning with respect to their neighbours were found to give rise to distortion on one side only (Fig. 2). This implies that the stones in the distorted region were positioned progressively away from the 'abnormal' one. Inspection of many mosaics showed that these directions point away from the mosaic centre (away from one end in the rare repetitively patterned corridor mosaics).

The relative severity of errors and irregularities increases with the distance from the mosaic centre, and this also indicates growth from the centre. Detailed investigations of the relative positions of cause and effect disturbances indicates that the mosaicists proceeded fastest along the four axial directions of the mainly square mosaics. This is corroborated by irregularities being generally greatest in the diagonal regions.

Some parts of patterns are slightly distorted by following the shape of a near-by motif. Because the motif lies on the side of the pattern that is nearer to the mosaic centre, 'centrifugal' growth is again indicated. The same thing is suggested by the locations of stones that are misaligned in the manner of the stone marked *X* in Fig. 5, for these follow the adjacent row of stones that lies nearest to the mosaic centre.

Analysis of patterns that are not centred on the mosaic centre shows the evidence to be less simple, but also points to growth by accretion away from the mosaic centre that proceeded fastest axially.

The lack of alignment between the peripheries of mosaics and the perimeters of the floors, which contrasts with the frequent accuracy of position of the mosaic centre relative to the floor centre, also indicates centrifugal growth.

Hinks suggests[7] that lack of relationship between mosaics and rooms is due to the mosaics being "casual ornaments". Blake's explanation[8], that the mosaic periphery was reconstructed during replastering of walls, is seldom applicable, and does not explain mosaics lying skew. Trial shows that using stones 'as they come' and starting from the centre often causes the mosaic perimeter to turn out skew with respect to the floor. To allow for this, it is best to make the mosaic smaller than the floor. That there existed a lack of concern that decorations should fit the room structure is apparent from the irregularity of geometric patterns painted on walls. Because the discrepancy between mosaic and floor limits is often filled by large stones, Blake's reconstruction proposal in part gave rise to the idea that the size of the stones increases with date.

Trial shows that the positioning of a mosaic stone is easy or difficult according to the number of stones bounding it. The difficulty also depends on both the positions of such bounding stones, and the sizes and shapes that they happen to be. It can be shown mathematically (to be published elsewhere) that the sequence of positioning stones that involves minimal difficulty is one of accretion; it can also be shown that this sequence should start at the mosaic centre (start at the mid-point of one end of 'corridor' mosaics), and should be fastest in the axial directions because of the typical arrangements of the patterns. This would explain why mosaics were apparently constructed in this manner: the mosaicists had been using the simplest method, which they presumably learnt from experience. Volunteers, ignorant of the findings here described, proceeded—after a while—in a similar fashion.

Comparison of the gaps between stones where special spacing might be likely (to cause closer coincidence with the intended patterns, that is, immediately preceding a boundary), with gaps where special spacing was unlikely, further indicated that the stones were not specially spaced. A similar investigation of the sizes and shapes of the stones corroborated that they were normally neither specially selected nor specially shaped. Triangular stones are rare in floors, but mostly occur in positions where they are better than approximately square ones (Fig. 1*D*). However, they are very variable in size and shape, and were apparently made by cracking a 'square' stone into two; trial shows this to be easy.

An investigation of gaps, the ways in which errors and irregularities occur, and of vertical sections of a further 83 mosaics, indicates that floor-mosaics were constructed *in situ*. This agrees with Toynbee's conclusion[9], which is based on signed mosaics, travelling mosaicists, figure compositions of local materials, variations in different mosaics reproducing the same scene, and finally decorative surrounds of fine workmanship which form units with the picture they enshrine. This contrasts with Ippel's proposal[10], prefabrication by sticking stones face down on to sheets, but he was more concerned with figure scenes.

The imperfect alignment of mosaics and room margins, and the ways errors and irregularities are distributed, suggests that mosaic designs were not marked out beforehand. This is also indicated by the presence of mistakes of a type which, had they been made in a plan beforehand, would surely have been rectified.

Trial shows that the use of haphazardly variable stones, more or less 'as they come', makes it virtually impossible to match a design on the floor accurately.

The use by the mosaicist of stones 'as they came' appears to result from the technique of not shaping them to suit. If unsuitable stones are rejected great wastage results,

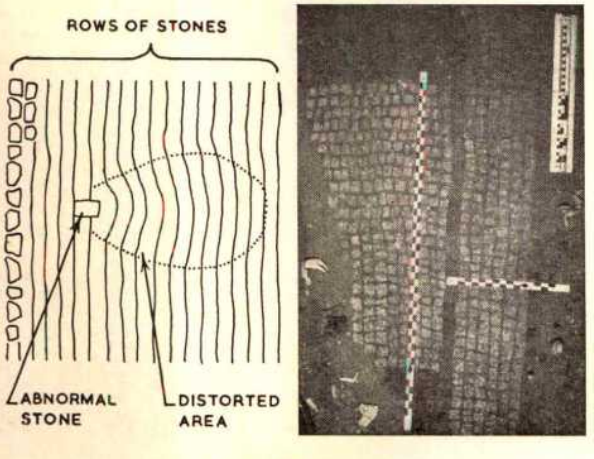


Fig. 2

which is unlikely, considering their probable source (to be published elsewhere). Trial shows that saving unsuitable stones for use later causes eventual acute difficulty.

It is a common belief that the mosaicists worked from 'pattern books' which showed the detailed positions for the stones. However, since 'pattern books' could not be matched by 'random' stones, it appears that, if they existed at all, they would simply be of a form useful to show to clients. Similar patterns exist on different mosaics, but consist of different stone arrangements. The existence of similar patterns is not itself evidence for 'pattern books', for the limited number of usable geometric patterns gives rise to a limited number of basically different results. This, and the fact that certain patterns were popular on other objects, would make it strange if many mosaics were not similar.

Observation : Pattern Sizes

In ancient floor-mosaics geometric patterns were produced from packing stones which vary in size and shape in a haphazard fashion within certain extremes; it would seem that these stones were apparently generally used without special selection, special shaping, or special spacing. Thus, if the hypothesis outlined at the beginning were correct, the mosaicists might have found by experience that the stones became more even at certain distances, and utilized this by basing their patterns on these particular sizes.

A pilot study showed that geometric patterns, taken from many different mosaics, nevertheless tend to fall into a few particular size groups. In some patterns only one dimension was needed for this purpose (for example, length $\alpha\beta$ in Fig. 1*A*), in others two or more (lengths $\alpha\beta$, $\gamma\delta$ and $\varepsilon\zeta$ in Fig. 1). It was found that each pattern in a 'size group' has one dimension of (virtually) equal length, despite great variations in pattern details (for example, length $\alpha\beta$ is constant throughout Fig. 1).

The existence of the 'size groups' may be explained by assuming that the mosaicists fixed the sizes of the patterns by using only a few alternative lengths, and that they used the same length for each pattern of the same 'size group'.

Because these equal lengths of different patterns are frequently composed of different numbers of stones, it appears that these distances were fixed by measurements and not by counting the stones. Equal lengths in the same pattern often consist of different numbers of stones (for example, Fig. 1*A*).

In a 'tracery' pattern of stones of slightly varying widths it might be expected that the rim of the pattern which was guided by measurements would be smoother than the other rim. In patterns which display one smoother rim, this is normally the one to which the measurement was made that was common to each pattern of the same 'size group'. Since these were usually the inner rims, it appears that the mosaicists normally proceeded by making measurements to these parts of their patterns. This agrees with centrifugal growth of the mosaics.

Some circular patterns tend to have a slightly square form (Fig. 3*A*). This effect could be correlated with the presence of an obvious stone located at the centre of the circular pattern ('obvious' because of its colour). Radial measurements of the circular pattern from the edges (see Fig. 3*A*) of this central stone were found to be more constant than those taken from any other central feature, real or imaginary. This suggests that the circular pattern was placed by measurements from the perimeter of the upper face of this stone, thus causing 'square' distortion. This is also indicated by the fact that this distance corresponds to the one that is common to each pattern of the same 'size group'.

'Square' distortion might be explained by mere packing of stones, apart from any measurements. However, examination of packing indicates otherwise. Moreover, a few cases were found in which one corner of the upper face of the central stone was incomplete, but was complete beneath the mosaic's surface. Thus the perimeter that could be used for measurements differs from the 'packing' perimeter of the stone. In such cases, the part of the circular pattern nearest the incomplete corner of the central stone is flattened, which indicates that distortions of circle patterns are a result of measurement rather than of packing.

This conclusion is supported by the observation that circular patterns around a real central point (for example, one formed by the meeting of four middle stones in a chequer pattern—Fig. 3*B*) are free from these distortions.

Comparison of circular patterns with a central point with those with a central stone (within the same 'size group') shows the former to be slightly smaller than the latter (Fig. 3), and by an amount equivalent to the size of the central stone. The explanation of this is that mosaicists probably used the same unit of length in both situations, measuring from the central point where it occurs, but from the perimeter of the central stone if one exists.

This suggests that the mosaicists preferred to measure from real features. Thus, where circular patterns lack both a central point and a central stone, and have instead a central motif devoid of suitable central feature, it is unlikely that the mosaicists used radial measurements. Diameters seem most likely, but the resulting 'circle' would tend to be an equi-diametric curve, as indicated in Fig. 4. This was, in fact, found to be the case.

Such 'equi-diametric' distortions again suggest that the pattern was not marked out before construction, for radii would then have been likely, obviating distortions of this nature. This consideration, together with the probable presence of the central stone at the time of making the measurements for circles exhibiting 'square' distortion, indicates that constructional measurements were made during the laying of the stones, and not beforehand as part of a preceding design.

Stones surrounding *emblemata* were found simply to follow the irregularities in the tray of the *emblema*, which indicates that the mosaic was constructed around it, as opposed to its being inserted into a hole left for its reception, as has been assumed by others.

Comparison of the eight radial measurements indicated in Fig. 3*A* on circles with a central stone was used to investigate ancient errors of measurement in these patterns. Similar observations were made on other patterns, and these in total show that the error was independent of the length of the measurement. This indicates that the mosaicists' measuring tools did not give increasing error over greater length, which excludes string, calipers (hinged), and 'spanning' by hands and

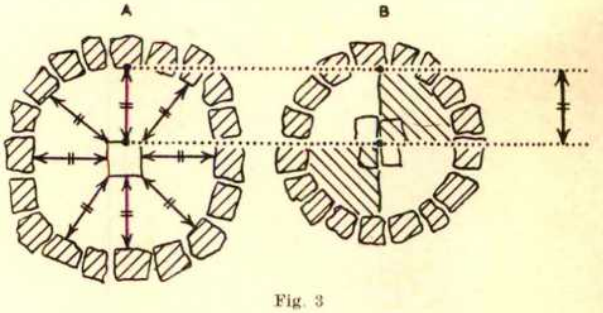


Fig. 3

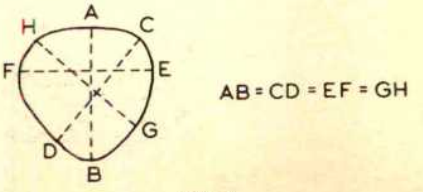


Fig. 4

fingers, but is strong evidence for rulers. The smallness of such ancient mensural errors also points to rulers, for those of the classical type that still exist (calibrated in still inadequately examined units) are mostly square in section with calibrations running around at least two faces, which reduces parallax error. That mosaicists used rulers is corroborated by the distortions evident in circular patterns already described here.

In concentric patterns where a circular pattern encompasses another, and no real central feature exists as a feature for measurements, 'equi-diametric' distortions will tend to occur in both patterns. If they were constructed independently by diameters, distortion of the outer pattern would not necessarily echo that of the inner pattern. Alternatively, if measurements for the outer were made from the inner, the outer pattern would follow the shape of the inner pattern. Since the latter is consistently the observed condition, it appears that the measurements for the outer pattern were made from the inner. This seems only natural when the inner pattern covers a large area and the outer pattern is only narrowly separated from the inner. Trial also shows that it is best to keep each constructional measurement short in order to avoid referring so far back in the mosaic that newly laid stones have to be trampled on when checking the zero of the ruler.

Pictorial mosaics of rectangular shape often exhibit opposite sides of unequal length and out of parallel; yet their outer bordering patterns lie at a uniform distance from their inner bordering patterns. Again, this is presumably a result of making the outer pattern equidistant from the inner. Because the innermost pattern is this shape (such that it could not have resulted from use of equal constructional lengths), it is unlikely that its form was itself guided by measurements.

In the case of more than two concentric patterns, it is likely that the constructional measurements for each pattern were made from the nearest preceding pattern. Again, measurements would probably be kept as short as possible. As with other patterns, evidence of the 'smoothest rim' and 'common' dimension type indicates that each encompassing pattern was normally laid by monitoring its inner rim. To decide whence, in the preceding pattern, this measurement was most likely to have been made, one can look where the already described 'common' dimension occurs. It is then found that this normally applies to measurements from the inner rim of the preceding pattern when the latter is narrow (Fig. 5*A*), but to measurements from its outer rim when it is wide (Fig. 5*B*).

The width of wide patterns was apparently similarly controlled by measurement (length *ab* in Fig. 5*B*), being often identical to the 'common' dimension of patterns of one of the smaller 'size-groups' (for example, length $\alpha\beta$ in Fig. 1).

The probable location of the ancient constructional lengths has become clear in many other geometric patterns.

If we accept that mosaic growth proceeded fastest in the four axial directions, then the first parts of each pattern to be reached in construction would have been those lying in these axial directions: consequently it would have been to these parts that constructional measurements would primarily have been made.

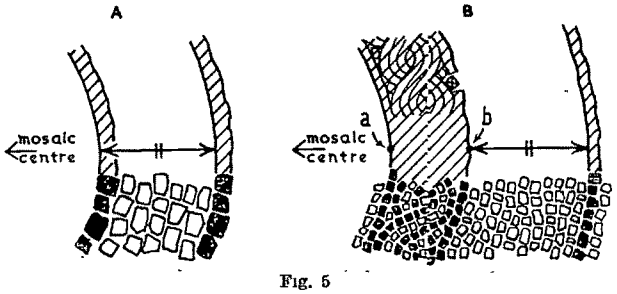


Fig. 5

Mosaic Units

To determine the probable values of the distances marked on the rulers of the mosaicists, a large number of distances was measured coinciding, as closely as possible, with the probable original constructional measurements on the mosaics. The frequency of their occurrence is shown in Fig. 6.

Measurement of more than 310,000 lengths produced the same distribution. The sample has since been increased with the same result. The vast majority of observed lengths are of closely similar values, forming ten major peaks (labelled *B*–$\bar{K}$ in Fig. 6), as well as many more minor ones. The total number of peaks formed is 106, but many of them are too small to appear in Fig. 6.

Each peak presumably represents one of the units of the mosaicists, the modal value of each approximating to the true value of each unit. The spread of each peak is a measure of the mosaicists' accuracy; to a small extent each is affected by my observational errors. If the relative heights of the peaks can be taken as a measure of the frequency of use of the various units, it is possible to equate the ten major peaks with the basic units of the ancient mosaicists.

Examination of Fig. 6 shows that several of the minor peaks occur at lengths which are multiples of the modal value of one or other of the major peaks. (These identifications are written sideways in Fig. 6.) These represent cases where patterns were occasionally made using multiples of a basic unit. Multiples of some major peaks give the same value as multiples of other major peaks. For example, the modal value of the minor peak at 36·0 cm is equally 3 *D* (modal value 6·0 cm) and 10 *C* (modal value 3·6 cm).

The modal values of the major peaks are: 2·4, 3·6, 6·0, 9·6, 15·6, 21·6, 25·1, 40·7, 65·8 and 106·5 cm respectively. These are the mid-interval values (class interval 0·1 cm). The standard deviation for each major peak is approximately 0·13 cm.

I shall call these ten basic lengths 'Mosaic Units'. 1·2 cm (peak *A* in Fig. 6) can also be counted as a basic unit, for it is probably important to the series in which Mosaic Units appear to lie. While in some mosaics this distance of 1·2 cm extends over several stones, it cannot exist in mosaics of stones larger than 1·2 × 1·2 cm.

Only minor peaks have been found at distances greater than Mosaic Unit *K* (106·5 cm), and this suggests that the rulers of the mosaicists were not normally longer than this. The most frequent lengths greater than 106·5 cm (peaks *l*–*o*) can be constructed with a ruler scaled in the Mosaic Units up to 106·5 cm.

Discussion

What little there is in ancient literature about the construction of mosaics largely stems from Vitruvius (first century B.C.). In the earliest extant version[11] of his work, which is believed[12] to have been written in the eighth century A.D., but which is equivocal and contains errors, a passage occurs, which in translation comments "over the *nucleus* accurately by *regula* and *libella* let the pavements be constructed whether *sectilia* or *tesseris*". The *nucleus* is the third foundation layer. *Regula* has been taken to mean a straight edge by some workers, but normally to mean a ruler. *Libella* is usually taken to mean a level. *Sectilia* probably refers to stones that were cut to shape, whereas *tesseris* almost certainly refers to the type of mosaic considered in this article. Perhaps it is to a Mosaic Unit ruler that Vitruvius refers.

Of the total sample of pattern dimensions observed, 89 per cent represent one or other of the Mosaic Units. Another 9 per cent are multiples of one or other of the Mosaic Units. A further 0·5 per cent are a fraction of a Mosaic Unit (usually ½ Mosaic Unit). Some of the remaining 1·5 per cent are identifiable as equivalent to ordinary units of measure current at the location

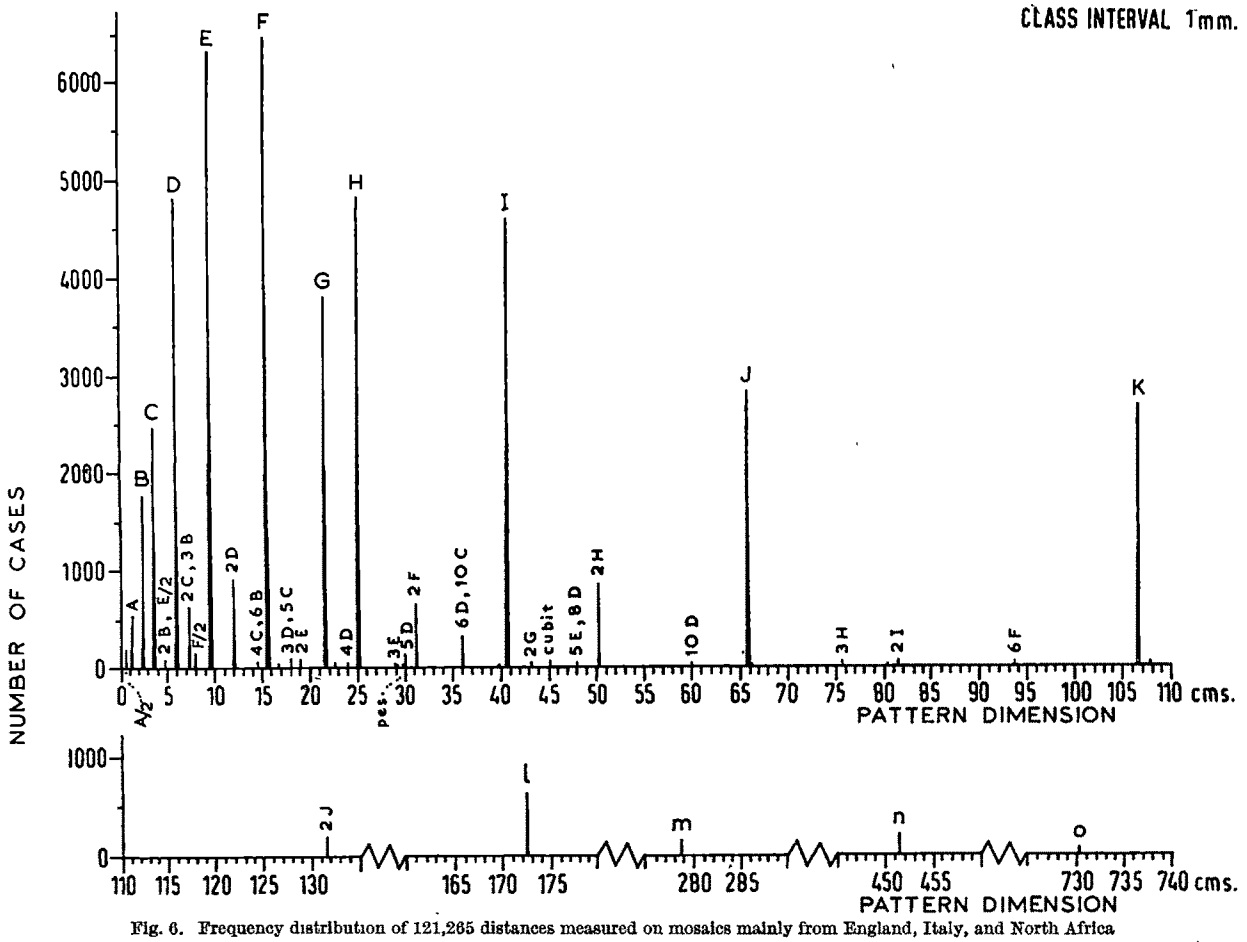


Fig. 6. Frequency distribution of 121,265 distances measured on mosaics mainly from England, Italy, and North Africa

and period of the mosaics in which such pattern sizes occurred.

A surprising result of these measurements is the general occurrence of the same absolute units, for at least a thousand years, and over a very large area. No likely relationship has been found between these Mosaic Units and known standard units of measure likely to be pertinent to the mosaic craft (its origins and development are uncertain). One might tentatively conclude that standardized tools—probably rulers—must have existed wherever classical floor-mosaics were made. Perhaps an original Mosaic Unit ruler will come to light.

Floor areas were frequently[13] fixed in terms of multiples of standard units. Because Mosaic Units are different from standard units, the mosaics would often, on completion, not have fitted the floor. This is another reason why they are smaller than the area available. The use of Mosaic Units over such a cultural and geographic extent, and for so long, suggests a practical reason for this rather than consistent subscription to a constant aesthetic. Some relation exists between the sizes of Mosaic Units and the most likely sizes for patterns, which would occur from the ways in which stones pack—that is, if one only considers common modal sizes.

Close agreement exists between the values of Mosaic Units and the easiest sizes for patterns, calculated from the hypothesis mentioned at the beginning of this article. That this may be the reason for the phenomenon of the Mosaic Units is supported by observations of the stones in adjacent rows in unpatterned mosaics. Every so often, many stones lie in line transversely through several of the rows, and the distances between such alignments frequently agree with the intervals which the hypothesis predicts. This aspect is at present being examined in a fundamental way, for it is possible that the packing hypothesis is a special case of a concept suggested here, that random disorder does not exist in reality.

If the mosaicists found, during the course of time, that their patterns tended to turn out most regular when made certain sizes, it is possible that, in order to take advantage of this, they made rulers with which to guide their construction.

It is hoped that when this thesis is published in full it will be of use in the investigation of other systems composed of aggregated varied parts. The work was supported in part by a grant from the Worshipful Company of Goldsmiths, from John Perryn's Charity, which enabled me to stay at the British School at Rome, and by two grants from University College, London. I thank Prof. R. Warwick (Guy's Hospital Medical School), Dr. H. Kalmus (University College, London), Prof. E. G. Turner and Prof. T. B. L. Webster (Institute of Classical Studies) for their help and encouragement. Construction of an automatic-studying machine for work on packing is being aided by gifts of machinery from I.B.M. and the British Broadcasting Corporation.

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OBITUARIES

Dr. J. B. Goodey

PLANT and soil nematodes have attracted few zoologists and it is all the more remarkable that the Goodeys, father and son, who trained, the first partly and the second wholly, as botanists, should have become world authorities on one aspect of the subject, namely, identification and classification. Basil Goodey joined his father at Winches Farm, St. Albans, the field station of the London School of Hygiene and Tropical Medicine, about a year before the Department of Nematology moved to Rothamsted Experimental Station. Until his father's death in 1953 he was mainly concerned with plant parasitic nematodes of the genus *Ditylenchus*, but afterwards he gradually assumed his father's role, published more than sixty scientific papers describing many new species and became increasingly interested in nematode taxonomy. He also undertook the revision of his father's books. *Laboratory Methods for Work with Plant and Soil Nematodes* was revised and enlarged three times. A supplement to *The Nematode-Parasites of Plants Catalogued under their Hosts* was produced and then, with the help of Dr. Mary Franklin and Mr. D. J. Hooper, the whole was completely revised shortly before he died. His greatest contribution to nematology, however, was the complete revision in 1963 of *Soil and Freshwater Nematodes* with descriptions and re-descriptions of many new genera and a new classification of major groups.

John Basil Goodey was born at Dorridge, Warwickshire, on May 10, 1914. Like his father he was a Quaker. During the early years of the Second World War he was engaged in Quaker relief work and for several years served as Clerk to the Society of Friends in Harpenden. He was educated at City of London School and University College, London, where he took his degree in 1937 and was Quain student in botany for the next two years. From 1943 until 1947 he worked in the Oil Seeds and Weed Control Unit first in the Imperial College of Science and Technology and later in the Department of Agriculture, Oxford. In 1961 he was awarded a D.Sc. degree of the University of London for work on plant nematodes and, in 1964, was elected a Fellow of the Institute of Biology. In 1962 he lectured at Cornell University, and in 1964 assisted in a nematology course, the first of its kind in India, at the Indian Agricultural Research Institute, New Delhi, under the auspices of the Rockefeller Foundation. From 1959 he served on the editorial board of the international journal, *Nematologica*, doing much to raise the standards of taxonomic papers by publishing in that journal "Data to be Considered, Observed and, Where Possible, Reported upon when Presenting Descriptions of New Species". His illustrations of species were models of clarity and essentially diagrams without unnecessary artistic embellishment. Over the past 2 years he had become interested in numerical taxonomy and had prepared for the computer lists of characters of major groups. He was happiest advising, teaching or serving others and, because of him, many students as well as experienced nematologists came to Rothamsted to learn to identify plant nematodes, or to work on the collection there, and samples for diagnosis were received from all over the world. Visitors from abroad were also welcomed in his home where, with Mrs. Goodey, they would spend the evening in discussions ranging from politics to literature and art.

While on his way to spend 18 months in New Zealand and Australia at the invitation of the New Zealand Department of Scientific and Industrial Research, he died of a heart attack on October 30, 1965, and was buried at sea. He leaves a widow, a daughter and two granddaughters.

F. G. W. JONES

Prof. Frank Allen

FRANK ALLEN, late emeritus professor of physics at the University of Manitoba, died on November 19 at Winnipeg, Canada, aged ninety-one. Born in New Brunswick of United Empire Loyalist stock, he was a fellow schoolpupil of the late Lord Beaverbrook. Like Beaverbrook and many of his generation, he held fierce religious and political beliefs; these were only tempered by time and by his love for science.

He graduated from the University of New Brunswick in 1895 with highest honours in physics and chemistry and a Gold Medal in Latin. He was appointed high school principal the same year at the astonishingly early age of twenty-one. In five years he had saved enough money to take him to Cornell, where he took his Ph.D. in 1902 for work on physiological optics, a subject which absorbed him for the rest of his life. It might well have been otherwise, for at Cornell he became interested in Oliver Lodge's newly discovered thermionic diode. He allowed himself to be dissuaded by Nichols, his professor, from investigating the effect of adding a third, or grid, electrode. A few years later Lee de Forest did add a third electrode and promptly patented the amplifying triode valve. The era of electronics was born and de Forest made a fortune. Thereafter, Allen frequently and ruefully warned his research students to be wary of taking the advice of any professor.

In 1904 he accepted the founding chair of physics in the new University of Manitoba and remained there all his life, attaining Fellowship of the Royal Society of Canada in 1912 and honorary doctorates of both Manitoba and New Brunswick.

With an annual research budget which would be spent by a modern physics department in an afternoon, he and his small group of enthusiastic students were forced to use themselves as research equipment. He wrote some sixty substantial papers, articles and books, mostly on the sensory reflex. He confirmed the Helmholtz trichromatic theory of colour vision, and accurately quantified the Fechner law of the intensity of visual stimulus. Then he turned to the other senses and showed, one after another, that they too followed the same logarithmic law. His methods were elegant and simple: the critical frequency of repeated stimulus, the least observable increment to a given stimulus and the effect of fatigue. All the senses, auditory, tactile, pain, pressure, heat, taste and muscular response, yielded to his persistence and ingenuity. Only the sense of smell defeated him; the olfactory reflex was too delicate and sensitive for the experimental techniques available to him. He never spared himself as subject; the senses of pain and taste, in particular, demanding considerable fortitude. He made much of his apparatus with his own hands, and brought a fine sense of craftsmanship to everything he did, whether it was making an instrument, carving a turkey or chopping wood, all three of which he much enjoyed.

He constantly preached the importance of science and the excitement of discovery, while for relaxation he delighted to write and converse in Latin, Greek and Hebrew. He outlived practically all his contemporaries and, indeed, many of his research students.

He is survived by his two sons and daughter.

J. F. ALLEN

NEWS and VIEWS

The Royal Society : Vice-Presidents

The president of the Royal Society, Prof. P. M. S. Blackett, has appointed the following vice-presidents for the year ending November 30, 1966: Lord Fleck, treasurer of the Royal Society, formerly chairman of Imperial Chemical Industries, Ltd.; Prof. A. A. Miles, biological secretary of the Royal Society, director of the Lister Institute and professor of experimental pathology in the University of London; Prof. M. J. Lighthill, physical secretary of the Royal Society and Royal Society research professor at the Imperial College of Science and Technology in the University of London; Prof. H. W. Thompson, foreign secretary of the Royal Society and professor of chemistry in the Physical Chemistry Laboratory, University of Oxford.

Chemistry in the University of Edinburgh : Prof. C. Kemball, F.R.S.

Prof. C. Kemball, at present professor of physical chemistry at the Queen's University of Belfast, has been appointed to the chair of chemistry in the University of Edinburgh in succession to Prof. T. L. Cottrell, who has been appointed first Principal of the University of Stirling. Prof. Kemball will take up his appointment on August 1, 1966. He was educated at Edinburgh Academy and at Trinity College, Cambridge, where he graduated in 1943 with first-class honours in the Natural Sciences Tripos. After war-time employment by the Ministry of Aircraft Production in the Department of Colloid Science in Cambridge, he was elected a Fellow of Trinity College in 1946, holding, in addition, a demonstratorship in physical chemistry from 1951. In 1954 he was appointed to the chair of physical chemistry at the Queen's University of Belfast, where he has also served as dean of the Faculty of Science and, from 1962 until 1965, as vice-president of the University. Prof. Kemball is distinguished for his work on the mechanism of heterogeneous catalytic reactions, leading to new knowledge of the nature and reactivity of absorbed radicals on metal film. He has been a pioneer in developing new techniques for the study of absorption phenomena and catalytic activity. His interest in these fields was aroused and stimulated by his association with Sir Eric Rideal at Cambridge and with Sir Hugh Taylor at Princeton, where he spent the academic year 1946–47 as a Commonwealth Fund Fellow. Awarded in 1951 the Meldola Medal of the Royal Institute of Chemistry as the most outstanding British chemist under the age of thirty, he won the Corday-Morgan Medal and Prize of the Chemical Society for 1958, and, in 1962, was the first non-American to receive the Ipatieff Prize of the American Chemical Society. He was elected a Fellow of the Royal Society in 1965.

Headship of the Plant Pathology Division, Ministry of Agriculture, and the Chair of Mycology and Plant Pathology in the University of Belfast : Prof. R. K. McKee

Dr. McKee, principal scientific officer at the John Innes Institute, Hertford, has been appointed to the headship of the Plant Pathology Division, Ministry of Agriculture, and the chair of mycology and plant pathology in the University of Belfast. He takes up this joint post in January 1966. Robert K. McKee, a native of Northern Ireland, was educated at Campbell College, Belfast, and at Cambridge, where he was an exhibitioner of Emmanuel College. After gaining his B.A. in 1938, he went to Trinidad to the Imperial College of Tropical Agriculture, where he carried out research on witches' broom disease in cacao, and lectured in mycology until 1943. After serving 3 years in the Royal Navy as a radar officer, he was appointed to the Agricultural Research Council potato storage station, Nottingham, to investigate the control of dry rot and common scab. Here, he discovered the toxicity of solanine to micro-organisms and the influence of solanine content on disease resistance in potato tubers. While at Nottingham, he was awarded a Ph.D. by the University and, in 1956, moved to the John Innes Institute to become the pathologist in the Potato Genetics Department. He first studied the inheritance of resistance to potato virus *Y* and potato blight in wild *Solanum* species and then carried out selection experiments towards breeding new scab- and blight-resistant potato varieties. While surveying some recent collections of South American potatoes, he discovered a new virus, Andean potato latent, which is unusual among potato viruses in having spherical particles. Dr. McKee has made valuable contributions to the methodology of his subject and has also been actively interested in the biochemical basis of disease resistance and the systematic distribution of steroidal alkaloids in *Solanum*.

Ministry of Power : Advisory Council on Research and Development

The Minister of Power, Mr. F. Lee, has appointed Dr. H. M. Finniston, managing director of the International Research and Development Co., Ltd., and technical director of C. A. Parsons and Co., Ltd., to be a member of his Advisory Council on Research and Development. Also appointed to this Council is Mr. P. A. Lingard, a full-time member of the Electricity Council since March 1965.

Production Engineering Advisory Service

In a written answer in the House of Commons on December 14, 1965, the Minister of Technology, Mr. F. Cousins, stated that he had decided to set up a Production Engineering Advisory Service to help the engineering and allied industries to increase their productive efficiency. This service would cover all aspects of engineering, from the improvement of engineering design to new and improved management and production techniques, including automation. It would be available to all engineering firms and would be operated on behalf of the Ministry by the Production Engineering Research Association in conjunction with the Ministry's regional offices. Mobile demonstration and advisory units would be stationed in each region and teams would be available to visit engineering works and show designers, foremen and production staff how to use the most modern production engineering techniques. The service would begin as soon as the mobile units could be prepared and staffed, and Mr. Cousins hoped it would be in operation in all regions within 12 months. Initially it would run for 4 years at an estimated net cost to the Exchequer of about £1 million.

Careers in Engineering

In a written answer in the House of Commons on December 13, the Secretary of State for Education and Science, Mr. A. Crosland, stated that he and the Minister of Technology were aware of the desirability of encouraging more young people to enter the engineering profession. An interdepartmental Publicity Working Party on Technology and Engineering had recommended a special publicity programme which included films for exhibition in schools, a number of travelling exhibitions and a

publication on careers in technology. In 1966 the Council of Engineering Institutions would carry out, with the support of the Ministry of Technology, a major survey of the distribution, deployment, remuneration and responsibilities of professional engineers, with the view of throwing light on the nature of the work which they perform.

Ministry of Transport : Advisory Council on Road Research

In a written answer in the House of Commons on December 13 the then Minister of Transport, Mr. T. Fraser, gave the terms of reference of the Advisory Council on Road Research as being to advise the Minister of Transport on the orientation and scope of research on roads and road traffic, including the design, construction and maintenance of the road system, traffic engineering, road safety and the interaction of the technical problems with economics, and to submit reports from time to time. The chairman is Dr. F. E. Jones and the deputy-chairman Prof. W. F. Cassie. The members include Sir Edward Dodd, Prof. G. C. Drew, Col. S. M. Lovell, Drs. J. P. Bull and A. R. Collins, and Messrs. J. Armour, A. C. Durie, S. H. Grylls, W. G. Harris, M. Holmes, O. A. Kerensky, M. Law, D. J. Lyons, J. S. McNeil, A. A. Shenfield, E. C. Williams and L. H. Wilson.

Ministry of Education and Science : Home and International Commitments

In reply to questions in the House of Commons on December 16, the Secretary of State for Education and Science, Mr. A. Crosland, said that the Government had agreed to participate in the Intersecting Storage Rings Project at the Geneva Laboratory of the European Organization for Nuclear Research, which is aimed at increasing the effective power of the existing equipment, and had offered to the Organization for evaluation, though without commitment, a site in Norfolk for the construction of a 300-GeV accelerator; it had joined the International Agency for Research on Cancer, and was now preparing to participate in the initial phase of the International Biological Programme. In 1965 provision had been made for an expenditure of £53·6 million for Research Councils and other science votes.

Fast Reactor Agreement between Japan and Britain

An agreement for an exchange of information and collaboration on fast reactor research and development has now been signed by Sir William Penney, chairman of the United Kingdom Atomic Energy Authority, and Mr. Kaneo Niwa, president of the Japan Atomic Energy Research Institute. Information to be exchanged concerns design and operating experience, basic physical and chemical properties of materials for use in fast reactors, reactor physics, and operating experience with certain fuels. The agreement will be effected by means of reports and visits; assistance in carrying out research and development will be given by procurement of materials and equipment and the use of facilities. There may be exchanges of staff from time to time. The progress of the agreement will be reviewed periodically at meetings between representatives of the Authority and the Institute.

United States–Yugoslavia Exchange Programme

The U.S. National Academy of Sciences and the Council of the Academies of Yugoslavia have announced plans for a programme of exchange visits by scientists of the two countries. The agreement, which covers a 3-year period, became effective from January 1 and provides for exchanges in all the disciplines in which the two Academies are mutually competent; natural sciences, including mathematics; engineering sciences; and behavioural sciences. The new programme is flexible in its provisions for both short-term visits by individual scientists to lecture, to conduct seminars, to survey research, or to exchange professional views and, for longer stays of up to 1 year, to carry out laboratory research or investigations in the field. Visits in each direction, totalling approximately 40 man-months per annum, are provided under the programme, with no necessary matching of either the number of exchanges or the scientific fields in which they occur. In addition to its provisions for the exchange of individual scientists, the agreement provides for co-operation between the two Academies in the field of scientific publications and documentation, including the exchange of information on research institutions and programmes and mutual assistance and advice on the exchange of books and scientific literature.

Register of Research in the Human Sciences (1962–65)

The third edition of the *Register of Research in the Human Sciences (1962–65)*, compiled by the Warren Springs Laboratory of the Ministry of Technology, has recently been published (Pp. vi + 353. London: H.M.S.O., 1965. £1 17*s*. 6*d*. net). This new edition is almost twice as large as its predecessor and contains more than four times as many entries as the first edition. The *Register* has been compiled on the basis of information received from more than 300 sources, covering research in the social sciences and in those fields of medicine, engineering, law, architecture, economics, history and geography which relate to the social sciences, and information is included for the first time on research in Northern Ireland. The layout of the *Register* is unchanged—each entry containing a brief description of the research project, research workers, approximate starting and completion dates of the project, interim and final results, parties concerned with the enquiry and other information. It is regrettable that a separate name index has not been provided.

Training the Creative Scientist

Dr. G. T. Seaborg's remarks on *The Creative Scientist: His Training and His Role* (Pp. 9. Oak Ridge, Tennessee: U.S. Atomic Energy Commission, Division of Technical Information Extension, Educational Materials Section (Dept. *SS*-1), P.O. Box 62, 1963) were originally addressed to the fourteenth National Science Fair at Chicago, in May 1963, but the pamphlet in which they are now published is still highly pertinent to the present situation. Dr. Seaborg's approach may be poles asunder from that of Prof. Kuhn, but his plea for greater care and interest in teaching science in its historical context in both schools and universities is fundamentally sound. Something on the lines of his pamphlet, adapted to the conditions in Britain, could contribute materially towards filling the vacant places in university science departments and towards fostering the supply of scientists with a wider outlook and greater adaptability.

Britain and the Scientific Industrial Revolution

In his lecture, "Britain's Part in the New Scientific Industrial Revolution" (Forty-third Earl Grey Memorial Lecture delivered at the University of Newcastle upon Tyne, May 17, 1964. Pp. 19. Newcastle upon Tyne: The University, 1965. 3*s*.), Prof. J. D. Bernal is concerned with the industrial and social changes arising out of the ideas which sprang from the scientific revolution that followed the discovery of X-rays in 1896. This industrial revolution is characterized by the removal of any limitation on the supply of power, and immense changes in the speed and feasibility of communication. The utilization of the digital electronic computer, the applications of advances in genetics and molecular biology in medicine and agriculture, and what he terms the 'research revolution', have all played a part in making science the most rapidly growing human activity. This growth of science will transform the whole world, and Prof.

Bernal holds that neither Britain's scientific effort nor her industry is adequate to face the change. Besides higher expenditure on scientific research, he urges both expansion and reform in education and a determined effort to avoid waste of trained manpower.

Overcrowding in Schools

Under the title *What Chance Has Your Child?* (A Study of the Effects of Overcrowded Classes in Our Schools. Pp. 15. London: National Union of Teachers, 1965. 6*d*.), the National Union of Teachers has issued a pamphlet by Miss K. Gibberd, to which Sir Ronald Gould contributes an introduction, describing the effects of overcrowded classes in terms of the children and teachers involved. In England and Wales some 75·1 per cent of all children in primary schools are being taught in classes of thirty or more, and 17·7 per cent in classes of forty or more, while in secondary schools 51·7 per cent are being taught in classes of thirty or more. Sir Ronald Gould believes that if anything there is greater need for smaller classes in the primary schools than elsewhere. Miss Gibberd does not indulge in facile generalizations and what she writes about the frustration of both teachers and children is the more impressive. She also notes a tendency, arising out of national parsimony, to calculate rigidly the needs of a school on the basis of one teacher and one classroom to every forty children and no more of either until another forty children are added to the school roll. She emphasizes the key importance of a sufficient number of competent teachers, and her sensitive presentation of the human issues should promote the public understanding, which is the only firm foundation for directing national resources to the appropriate end.

Tracking Hurricanes

Britain is fortunately spared the terrible ravages of the giant tropical storms which may, and often do, sweep across the islands of the West Indies and the Gulf of Mexico, extending along the North American coast. The critical period of each year is from June to November, when these hurricanes, mostly born in mid-Atlantic, gather velocity and, following unpredictable, haphazard courses, roar across the Caribbean Islands and adjacent mainland, leaving death and destruction in their trail. In years past there was apparently little in the way of organized scientific study of these hurricanes, particularly as a means of forewarning people in those places likely to be in their path, in order that, given time, preparations could be made and safety measures taken accordingly. Often, tragically, there was no time, such is the speed and strength of these furious storms. To-day, tracking of hurricanes has evolved into a highly organized branch of meteorology; search planes, radar, television-equipped *Tiros* satellites, and an international communications system are used to follow and report every whirling storm of wind and rain as it develops. Broadcast bulletins give advance warning to enable residents of threatened areas to take steps to protect lives, homes and property. Public education in the behaviour of hurricanes has proved to be the best means of reducing their toll. A brief account of this vital work is contained in a short article entitled "Tracking the Hurricanes" (*The Lamp*, Standard Oil Co. (New Jersey), New York, 47, No. 3, 1965). The Esso Standard Oil S.A., Ltd., the parent company's marketing affiliate in the West Indies, the Bahamas, Bermuda, Central America and Panama, in 1964 decided to help promotion of hurricane safety in the many widely scattered islands and regions where it operates, by the preparation of a hurricane tracking map for free distribution by Esso dealers. The object of this publication was to make it possible for customers to follow broadcast storm reports, mark a hurricane's position by latitude and longitude, thus plotting its course from hour to hour. The article included information on the meaning of Weather Bureau returns, explanation of a storm's behaviour, and suggested safety precautions. The circulation of this map exceeded 200,000; newspapers also reprinted it. The article includes a small-scale reproduction, as a grim reminder, of the paths of some destructive hurricanes of the past (1938–64); this was included with the original map in question. It is noted that similar hurricane maps were made available in 1965 and presumably the same facilities will operate in future years; this is a most praiseworthy and rewarding enterprise on the part of the oil company concerned.

The Glaxo Volume

The Glaxo Volume is the title of a most attractive publication which is described as "An occasional contribution to the Science and Art of Medicine", the twenty-ninth issue of which is now available (Glaxo Laboratories, Ltd., Greenford, Middlesex. Pp. 48. 29, 1965). At the outset it is pointed out that articles in *The Glaxo Volume* are contributed by medical and other scientists, including some of the technical staff of Glaxo Laboratories, Ltd. The authors remain anonymous for professional reasons. The first article is entitled "Fears for Food" and deals briefly with the necessity of discarding the concept of what is 'natural' about our foods and diets, and of considering, in the light of modern medical and nutritional science, what is wholesome and what is safe. The question of the use of food additives is discussed; this poses the problem of how much of the food additives or residues the citizen is likely to consume in the ordinary course of events and what is going to be the effect of three, or six, or even twenty times that amount. It is pointed out that even with the present limited number of 'permitted' additives or residues, monitoring their presence or effects in human or animal foods "is a pretty forbidding task". The second article concerns "The Blood-Clotting Mechanism". This discusses what is known as the 'classical' theory (Morawitz, 1905), the 'augmented' classical theory (Owren, 1947), some information from haemophilia, opposing theories, the 'sequential' theory, evolution of the clotting mechanism, and the 'derivative' theory. This article includes some excellent explanatory diagrams in colour appropriate to the text. A third article is entitled "Pregnancy and Plumpness". The belief that pregnancy predisposes to obesity is widespread but ". . . singularly little is known about the nature of the weight gained during pregnancy or about the natural history of body weight during the childbearing years". The problem is considered under the headings of weight gains during pregnancy, physiological storage of fat, variations in the pattern and effect of age and parity on body-weight, and concludes with some practical considerations. In marked contrast is the article "Art in Bedlam", a short account of one Richard Dadd (1817–86), a painter whose imagination developed unusually during a long period in Bethlem Hospital (nicknamed 'Bedlam'); a reproduction of one of his paintings "The Fairy Feller's Master Stroke" (in colour), is shown The fifth article is "Genetics and Drug Response", considered under the subheadings: blood groups, taste testing, haemoglobin 'Zurich' and sulphonamides, *X*-chromosome gene deficiencies and acetylation of isoniazid and other drugs. This article comes to the conclusion (*inter alia*) that "Unexpectedly wide variations can occur between responses of different people to the same dose of a drug. New drugs in particular should therefore be used with appropriate caution". Finally, an amusing, if not satirical, article entitled "Men are Not Machines" brings to a close this excellent publication which one could wish was more than an occasional contribution to medical science.

Solution to Waste-handling Problems

There are many problems inherent to raw water clarification, conditioning of slimes and sludges, and other industrial and sanitary processes. Most cases, indeed, where suspended material has to be removed from aqueous

systems, demand time, research and trial for their effective resolution. A new polymeric flocculant known as 'Primafloc *C*-7' is now available in commercial quantities for such purposes and a brief account of its constitution, merits, and properties is to be found in a short article entitled, "A Solution to Waste-handling Problems" (*Rohm and Haas Reporter*, Rohm and Haas Co., Philadelphia (London, Lennig Chemicals, Ltd., 26–28, Bedford Row), 23, 5, September–October 1965). This new product is a water-soluble cationic polyelectrolyte, supplied as a free-flowing, slightly hygroscopic powder, one of the latest in a series of such compounds to be offered by Rohm and Haas. It readily dissolves in water at a concentration of 2–6 per cent; before use, this solution is normally diluted to a working concentration of about 0·1 per cent. It is claimed that, in the clarification of turbid waters, 'Primafloc *C*-7' can be used as a prime coagulant; addition of inorganic coagulants such as alum or ferric sulphate is unnecessary. Furthermore, addition of 1–10 p.p.m. of this compound, together with a suitable clay, will rapidly and effectively remove suspended solids, an advantage where restrictions preclude the use of inorganic coagulants. If inorganic coagulants are used, 'Primafloc *C*-7' (0·1–1 p.p.m.) can greatly increase floc growth and settling rates, thus improving final water clarity. Since most naturally occurring suspended particles have a negative surface charge, causing them to repel one another and remain suspended, 'Primafloc' cationic polyelectrolytes, containing as they do a larger number of positively charged sites, work on the principle of neutralizing these negative charges, causing particles to coagulate, and thus to settle. There are other 'Primafloc' polyelectrolytes available for various purposes in waste-handling problems.

Procter and Gamble, Ltd.

THE internationally known Procter and Gamble organization comprises not only the parent and subsidiary companies in the United States, but also companies operating in Belgium, Canada, France, Great Britain, Italy, Mexico, Peru, Philippines, Switzerland, Venezuela, and West Germany. The principal products manufactured by this group are the familiar retail brands of synthetic detergents, edible shortenings and margarines for the bakery trade, industrial soaps, textile brands of soap in various forms, certain building trade specialities, and other products including refined and hardened edible oils, fatty acids, glycerine, fat additives for animal feeds, etc. All these products are manufactured and sold in Great Britain by Procter and Gamble, Ltd., whose financial statement for the year ended June 30, 1965, has been published (Procter and Gamble, Ltd., *Financial Statement for the year ended 30th June 1965*. Pp. 16. Newcastle upon Tyne: Procter and Gamble, Ltd., 1965). Apart from the commendably lucid statement of the financial aspects of this company's affairs, which has previously been commented on in these columns (*Nature*, 204, 1257; 1964), this report contains much of both technical and economic interest. The emphasis in this report is on the functions and activities of those members of the Company whose task it is to buy the raw materials, goods and services needed in the course of day-to-day operations. Under the heading "Buying Better Value for Consumers" are discussed new products and new processes, the buyer's broad responsibilities, product improvement, transport and international operations. Transport is particularly emphasized in this brochure, which contains another series of most pleasing illustrations in colour of buying and shipping activities, the work of David Knight, who contributed so much to the artistic value of the 1964 statement. On the research side, a new extension of the Longbenton research laboratories has made it possible for all major research departments to be brought under one roof. The job of the Research Division is to ensure that every product is formulated to give maximum performance and appeal in its own field. One instance of this may be quoted: in the past 16 years, the Division's home laundry carried out the equivalent of 80,000 weekly washes, equal to 1,600 years of domestic washing experience for one household; its controlled test methods are based on present-day domestic washing habits as established by consumer research. It is noted that during the year under review, the Company has co-operated fully with the Monopolies Commission in their investigation of the household detergent industry.

Soils and Foundations

MOVEMENT resulting from interactions between soils and foundations was the subject of a recent publication by the Building Research Station, a note of which appeared in *Nature* (208, 1163; 1965). A further publication in the same series outlines the soil and other investigations necessary in assessing the suitability of sites for new buildings and is entitled *Soils and Foundations*: 2 (*Building Research Station Digest* (Second Series), No. 64. Pp. 6. London, H.M.S.O., 1965. 4*d*.). The need for thorough site investigation when any building work is envisaged which may be potentially vulnerable to conditions existing below ground-level is a prior charge on the project, and cannot be too strongly emphasized; this applies equally to alterations and extensions of existing work as to entirely new schemes. The comparative scarcity of building sites available for large projects, especially in built-up industrialized areas, has led to the use of sites which may have been avoided in the past for one reason or another but have now come up for serious reconsideration; in such circumstances early re-appraisal of the site is imperative, so that any special measures that may be required to deal with difficult conditions can be planned at the outset. This publication outlines the main stages to be undertaken in any investigation of site conditions; these are, first, information available off site (appeal to the local authority, Ordnance Survey maps and records from local sources for knowledge of soil and general conditions, for example, infilled ponds, ditches, streams and disused pipes). Secondly, it is necessary to undertake site reconnaissance, which should include topography, possibility of flooding, groundwater-level and vegetation as a clue to the nature of sub-soil. The third stage embraces site history, whether virgin land or a previously used site. The fourth covers soil investigations on site, which means those concerned with relatively simple observations or tests, and, where necessary, full-scale soil mechanics to determine the strengths and deformation characteristics of the soil under load and to identify conditions in which soils are otherwise susceptible to movement. *Soils and Foundations*: 2 contains a useful table of soil identification, a summary of the scope of site investigations both in plan and in depth, details of information required from any one site, and notes on sampling (use of post-hole auger, etc.). An appendix on various sources of information about any particular area concludes what is undoubtedly a most valuable guide to an often complex problem of site investigation.

Bibliography on Religion and Science

A BIBLIOGRAPHY on religion and science, compiled by A. Maltby (The Library Association. Special Subject List No. 46: *Religion and Science*. Pp. 36. London: The Library Association, 1965. 10*s*.; L.A. members, 7*s*. 6*d*.) is arranged in three sections covering, respectively, the history of the conflict between religion and science prior to the nineteenth century; the nineteenth century struggle; and the relation between religion and science in the twentieth century. Nearly 200 of the 238 entries, some of which are annotated, fall into the third section. There is an index of authors. Although the bibliography appears to be representative, the basis of selection is not stated and there is one startling omission: A. N. Whitehead.

Postgraduate Agricultural Studentships

A LIMITED number of postgraduate studentships are to be awarded by the Ministry of Agriculture, Fisheries and Food and the Department of Agriculture and Fisheries for Scotland for the academic year beginning October 1. The studentships are available in the various branches of husbandry, horticulture, farm management, agricultural or horticultural economics, agricultural or horticultural statistics, marketing, agricultural and dairy engineering (including farm mechanization and farm building), rural estate management and agricultural science. Applicants normally resident in England, Wales and Northern Ireland can obtain further information and forms of application from the Ministry of Agriculture, Fisheries and Food, Room 231A, Great Westminster House, Horseferry Road, London, S.W.1. Applicants normally resident in Scotland should apply to the Department of Agriculture and Fisheries for Scotland, Room 53, St. Andrew's House, Edinburgh, 1. The closing date for receipt of completed application forms is February 28. Awards are also available to postgraduate and post-doctoral students of agricultural science under the NATO Science Studentship and Fellowship Programme, which is intended to stimulate the exchange of students between member countries. The scheme is administered for United Kingdom students by the Science Research Council, State House, High Holborn, London, W.C.1, to which application should be made by March 1.

Medical Research Council: Industrial Psychology Research Unit

THE Industrial Psychology Research Unit of the Medical Research Council, which was formerly housed at University College, London, has now moved to the headquarters of the National Institute of Industrial Psychology, 14 Welbeck Street, London, W.1. Dr. C. B. Frisby, director of the Institute, will take over the honorary direction of the Unit from Prof. G. C. Drew. Aspects of the work of the Unit, which has been concerned with investigations of shift work, incentives, absenteeism, labour turnover and industrial accidents, will continue to appear in *Occupational Psychology*.

University News:

Birmingham

THE following appointments have been made: Dr. M. J. Chamberlain (lecturer in the Department of Experimental Pathology); Dr. G. Harris (Senior Research Fellow in the Department of Experimental Pathology); Dr. R. Jefferis (Research Fellow in the Department of Experimental Pathology).

Edinburgh

THE following appointments have been made: *Senior Lectureships*, A. M. M. Wilson (bacteriology); Dr. A. D. S. Barr (mechanical engineering); *Lectureships*, R. A. Jones (animal health); Dr. J. Godfrey (zoology).

London

DR. LEWIS WOLPERT, reader in zoology at King's College, has been appointed to the chair of Biology as applied to Medicine tenable at Middlesex Hospital Medical School. The title of professor of experimental pharmacology has been conferred on Dr. J. R. Vane in respect of his post at the Institute of Basic Medical Sciences.

Newcastle upon Tyne

T. M. FROST has been appointed lecturer in the Department of Mechanical Engineering as from January 1.

Sheffield

DR. R. J. MOFFETT has been appointed lecturer in applied mathematics.

Announcements

MR. J. P. M. BRENAN has been appointed deputy director of the Royal Botanic Gardens, Kew, and keeper of the herbarium and library, as from December 1, on the retirement of Dr. C. E. Hubbard.

THE Council of the Institute of Metals has made the following awards: *Institute of Metals (Platinum) Medal for 1966*, to Prof. F. A. Forward, Privy Council Office, Ottawa, for his services to the non-ferrous metal industries; *Rosenhain Medal for 1966*, to Mr. A. Cibula, British Non-ferrous Metals Research Association, for his contributions in the field of physical metallurgy.

APPLICATIONS are invited from members of the Plastics Institute for the award of the Novadel Prize for 1965–66. The Prize, awarded annually by the Plastics Institute and worth 100 guineas, will be given for the best essay or report received before September 30, 1966, on some aspect of the reinforced plastics industry. Further information can be obtained from the Secretary, the Plastics Institute, 6 Mandeville Place, London, W.1.

A SEMINAR on "Planning and Organization of Science Research in Developing Countries", arranged by the Science of Science Foundation, will be held at the Ciba Foundation on March 14. Further information can be obtained from the Science of Science Foundation, c/o the Ciba Foundation, 41 Portland Place, London, W.1.

A SYMPOSIUM on "Non-elastic Processes in the Mantle", arranged by the Upper Mantle Committee of the International Union of Geodesy and Geophysics, will be held in the University of Newcastle upon Tyne, during February 21–25. Further information can be obtained from Dr. D. C. Tozer, School of Physics, University of Newcastle upon Tyne.

THE annual general meeting of the North of England Section of the Society for Analytical Chemistry will be held in Manchester on January 29. The meeting will be followed by the presentation and discussion of a paper entitled "Thermal Analysis with reference to Inorganic Hydrates". Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A SHORT course on "Recent Developments in Polymer Technology" will be held at the Bradford Institute of Technology during February 25–26. Lectures will include aspects of newer polymers; die design; polymer reinforcement; testing for end-use performance; developments in epoxide and polyurethane polymers. Further information and forms of application can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

A CONFERENCE on "Recent Progress in Physical Optics" will be held in Paris during May 2–7, in association with the seventh general assembly of the International Commission for Optics. Further information can be obtained from the Secrétariat du Congrès CIO 7, Institut d'Optique, 3 Boulevard Pasteur, Paris 15. Funds have been made available for travel grants for: (*a*) 'young scientists'; and (*b*) other scientific workers, to attend the conference. Applications for such grants should be made, before January 20, to the Chairman of the National Sub-committee for Optics, the Royal Society, Burlington House, London, W.1.

ADDENDUM. In the article entitled "Stimulation of the Uptake of Water and Ions by Indolyl-3-acetic Acid: its Dependence on Nucleic Acid and Protein Synthesis", by R. Mitra and S. P. Sen, which appeared on p. 861 of the August 21, 1965, issue of *Nature*, the explanations of the symbols used in the figures were omitted and are as follows:

Fig. 1. ●, IAA; ○, actinomycin *D*.
Fig. 2. ○, IAA; △, porphyromycin; ●, actinomycin *D*.
Fig. 3. ○, IAA, ●, actinomycin *D*.

THE USE OF MANPOWER

ONCE more the annual conference of the Institute of Personnel Management has been concerned with an issue of vital importance to the national well-being. Held at Harrogate during October 7–9, the conference was attended by more than 1,000 members and visitors from all parts of the world and considered how Britain should make the best use of manpower.

In an opening address, the Minister of Labour suggested ways in which productivity could be improved to achieve the 25 per cent increase required in the National Plan, without the 400,000 extra workers who would appear to be required and who will not be forthcoming. First, he suggested increased investment in labour-saving plant and machinery. Innovations, such as numerically controlled machine-tools, automatic welding techniques and automatic lathes, are all helping the engineering industry to produce more goods with proportionately fewer workers. Automation is being introduced to the coal mines, where considerable increases in productivity are being achieved through the use of remotely controlled faces. Computers are being used for many routine office jobs and an increasing number of firms appreciate that computers can also be used for advanced design work and for production control.

Secondly, if we are to take full advantage of technological progress, it is inevitable that some industries will decline, and release workers. Other industries will expand and will need these workers. There should be no obstacles to this movement. Firms which keep workers they do not need help neither themselves nor the country, nor—in the long run—the workers themselves.

Thirdly, the deployment and use of our available manpower resources should be critically examined. This is equally important at all levels—on an industry basis and in the individual firm, as well as over the economy as a whole. A number of industries have already estimated possible increases in output per head if various obstacles to the efficient use of labour can be overcome. In a number of other industries, management organizations and unions are together examining the measures which must be undertaken to ensure a more effective use of manpower. It is at the level of each factory and office that the problem must finally be tackled.

What are the obstacles to achieving greater efficiency in the use of labour? A great deal is heard about restrictive practices such as unnecessarily high manning scales, restrictions on the introduction of new machinery or working methods, on the entry of trainees, and insistence on outmoded demarcations. They cannot be tolerated in the battle for economic objectives. They must be vigorously tackled by both management and unions. In recent months there has been a significant increase in the number of agreements between management and the unions allowing for a greater flexibility of working and the removal of practices which impede efficiency.

Deliberate restrictive practices are, however, only a part of the problem. A much greater problem is the ready acceptance of traditional ways of doing things. Industry must be prepared to reappraise objectives and the means of achieving them. The initiative for this rests primarily with management although joint productivity committees also have a most important part to play. A well-developed system of joint consultation is essential to the orderly development of progressive ideas.

There is also need not only for rigorous scrutiny of the deployment of manpower at present, but for a forward look. The report on the metal industries published recently by the Manpower Research Unit of the Ministry of Labour showed that fewer than one in four of the firms approached made manpower forecasts for more than two years ahead. Half the firms appeared to do no forecasting at all. A firm that wants to develop a systematic training programme—any firm that is planning to expand its production or introduce major technical changes—cannot afford to neglect the vital element of manpower in its forward planning.

The Government itself recognizes that a firm cannot plan ahead if it feels at the mercy of outside forces, not knowing what the Government and the firms which are its customers and suppliers intend to do. This is one of the reasons why the National Plan has been published.

The Government also recognizes that it has an obligation to help men to adapt their skills or to learn new skills. During the past two years, the number of places in Government Training Centres has been more than doubled and the number of centres has been increased from 13 to 30, providing, by the end of 1965, 6,000 training places. A further expansion, bringing the number of centres to 38 and places to 8,000, has been announced. When all the expansion plans have been completed, the centres will be capable of producing some 15,000 trained men a year.

But this is only a small contribution to the training that is needed, and industry must still play by far the greatest part in this. The Industrial Training Act was passed to encourage employers to accept this responsibility. Nine boards are now established and by early 1966 the boards will cover some 8 million employees—one-third of British industry. The Government must also do everything possible to minimize hardship caused by necessary changes of employment.

A further obstacle to growth is the present regional imbalance in the economy. While firms in the South and Midlands are plagued by shortages of labour, unemployment in areas like Scotland, Wales and the North is still well above the national average. Financial assistance is being given to firms which build or expand factories in Development Districts and control is being exercised over the issue of Industrial Development Certificates in areas where the demand for labour is high.

Mr. Gunter was followed by D. A. C. Dewdney, vice-chairman of the National Board for Prices, Incomes and Productivity, who stated that responsibility for employment in British industry has been much too fragmentary. One part of an organization decides that it needs so many people of such-and-such grades to do a particular kind of work. Then another part of the organization does the hiring, looks after the workpeople when they have been employed, and probably carries out the negotiations with the unions. The processes of employing are thus divorced from those of deciding what the employed person is actually supposed to do, and management all too rarely asks itself the really fundamental question—why am I employing this man at all? It is extraordinary that this attitude should have persisted into the post-war years, when the problems have not been those of shortages of work but shortages of labour. This is reflected in the replies of a number of industries to the Government while it was preparing the National Plan, when many industries admitted to having under-employed labour resources. Such a situation will have to be radically changed if national ambitions for economic growth are to be fulfilled. Productivity is at the heart of the policy of which the National Board for Prices, Incomes and Productivity is the instrument. Productivity is one of the three complementary and inseparable themes of the policy,

all three components of which have been steadily neglected in the past. This neglect represents an idleness of habit which it is the purpose of the policy, and the Board, to change.

Productivity cannot be separated from the problems of prices and incomes and, in the short run, our present position requires that demands for increases in incomes be moderated. If they are not moderated through the exercise of self-discipline, other factors will take over and moderation will be imposed. The usual alternative to self-restraint has been found to be a rise in unemployment. The only other method of restoring equilibrium is strict physical control.

It has become increasingly clear that the problems with which we are faced are both economic and social. The diagnosis is a matter of economics, but the solution is much more social and educational.

The main role of the Board should be as a part of the educational process. Its reports should not merely be admonitory but constructive. A second requirement for the reports is that they should be of as wide a general significance as possible.

Mr. Dewdney also made it clear that in order to implement the prices and incomes policy with institutions as they now stand, management must take the initiative in trying to balance the expectation of the people of Britain for better things against our ability to earn them. Such initiative will be welcomed by the Trade Unions.

Sir John Hunter, chairman of the Central Training Council, discussed the role of training in a technological age. He indicated that the most important steps in the approach by management to training policy are:

(1) Training is an integral and essential part of management function. Top management must therefore be closely involved in development of training policy; and basic responsibility for implementation of policy is a matter for line management—not to be devolved on training staff, and conveniently forgotten.

(2) A survey of training requirements should be undertaken. This involves analysing the responsibility, skill and knowledge of different jobs, and an examination of the extent to which staff discharge them inadequately. Are such problems as high turnover, production bottle-necks and lack of promotable staff due to lack of training?

(3) Management needs to be able to call on expert advice and assistance in analysing job and training requirements, and implementing training programmes.

(4) Is training effective? There should be a regular check on training programmes to ensure that staff get what help they need to carry out their responsibilities satisfactorily.

Among the many problems to be faced is that of management development. It is generally recognized that far too many firms adopt a haphazard approach to management recruitment, development and succession. This is not merely a matter of prestige courses: there must be a planned programme which starts with selection of potential managers, and includes relevant and challenging experience on the job, regular reviews of progress and development of specific required skills and knowledge through carefully chosen courses (internal and external).

A second key problem is the need for training officers. The Boards will have to make a big effort to train considerable numbers of training advisers and training officers capable of providing firms with expert support in the development and implementation of training policy. This effort will involve introductory courses for postgraduate students and for people with suitable industrial experience, arrangements to enable relatively new or inexperienced training officers to gain experience in firms under the supervision of a senior training officer, specialist courses in technique and particular subjects for those who are already training officers, and advanced courses to enable experienced training officers to study new methods and undertake research.

The role of the universities in a technological age was outlined by Sir John Cockcroft, Master of Churchill College, Cambridge, who, after indicating the country's great lack of and need for technologists, suggested that there are different varieties of technologists, a broad spectrum requiring different training. There are, first of all, the technologists whose major role is to force the pace of technological change. Their work is predominantly intellectual and requires a deep knowledge of science. They require a strong scientific base in their undergraduate training and good postgraduate training. The second broad group of technologists are those who design and build engineering systems and make them work. Good design engineers are the most scarce of all categories and the role of the university in helping to produce them has been the subject of a recent enquiry, on behalf of the Department of Scientific and Industrial Research, chaired by G. B. R. Fielden. In the evidence given to the Committee, there was no consensus of opinion about how, or to what stage, design should be taught in the education and training of an engineer, and a large number of approaches to the problems are being tried in universities and colleges.

The Committee came to the conclusion that the 'sandwich' course is the most suitable preparation for a career as a professional design engineer. This was the type of course first adopted by the Colleges of Advanced Technology. The Committee, however, felt that it was essential that the period spent in industry by these students should be tested on what they learned during their industrial training. An alternative to the 'sandwich' course is for an engineering student entering a university to spend a year in industry before his entry, and to spend part of one long vacation in a works. In this way he will obtain a better three-dimensional feeling for the equipment he will be hearing about in the more analytical lectures. The Fielden Committee also supported experiments in teaching design at postgraduate levels, which are under way in the University of Cambridge and Imperial College. Manchester College of Science and Technology is also carrying out advanced training in machine tool design, and the College of Aeronautics is giving training in design of aircraft and aero-engine industries.

In order to teach design, it is essential that the teachers have had some personal experience of design work in industry, and since the best designers obtain salaries in industry far higher than the universities can offer, it is important that universities should obtain the services of industrial designers on a part-time basis by appointing them as extraordinary or visiting professors, readers or lecturers.

Sir John also examined the way in which the universities link up with industry to carry out research projects and to provide suitable courses in management education. The latter was discussed more fully in a sectional meeting when A. F. Earle, director of the London Business School, described the philosophy on which the School is being founded and the types of courses which it is planned to commence in the coming year. There will be a twelve-week course for men aged 28–35 in middle management positions, one of six weeks' duration for men of 35 years upwards in more senior management work, and a postgraduate course of two years' duration leading to a masters' degree designed for university graduates within one or two years of having completed their first degrees in any subject. The post-experience courses will be strenuous and will be aimed at the cultivation of analytical powers and widening the acquaintance of the students with business problems beyond their range of experience. The postgraduate course will not produce a ready-made manager—that requires extensive on-the-job experience and maturing personal qualities—but it should ensure a man with broad analytical powers and business knowledge which should enable him to travel up the manage-

ment ladder more quickly and to a greater height than would otherwise be the case.

In another sectional meeting R. Lahnhager, director of the Swedish Council for Personnel Administration, provided information about the way Sweden encourages mobility of labour. Much emphasis is placed on the relocation of industry itself and the use of investment funds to stimulate this.

In a provocative paper on the role of the personnel manager in technical change, Prof. Lupton of the University of Leeds indicated that, in the attempt simultaneously to maximize welfare and technical and economic efficiency, certain administrative techniques have been developed and are widely used in large organizations with well-developed personnel functions. He suggests that this is to put the cart before the horse. Management should decide whether there are any alternatives which might, while offering high technical advantages, also minimize the adverse social and psychological consequences. Or it might well be advantageous, if there is time to work out the possible social and psychological consequences of a number of alternative ways of introducing change, to discuss these with the people who are going to be involved, and their representatives.

All this implies the presence, when decisions are being made about technical change, of someone with expert knowledge of the organization as a social system, and who is aware of the complexities and subtleties of the relationship between technology and organization structure. Such a person will be able to predict more accurately than others the effects of projected changes in technique. The final course of action will then be based not only on economic and engineering data and methods of analysis, but on social and psychological data and their analysis, and will be a choice which satisfies all three criteria. To perform this additional function adequately demands an expertise to be learned in addition to the skills already demanded from the personnel manager.

H. Clegg of Nuffield College, Oxford, discussed the significance of wage drift in relation to an incomes policy. Wage drift can undermine an incomes policy in two ways. Incomes policies invariably attempt to control wages and salaries through the settlement of wage rates and salary rates. But rates are not incomes. Earnings are incomes: and if earnings rise faster than rates through a process of 'drift', to that extent incomes policy is ineffective. Drift is also uneven in its incidence. To some workers it can build up a pressure of resentment among the less-fortunate who demand compensating adjustments of rates. To meet these demands means further departures from the policy; to refuse them may lead to an explosion which could destroy it.

But can the drift be controlled? There is talk now of putting statutory authority behind an incomes policy, but an examination of the elements which go into drift is enough to show that this would not be easy. Quite apart from the question of sanctions, the controls would have to apply to overtime, to the various systems of payment by results, to job evaluation schemes, to promotion and up-grading and so on.

To foresee whether wage drift will become a serious obstacle to the success of incomes policy in Britain, we would have to know whether the policy is going to establish its grip on rates of salaries and wages. As yet, it has not got this far, but some of the cases which have come before the Prices and Incomes Board have directed their attention to the problem of drift, and these give some indication of the way they may try to deal with drift if that stage is reached. If the Prices and Incomes Board were to encourage a view that drift is permissible wherever it is accompanied by an equivalent rate of increase in productivity, it could lead an incomes policy sadly astray.

A more discriminating approach to the problem of mobility of labour was suggested by Dr. Hilda R. Kahn, senior lecturer in the Department of Social Administration, University of Hull. Some immobility is an entirely natural phenomenon in an affluent society. When the alternative was near-starvation, people could be expected to be almost infinitely mobile. But when a country has reached an advanced stage of socio-economic development —providing reasonable living standards, as well as a welfare state, for most—a certain reluctance to uproot oneself is a natural consequence. And it is perfectly legitimate for a society to opt for some immobility in preference to faster economic growth, provided it does not simultaneously insist on paying itself the higher income which faster growth would have made possible, but which its preference for immobility has not.

Nor is the British population nearly as immobile as has often been assumed. Thus, major changes have occurred during the post-war period in the size of important industries such as agriculture, mining, textiles and distribution; there are between two and three million job-changes each year in manufacturing industry alone; and a considerable amount of inter-regional migration has likewise taken place in recent years. Much of the two-way traffic between regions, however, has merely the effect of cancelling itself out, while some of it is actually harmful in that it has produced excessive congestion in certain parts of the country—costly in both social and economic terms. Similarly, an uncritical emphasis on mobility may accelerate mere labour turnover, and the crucial problem remains of reconciling the twin desiderata of good workplace relations and mobility. All this suggests that it is 'purposive mobility', rather than mobility as such, which policy must seek to encourage.

The aim of a mobility policy should be to seek to achieve the optimum distribution of the country's manpower through the guidance of school-leavers and the voluntary mobility of adults to the greatest possible extent. Apart from the many measures in kind which this entails—in the sphere of housing and re-training, for example—it may pay us to evolve, in addition, a system of substantial cash bonuses, to accrue to workers prepared to move where they are wanted. In view of the insistence on the maintenance of traditional relativities which has become so prominent a feature of post-war collective bargaining, it is doubtful whether we shall ever get a wage structure sufficiently flexible to further the cause of 'purposive mobility'. The proposed payments would therefore have to be operated by the Ministry of Labour via a drastic extension of its present Resettlement Transfer and analogous schemes Such 'mobility bonuses' might be used to aid all forms of desirable re-deployment, and would be alike more acceptable, more effective and quite possibly also less costly than redundancy. Such a system could also claim to be wholly fair, since it would specifically accrue to those serving the national interest.

Finally, Prof. T. Malm, of the Schools of Business Administration, University of California, showed very clearly the need for a new type of personnel administration to serve the needs of rapidly changing industry. In Britain and the United States as well as elsewhere, enterprises have been affected by varying degrees and forms of mechanization, automation and computerization. While most are still far from 'full automation', executives and workers at all levels have been seriously affected—for better or worse—by new products and processes, and by reorganization of the enterprise, the work group, or the individual job, resulting from technological change. All too often, the personnel officer has not participated fully in planning for the implementation of automation or electronic data processing, only to be called on later to 'put out fires' when human and organizational stresses and strains make clear the need for improved manpower programmes and policies. While personnel and industrial relations specialists have been leaders in 'human relations', in general they have lagged in their appreciation and application of operations research and management

science. Much needs to be done to improve the analysis of personnel policies and practices; the personnel officer needs fuller understanding of and participation in operational research and management studies to solve his own problems more effectively, and to contribute more positively to planning and decision-making, to administration and management in the enterprise as a whole.

T. H. Hawkins

PREPARATION AND STANDARDIZATION OF ISOTOPIC TARGETS AND FOILS

STABLE and radioactive isotopic films and foils of precise composition and dimensions are needed as targets for a variety of particle accelerators for nuclear data measurements; accurately defined films of α-active actinides are also needed as fission standards for reactors. Such standard targets are made in a number of laboratories in different countries and there was a clear need for closer co-ordination with the users in order to relate the limits of chemical purity and dimensional tolerance desirable to those practically attainable. To meet this need and also to promote an interchange of ideas and techniques a seminar was held at Harwell, Didcot, during October 20–21, 1965. The hosts were the Electromagnetic Separation Group of the Atomic Energy Research Establishment (A.E.R.E.) and the Special Techniques Section of the Nuclear Physics Division of the Atomic Weapons Research Establishment (A.W.R.E.).

The seminar, of about seventy scientists, was strongly supported by the Central Bureau of Nuclear Measurements of Euratom, Geel, Belgium, and the isotopic Target Preparation Centre of Oak Ridge National Laboratory (O.R.N.L.), Tennessee. Participants from laboratories in eight other countries also contributed to the 30 papers and the discussions.

Isotopic targets, which may be required on backings or self-supported, generally range in thickness from a few μg/cm² to several mg/cm². A wide variety of chemical techniques such as vacuum evaporation, electrodeposition and electrospraying, and metallurgical methods are used, but problems arise owing to the small amounts of expensive isotopic material available, the risks of isotopic contamination and the need for high chemical purity and exactly known thicknesses. Chemical conversions and purifications on the sub-gram scale may have to precede the target-making process.

During the first day the meeting considered the problems involved in these methods, means of improving process efficiencies and the effects of impurities. G. H. Debus of the Geel Laboratory emphasized the difficulties involved in obtaining exact and realistic specifications from users, in preparing and transporting samples, and obtaining from users adequate comments on the behaviour of the targets which would help future preparations. Other contributions from the Geel Laboratory discussed non-uniformity of deposits and isotopic fractionation during evaporation. G. T. Arnison of A.W.R.E. analysed the experience gained using electron bombardment techniques instead of resistance heating for vacuum evaporation. The description by W. Parker of the Chalmers University of Technology, Gothenburg, of the novel method of 'molecular plating' from organic solutions, which appears especially useful for making actinide targets, excited much interest. Experience with established methods for making α-active sources was reviewed by Mrs. Glover of A.E.R.E. and J. Champion of the French Atomic Energy Commission.

For many applications unbacked targets are desired, and several laboratories use metallurgical techniques to make isotopic metallic foils of thickness down to below 1 mg/cm². The end-product of electromagnetic isotope separation is normally the oxide, and methods of efficiently converting small quantities of the valuable isotopes to the metal were described. A range of foils of the isotopes of the rare earth metals, reactive alkaline earth metals and some of the actinides has been prepared by simultaneous reduction-distillation followed by rolling or evaporation at O.R.N.L. Some of these were shown by E. H. Kobisk, who also considered the merits of ultra-high vacuum techniques as a means of obtaining high purities. The techniques used at the Argonne National Laboratory for rolling films down to thicknesses of a few microns and at Geel for high-frequency levitation melting and evaporation were described. This latter method avoids contamination by crucible material and can be used for metallic samples of 3–100 mg. J. B. Reynolds of A.E.R.E. listed the thin stable isotope metallic foils now made at Harwell by rolling techniques and showed how the deformities and thickness contours in a rolled foil had been measured by attenuation of 5-MeV α-particles. The problems of preparing targets by very small-scale electroplating were discussed by several speakers.

The second half of the meeting was concerned with measurement and standardization. F. A. Howe, A.W.R.E., introduced the topic with a critical survey of the non-destructive methods for determination of film thickness which were applicable to nuclear targets. Methods used at Geel for precise standardization of the chemical and physical properties of targets were reviewed by H. Moret and K. F. Lauer and some specific procedures were described by other speakers. This aspect of thin-film preparation will undoubtedly receive increased attention in the future, and the first seminar on the subject was particularly useful in making the participants aware of the many causes of errors which can exist in the determination of purity, uniformity and thickness of isotopic targets. Both makers and users will undoubtedly be more cautious in the future and will understand each other's problems better. More efforts will be made to introduce monitors to follow the continuous build-up of targets and to control and measure the chemical and physical characteristics of the films and foils.

Copies of the seminar programme and abstracts and, later, the record of the proceedings can be obtained from the following address: Electromagnetic Separation Group, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire.

M. L. Smith

THE NATIONAL INSTITUTE OF OCEANOGRAPHY

THE National Oceanographic Council met for the last time on October 20, 1965, and decided to petition the Queen to withdraw the Royal Charter granted in 1950. This was the final stage of the change-over of the responsibility for the National Institute of Oceanography from the National Oceanographic Council to the recently formed Natural Environment Research Council. Mr. J. P. W. Mallalieu, Parliamentary Under-Secretary of State for the Royal Navy and chairman of the National Oceanographic Council, while thanking the Council for their work over

the past 15 years in promoting the study of oceanography in Britain, referred to the growing public recognition of the importance of oceanography.

During the Second World War, very much as a consequence of the remarkable work on waves carried out by Dr. Deacon's group at the Admiralty Research Laboratory at Teddington, it was felt by many people that there should be a more concerted attack in Britain on research into the physics of the sea, and eventually, in 1949, after discussion between the Advisory Council on Scientific Policy and various Government departments the National Institute of Oceanography came into being with Dr. G. E. R. Deacon as director—the Royal Charter being granted to the National Oceanographic Council in the following year. The whole scheme provided for: (1) A Royal Charter incorporating a National Oceanographic Council with the object of advancing the science of oceanography in all its aspects, under the chairmanship of the Civil Lord of the Admiralty, and consisting of representatives of Government departments, the learned societies, the universities and those Governments of the Commonwealth that might be expected to give financial support. (2) An executive committee to implement the policy of the Council. (3) A National Institute of Oceanography to carry out the work of the Council.

Over the past 16 years the money has been provided more or less in equal proportion by the Development Commission and by the Admiralty, and there have been welcome but relatively small contributions from various Commonwealth countries. The income in 1949–50 was just over £100,000, in 1963–64 some £400,000. The nucleus for the investigation of the physics of the sea was Dr. Deacon's wave group; the nucleus of the biology department was the long-existing Discovery Investigations.

The National Oceanographic Council held its final meeting at the National Institute of Oceanography in Wormley, Surrey, and the occasion was marked by a display of exhibits illustrating the work. A press conference was held, chaired by Mr. R. E. Prentice, Minister of State for Education and Science, assisted by Dr. Deacon and by Prof. M. J. Lighthill, the chairman of the Oceanography and Fisheries Committee of the Natural Environment Research Council. Awareness on the part of the public was reflected in the emphasis which many of the exhibits placed on the user, particularly one showing over 120 enquiries from civil engineers, port authorities, etc., who have consulted the Institute in recent years for help about wave conditions and who need advice, data or instruments. Such knowledge of waves is essential to those who build and operate ships, design hovercraft, drill for oil, build ports and other coastal installations, and also to those who attempt to control coastal erosion. This display particularly illustrated the statement of the director, Dr. Deacon, that "The Institute's main purpose is to improve understanding of the basic physical, chemical and biological processes that govern all events in the ocean, and, while taking every opportunity to increase knowledge, to help as much as possible in applying it". For many years the wave group at the National Institute of Oceanography has been recognized as second to none in the world both in the study of the theory and statistics of waves and in the design of instruments for collecting measurements. The practical value of such academic research has become increasingly apparent to those who consult the National Institute of Oceanography. One major wave project in recent years has been the design of a series of buoys to measure the direction in which waves are travelling, the most recent design being a cloverleaf buoy which measures the pitch and roll of three floats held at the corners of an equilateral triangle. The direction of alignment of the buoy and the vertical acceleration at the centre of the triangle are also measured. The buoy and the ship are linked by a 26-core cable for carrying electrical power to the buoy and for bringing back reference signals and outputs to a multichannel recorder and data logger. From these records the directional spectrum of the sea waves can be computed. These results are of importance both to wave theoreticians and to the British Ship Research Association for ship motion studies. That pure research can result in wave forecasting which may save a ship time in crossing the Atlantic, or otherwise improve its efficiency, is a vindication of the purpose of the Institute in studying fundamental problems of the ocean.

Another basic problem is the study of deep circulation of the ocean, with its concomitant idea of the transfer of energy from one part of the ocean to another. Since their development at this Institute ten years ago, neutrally buoyant floats have been used to measure currents at great depths in the sea and have radically changed our ideas about the movement of the deep water. The deep currents have been found to be faster and more variable than had been supposed—fast enough to have practical implications for problems of waste disposal in the deep sea and for the design of deep moorings. These variable currents may play an important part in the general circulation of the ocean, similar to that of depressions and waves in the atmospheric circulation. One display representing examples of the deep current observations in the North Atlantic Ocean showed a fast south-flowing current close to the continental slope off North America. In mid-ocean, average speeds of 10 cm/sec at great depths were found and there was evidence of eddies some 100 km across. A narrow streak of water was discovered travelling at nearly 1 knot at 4,000 m. It also appeared that the currents at such a depth were faster than those at mid-depth (2,000 m) and moved in a more nearly meridional direction.

Much of the information about deep-water masses and their movements is obtained by the classical and still essential method of making observations at hydrographic stations, samples of water being collected at various depths with simultaneous temperature recordings, and this was part of the required international survey of the physical oceanography of the Indian Ocean. A part of the hydrographic work of the third cruise of the R.R.S. *Discovery* in 1964 during the International Indian Ocean Expedition was shown by a display of sections of salinity, oxygen and temperature at different seasons along 58° E. Besides the usual water-sampling and net hauls on stations in the Indian Ocean, relative currents were measured using propeller-type current meters in the near-surface water. Two of the most striking features were the equatorial undercurrent and the Somali current, which were both particularly well developed at the time they were investigated.

Another phenomenon associated with the Somali current, recorded as flowing north-east at nearly 7 knots, was the exceptionally cold coastal water. The association of recently dead fish with this upwelling was remarked on in the display of the Institute's biological section as was the upwelling from a different cause off the Arabian coast, investigated particularly in its association with the return of nutrients from the deep water to the surface. This was one aspect of a prominent part of the biological research, the investigation of the fertility of the oceans. Aspects of the organic cycle investigated at the Institute include photosynthesis, the growth of phytoplankton and its dependence on nutrients and chlorophyll, and the taxonomy and distribution of zooplankton, which has been investigated mainly by sampling and recently by echo-sounding. The R.R.S. *Discovery*, the main tool of the Institute, is at present at sea in the North Atlantic on a biological cruise, co-operating with visiting scientists from the United States, in an attempt to use underwater sound as a method to study the distribution of marine animals. At all times from the period of the Discovery Investigations to the present some members of the biological group have investigated certain animals in detail, most notably the common food of Antarctic

whales, *Euphausia superba*, which was the work of the late J. W. S. Marr. One of the most active investigations to-day is that of the squid, known as the diet of sperm whales, but also one of the most numerous and widely distributed animal groups in the sea. The Whale Research Unit showed exhibits representing its work with the biology of the larger species of whale of commercial importance, the blue, fin, sei and humpback whales and the sperm whale; as in the days of the Discovery Investigations the immediate purpose is to provide scientific information to help in the regulation of the whaling industry, but in the long run to aid the conservation of whales. Most of the work of the Whale Research Unit is therefore concerned with the distribution and migration, breeding cycles, feeding and population structures of these large species of whales.

Associated with the hydrographical and biological work is the work of the chemists (the 306 hydrographic stations on the third cruise of the R.R.S. *Discovery* resulted in about 8,000 water samples on which some 27,000 analyses were made while at sea). On show at the Institute were two particular projects in chemical oceanography, one concerned with a study of the major constituents and nutrients in sea-water, and the other concerned with certain related physical properties, such as electrical conductivity and density.

The three-dimensional and time-varying complexity of the ocean might make investigation of the sea-floor seem comparatively straightforward, but time variations can occur there as well. One exhibit showed that skilled interpretation of sonar records can indicate the transport path of sediments on the continental shelf in waters around the British Isles. Another exhibit of a detailed investigation of a part of the deep sea-floor showed how a turbidity current, formed from land-derived material accumulating on the edge of the continental shelf and precipitated by some catastrophe down the continental slope, may have levelled out an abyssal plain by deposition of sediments in the Bay of Biscay and then eroded a connecting channel to a lower abyssal plain west of Portugal. Such dynamic sea-floor studies are unusual, the greater part of the work being on relatively stationary features now known from the painstaking accumulation of echo-sounding records (involving the design at this Institute of a deep sea 'precision' echo-sounder with an accuracy of 1 fathom in 3,000 fathoms) together with photographs of the deep sea-floor made with a National Institute of Oceanography underwater camera. These show such various phenomena as long ripples formed by bottom currents, boulders covered by a coating rich in manganese, steep slopes in channels and on sea mounts and possible basaltic lavas from the Mid-Atlantic ridge. Cores are obtained from both the deep and shallow sea-floors, and further geological information on faults, folding and possible lithological structure of the sea-bed in shallow waters is obtained from the interpretation of sonar records. (It is evident that this shallow-water geology is of interest to exploratory oil companies.) One of the projects recently started at this Institute is the development of a geological long-range sonar for use in deep water.

The National Institute of Oceanography took an active part in the sea-floor studies of the International Indian Ocean Expedition. On all three cruises a continuous echo-sounding record was obtained; the second cruise was particularly concerned with the geophysics of the sea-floor and has helped to answer questions about the formation of mid-ocean ridges and their relation to continental drift. One exhibit showed the possible development of the Gulf of Aden as a result of the splitting away of the Arabian Peninsula from the African continent some 20 million years ago. In a sense, the Indian Ocean work was a profitable digression from the work in the Atlantic. A contoured bathymetric chart of the north-east Atlantic compiled from soundings collected in the Hydrographic Department of the Navy (many of them taken by the R.R.S. *Discovery II* and the R.R.S. *Discovery*) showed clearly the structure of the Mid-Atlantic ridge and its median valley, the complexity of the continental shelf, and the recently explored deeps in a line with, and some four hundred miles west of, the north coast of Spain. This chart also amply demonstrates how it might profitably be used for navigation in conjunction with a precision echo-sounder. In itself, as it now stands, it could be of considerable use, involving possibly large financial savings, to those who lay cables across the sea-floor.

Much of the work at the National Institute of Oceanography is unique, particularly the electronic analogue model of the North Sea, which takes into account such variables as the topography of the sea-floor and the wind pattern, and which can predict storm surges with a reliability depending on the accuracy of the weather forecast. This is but one more instance of what was very evident in most of the exhibits, the application to-day of work which some years ago was of academic interest only.

Many of the exhibits showed that, as yet, many projects are necessarily incomplete; much of the work is as yet unpublished; the gear is constantly being improved; the data obtained, despite the use of computers, prove all but intractable in quantity to the very few scientists who wish to use them, and who are yet aware that these very observations are pitifully few in relation to the size of the ocean. The most urgent need is for more people to tackle the present problems, let alone those problems which will become apparent as more gradually becomes known about the ocean. Necessarily, most of the exhibits showed the work of the past 16 years, but some included indications of that planned or hoped for in the future, such as the thermistor chain, an instrument which should be particularly useful for measuring internal waves from ships underway at speeds up to 12 knots, and depths up to 2,000 ft. The temperature-sensing units will be towed at 50 ft. or 100 ft. intervals on a single-core double-armoured cable. It is hoped also to do more work on the results of the two seismograph stations, one at Wormley and one at Haslemere, and by analysing the six outputs from these stations, to obtain the two-dimensional spectrum of microseisms at any instant of time; this will give information as to the direction of arrival of the microseisms. Their relationship to sea-waves generated in storm centres has already been clearly demonstrated. More information, too, can be obtained from the pressure telemeter designed for use on the edge of the continental shelf, this instrument having been planned to measure the very small pressure fluctuations on the ocean floor caused by the passage of surface waves overhead.

Other exhibits, more complete in themselves and distinct in their applications, ranged from the simple devices (such as jelly bottles for measuring currents, especially at the bottom, which are of particular interest to fishermen and civil engineers) to the sophistication of 'vorticity waves'. It is suggested by theory that if a mass of fluid such as the sea or atmosphere is rotating nearly uniformly an unusual type of wave motion can be projected through the fluid. A model was demonstrated showing such vorticity waves set up in water in a rotating cylindrical vessel.

When the Royal Charter was granted in 1950 there were thirteen listed objects for which the Council was established and incorporated; the first, and necessarily foremost, of these was "to advance the science of oceanography in all its aspects", and the engineering section of the Institute, in addition to designing and making the prototypes of almost all the instruments used at the Institute, both in the laboratory and at sea, has provided instruments and equipment to other laboratories throughout the world. Much of the gear is required in too small numbers to be produced commercially and in addition there is already in the Institute an accumulated knowledge of the difficulties caused by high pressure at great depths in the ocean

and by the corrosive properties of sea-water. About forty projects were displayed as exhibits by the engineering section both as instruments and by working drawings, out of some 120 designed in the past sixteen years. Some of the more recent instruments have been briefly referred to here. The stabilized narrow-beam transducer is at present being used in the R.R.S. *Discovery*; this is part of a 36-kc/s underwater acoustic echo-ranger used for work on fish-detection problems and marine geological investigations. The axis of the acoustic beam is perpendicular to the fore-and-aft line of the ship and may be rotated from the horizontal to the downward vertical. Gyro stabilization is provided against the roll of the ship; beam width and side lobe patterns are adjustable. The range is about 800 yd. and scanning is achieved by steering the ship past the features to be investigated. A more recent project is the towed vehicle, which houses a transducer array for the long-range geological slanting sonar briefly mentioned here as being intended for use in deep water, which will work at a much lower frequency and will necessarily be larger than the sonar used in shallow water. It is intended that the vehicle will be towed at a depth of about 400 ft. and, in order to obtain adequate signal levels for the sound scattered back by the sea-floor, each of the 144 transducer elements will transmit 400 W of acoustic power. In connexion with the thermistor chain a helical traversing capstan is being developed as the traction member of a deep-sea hoist for handling faired electric cables. The capstan is so constructed that the cable advances across its surface with a purely rolling action, free of slipping or sideplay; there are no unbalanced forces to cause the path to be altered.

Yet another stated object of the Royal Charter was to collect and maintain an oceanographic library and, starting in 1953 with the welcome nuclei of earlier collections, the National Institute of Oceanography Library has now grown to be one of the major oceanographic libraries, containing about 3,500 text-books, 25,000 reprints and 7,000 unpublished reports all cross-indexed for easy reference, together with an annual intake of about 400 periodicals. There is a specialized collection of charts and atlases and a fine collection of scientific reports of oceanographic expeditions. The publications of the Institute, the *Collected Reprints* and *Discovery Reports*, are distributed on a world-wide basis, preferably with an exchange arrangement In a year the library lends some 3,000 publications, about 2,500 to scientists at the Institute and the others to many outside libraries.

The work of the Institute over the past 16 years was represented by these exhibits; the general line for the future was discussed at the press conference. It was reassuring to hear from Mr. Prentice that both the growth and continuity of the present work of the Institute would be maintained under the Natural Environment Research Council and encouraging to hear Prof. Lighthill comment on the fact that, although little money had been spent on the National Institute of Oceanography, yet the return had been good value; the amount of money spent on research is not necessarily in direct proportion to the results.

MARY SWALLOW

INSECT BEHAVIOUR

THE Royal Entomological Society of London continued its series of biennial symposia by organizing a meeting on "Insect Behaviour" which was held at the Imperial College of Science and Technology, London, during September 23–24, 1965. The meeting was opened by Mr. E. O. Pearson, the president of the Society, who pointed out that the objective of this series of symposia was to provide authoritative surveys of recent work in the chosen field for the information of entomologists generally, and the very high attendance at this meeting emphasized that these symposia fulfil a real need.

Prof. Birukow (Gottingen) spoke on orientation behaviour and factors which influence it. This particular aspect of insect behaviour is engaging the attention of several German entomologists, and Prof. Birukow was able to indicate the way in which past attempts at the systematization of the subject contrasted with more recent approaches such as those of Jander. He described the capacities and physiological mechanisms of lower levels of orientation behaviour (the so-called baso-taxes) and pointed out that higher orientation behaviour may have evolved from these. The variability noted in orientation behaviour results principally from adaptive variability which ensures that an animal can never become 'a slave to its own orientation mechanisms'. Insect orientation behaviour can be adjusted by the interaction of innate and learned patterns, but in complex behaviour it is rarely possible to discriminate between these two sources. Prof. Birukow gave details of his interesting work on light orientation in *Calandra granaria*, which is affected by biological rhythms and meteorological factors.

Dr. P. S. Corbet (Ottawa) gave an account of the role of rhythms in insect behaviour, and from this wide field took as his main theme the factors which influence field periodicities. Particularly valuable were his clear definitions of the terms used to describe periodic behaviour and his elucidation of the relationships between them. He pointed out that the causal analysis of field periodicities in terms of their endogenous and exogenous components had been neglected because integration of field and laboratory work on periodic behaviour had seldom been attempted. He underlined the need for investigations of the periodicity of single processes in the laboratory under controlled conditions and also in Nature, preferably in several situations. He particularly emphasized the need for entomologists to recognize the pervasiveness of rhythmicity as an experimental variable. He instanced the response to sex pheromones, the amount of body protein and the intensity of virus transmission as processes under control of circadian rhythms which, therefore, show different values at different times of day and night. He suggested that a description of any such process should properly include an account of its periodicity, particularly when this affects its measurement or detection.

Dr. P. T. Haskell (London) discussed flight behaviour, first mentioning factors inhibiting or limiting to insect flight, particularly the effect of wind on height of flight and orientation. He went on to outline the general aspects of flight behaviour in respect of food-seeking, homing and territorial flight, defence and predator avoidance, courtship and mating, oviposition and migration and dispersal. He pointed out that there seem to be certain basic patterns which are utilized in different types of behaviour such as, for example, food-seeking and oviposition, which pose the same fundamental problem to the insect, that of homing on a target. He described recent work on mechanisms involved in anemotactic behaviour released by olfactory stimuli, which are important in relation to mating, food and oviposition behaviour, and queried theoretical deductions which supposedly rule out tropotactic orientation to an odour source.

Prof. V. G. Dethier (Pennsylvania) dealt with feeding behaviour and pointed out that in the past decades the emphasis in this field of investigation had altered from description to an investigation of mechanisms. He examined the present-day controversies as to whether

insect relationship to food plants is dictated by token or nutrient stimuli, whether oligophagy is based on attraction or repulsion and whether taste or smell is more important. Behavioural, and particularly physiological, experiments seem to indicate that all these factors are involved. The inflow of data to the central nervous system relating to stimulation by food plant substances is complex, and hence neither the mechanism of discrimination nor the basis of preference can be a simple one. Prof. Dethier pointed out that the regulation of the initiation, termination and frequency of feeding had received less attention than the problem of selection. In some insects, for example the fly, the process is driven by sensory input and is terminated by sensory adaptation, while the frequency of feeding is controlled by an as yet unknown mechanism; on the other hand, in other insects such as the locust, endogenous central activity appears to influence the initiation of feeding.

Dr. A. Manning (Edinburgh) described sexual behaviour and defined it as "specialized behaviour patterns which form the normal preliminaries to mating". He pointed out that elaborate sexual behaviour occurs in some insects but not in others and raised the problem of 'female coyness'. He considered three possible functions of courtship: appeasement and synchronization, physiological maturation, and sexual isolation, and concluded that various combinations of the foregoing functions must have been involved in the evolution of 'female coyness'. He felt that a direct origin of the movements used in courtship from modified pre-copulation movements was often plausible, and pointed out that the ritualism of insect sexual behaviour had followed a course very similar to that found in vertebrates. As to the organization of sexual behaviour, he emphasized that the data were so meagre that little generalization could be made, but that sexual behaviour seems to depend more on the sex of the brain than on that of the lower centres.

Dr. J. D. Carthy (London) spoke on insect communication, which could be said to occur whenever a sensory signal caused a change in the behaviour of an animal. He pointed out that in most cases the receiver is informed of the physiological state, the sex, the species and often the position of the sender, that the language is often private to the species and that dialects may occur which are specific to a group of individuals in a neighbourhood. He examined the range and type of visual, acoustic, tactile and chemical communication and brought forward examples showing how these may be combined to increase the total sum of information passed.

Prof. E. O. Wilson (Harvard), in dealing with the behaviour of social insects, deliberately minimized the functional aspects of this behaviour in order to pay close attention to theoretical considerations of its evolution. In examining its origin, he supported the view of Hamilton that true sociality, mostly limited to the Hymenoptera, had arisen because of the haplo-diploidy prevalent in that order, which, since it resulted in greater genetic similarity between siblings than between parents and offspring, favoured the development of altruistic behaviour. Sociality in the termites, he felt, was associated with obligatory protozoan symbiosis, thus taking the view that true sociality was made possible through pre-adaptations which themselves had nothing to do with sociality. Prof. Wilson went on to examine fitness in insect societies and indicated, for example, that worker inefficiency, including mass behaviour that is the opposite of social facilitation, is widespread in the social insects and yet makes a positive contribution to colony fitness. On balance, however, he concluded that the question of altruism above the individual level remains open.

The final paper was given by Dr. J. S. Kennedy (Cambridge) and the arrangements for this were of an experimental nature in that Dr. Kennedy was asked to talk on "Some Outstanding Questions in Insect Behaviour", drawing largely on points raised by the preceding speakers and thus promoting wide-ranging general discussion to end the symposium. Dr. Kennedy treated four main topics: the origin of social behaviour, communication and 'female coyness', orientation behaviour, and the control of responsiveness. As regards the origins of social behaviour Dr. Kennedy disagreed with Prof. Wilson and suggested that the long-standing theory of parental care as the basis of social behaviour was still adequate. The ensuing lively discussion touched on the value of parental care, the influence of food-sharing, the significance of symbiosis and the evolution of altruistic and selfish behaviour. On balance, while it was conceded that the phenomena of haplo-diploidy and symbiosis may have played some part, parental care won the day as being likely to afford a sufficient basis for the evolution of sociality.

As regards 'female coyness', Dr. Kennedy asked if this was widespread and enquired whether 'male coyness' was also involved, and whether in any case the term was not a misinterpretation of the delaying function of the behaviour which may be necessary so that both males and females can complete internal processes essential to mating. The discussion emphasized the paucity of data on the subject, although the interesting suggestion was made that there was selective value to the behaviour if it was considered as an extension to normal pre-copulatory communication which establishes specific recognition.

On orientation, Dr. Kennedy asked whether the system of Jander did in fact correspond to the 'natural structure' of the behaviour; the discussion did not elucidate this point and served to emphasize the continuing lack of any adequate structure for the classification of insect behaviour.

The final problem discussed by Dr. Kennedy was the control of responsiveness in insects in various behavioural situations, and raised the important issue of the separation of endogenous activity, or 'drive', from reflex activity—the stimulus-response system. The general tenor of the discussion was that the past approach to this problem, based on an 'either–or' choice of these two concepts, had been misleading; the actual system probably involved characteristics of both.

All the papers and the ensuing discussions are to be published shortly in a fully bound volume by the Royal Entomological Society.

P. T. Haskell

FOREST NURSERIES

Forest nurseries became important in British forest practice with the introduction of exotic conifers in the latter part of the eighteenth century for large-scale reforestation work on private estates. But they assumed even greater importance after the creation of the Forestry Commission in 1919 with its declared policy of afforestation. At the present time the plant requirements of the State forest service and of private estates and commercial enterprises are enormous and nursery practice has become a scientific matter. This has developed greatly in the past twenty years. Before that, nurseries were mainly the agricultural type where fertility was maintained by farmyard manure in some cases but mainly by ploughing-in green crops and the use of fallows and rotational practice. However, it was found that the best conditions were not being obtained for growing coniferous seedlings. These were required in greater quantities than ever and in less time than the usual 3–4 years to produce a plant suitable for afforestation purposes. Some experimental work had been done by the Forestry Commission, but in 1944 it

was decided to set up a "Sub-Committee on Nutritional Problems in Tree Nurseries" the terms of reference of which were "to assess the problems underlying the nutrition of forest trees in nurseries and to suggest any desirable extensions already in progress".

The account of the work carried out between 1945 and 1962 by a joint research effort between staff of the Rothamsted Experimental Station and the Research Branch of the Forestry Commission is embodied in *Forestry Commission Bulletin* No. 37 *.

Volume 1 comprises the text and the main tables and Volume 2 contains detailed supporting tables, but this second volume is not needed for reading the first. The initial volume contains three main parts. First, there is a historical account of nursery practice in Britain since the 1920's. The objects and scope of the investigation are defined and notes are provided on sites and experimental methods. The second part, and by far the largest, is concerned with seed-bed experiments and these are well described and supported by adequate tables. The third part deals with experimental work on transplants and this required extension experiments.

Histories of individual nursery sites and notes on sylvicultural techniques are included in the appendix and there are useful supplementary papers on microfauna and microflora. There are eight pages of coloured plates and they can only be described as superb. They bring out in a most certain way what each plate is meant to show. Indeed, the whole publication maintains that high standard associated with Forestry Commission bulletins.

Volume 2 will have a limited demand, but Volume 1 is a valuable contribution to the understanding of forest nursery problems and to the improvement of techniques in raising young trees for afforestation and re-forestation purposes. As planting is likely to be the means most used for many years to come in regenerating Britain's forests, it is obvious that good forest nursery practice is of the greatest importance. C. J. TAYLOR

* Forestry Commission. Bulletin No. 37. *Experiments on Nutrition Problems in Forest Nurseries*. By Blanche Benzian. Vol. 1. Pp. xi + 251 + 25 plates. 50*s*. net. Vol. 2. Pp. v + 265. 20*s*. net. (London: H.M.S.O., 1965.)

PERUGIA UNIVERSITY MEDICAL SCHOOL

NEW LECTURE HALL OF PATHOLOGY

By PROF. LUCIO SEVERI

Director, Institute of Morbid Anatomy

ON June 27, 1965, Lord Florey, president of the Royal Society of London and Provost of Queen's College, Oxford, opened the new Lecture Hall of Pathology in Perugia, Monteluce. At the same time he delivered the first Cesare Massari lecture entitled "The Anatomy and Physiology of Small Blood Vessels".

The ceremony was attended by the Magnifico Rettore of Perugia University, members of the Perugia University Medical School, participants in the third Perugia quadrennial international conference on cancer ("Lung Tumours in Animals"), and other distinguished guests from many Italian universities.

Science in Perugia

It Italy, as elsewhere, there is an ever-growing and deeply felt concern about the training of scientists and technologists. In Perugia, scientists and technologists are graduating in large numbers, certainly in greater numbers than at any other time. These numbers, however, are not satisfactory, since scientists and technologists comprise only about one-third of the student population—the lowest point of a progressive percentage decrease over the past 26 years. The numbers of students in pharmacy, agriculture and veterinary medicine are not increasing, although these faculties are traditional in the University of Perugia; however, the Faculty of Science, founded during the academic year 1951–52, is steadily developing. It is difficult to give figures, as classification is, to some extent, subjective, especially in a university where everyone has his own ideas on how disciplines should be grouped together. Nevertheless, Table 1 gives some figures.

Of the figures given in Table 1, scientists represent the proportions shown in Table 2.

Medicine in Perugia

The Perugia University Medical School is fortunate: although it is situated within the city boundaries, it lies on Monteluce hill (1,500 ft. above sea-level) facing east; it dominates the valley of the Tiber and looks on to Assisi and the Appenines (snow-capped for the greater part of the year). The development of medical studies has followed a normal pattern, as can be seen from Table 3.

The Medical School has steadily expanded its facilities since the end of the Second World War. New buildings have been provided for those departments dealing with general pathology and morbid anatomy, orthopaedics, paediatrics (with a Poliomyelitis Department), surgery and dentistry. A large six-storeyed block has been built for the Institutes of Biology, Physiology, Anatomy, Pharmacology, Forensic Medicine and Hygiene. Small lecture rooms have been set aside for seminars and tutorials in each Institute and there is a large common lecture hall sufficient for 300 students. Probably the most interesting development in the field of research in the Perugia Medical School has been the growth of the Division of Cancer

Table 1. TOTAL STUDENTS (ALL SUBJECTS)*

	Undergraduate	Postgraduate	Total
1938–39	615	—	615
1949–50	2,558	—	2,558
1954–55	2,606	86	2,692
1959–60	3,906	289	4,195
1964–65	6,130	248	6,378

* The student enrolment figures quoted in the text were taken from the University of Perugia Year Books for the academic years 1938–39, 1949–50, 1954–55, 1959–60 and 1964–65.

Table 2

	I	II	III	Total scientists	Total students	Total scientists expressed as a percentage of total students
1938–39	—	263	201	464	615	75·4
1949–50	—	1,242	464	1,706	2,558	66·7
1954–55	142	1,019	346	1,507	2,692	56 0
1959–60	273	1,090	337	1,700	4,195	40·5
1964–65	496	1,356	315	2,167	6,378	34·0

Group I. Students in mathematical, physical and biological sciences, with degrees in biological sciences, natural sciences and chemistry.
Group II. Students in medical sciences (including medicine, pharmacy and veterinary medicine).
Group III. Students in agriculture.

Table 3. STUDENTS IN MEDICINE

	Undergraduate	Postgraduate	Total
1938–39	177	—	177
1949–50	579	—	579
1954–55	617	86	703
1959–60	607	289	896
1964–65	842	248	1,090

Research[1] which is housed at Monteluce, although its personnel are involved only in a tenuous way with the University.

Medical teaching requires both large and small lecture theatres, but this need has not yet been met at Perugia.

The New Lecture Hall of Pathology

In Italian medical schools, the teaching of pathology is divided into two parts: general pathology for students in their second and third years, and morbid anatomy (or pathological anatomy, including pathological histology) for students in their fourth and fifth years.

In Perugia, the two teaching programmes have always had a common lecture hall, and during the period from 1927–28 until 1949–50, this was in the old Institute of Morbid Anatomy and seated only about 40; then, when the two Institutes transferred in 1950–51 to a new building where they have so far been located, they used a lecture hall which accommodated about 135, but this was demolished in the spring of 1964.

The Lecture Hall of Pathology has now been rebuilt between a retaining wall (about 15 metres high) and the back wall of the Institutes of General Pathology and Morbid Anatomy. Its ground plan is in the form of a trapezium with its shorter parallel side abutting on to the Institutes. The floor level of the new Hall corresponds to that of the old one, and to the ground-floor level of the Institute of Morbid Anatomy.

The building has three storeys including a basement, and between the ground and first floors is the lecture hall itself. This opens off the main entrance of the Institutes, from which run two lateral corridors. These lead to the hall and, below the hall, to the dressing-rooms and toilets. The right-hand corridor also leads to the outside on the east; the one on the left ends in a stairway which leads into the garden on the west side and basement.

At the back of the area below the hall are two stairways, which ascend to the first floor where the hall can be entered from two sides. From a stairway on the left a narrow corridor gives access to the large central projection room and four small cabins, two on either side; these can be used for simultaneous translation. The hall is 20·4 m long, and the dais area projects into the old building for 3·6 m. The 340 seats are placed in 12 ascending rows, with one row at floor level, and rise steeply in three large blocks—the two side blocks being at a slight angle to the central one. Two stairways divide the side and centre blocks. The top (13th) row is 4·6 m from floor level.

Lighting within the hall is both artificial and natural; daylight comes in from six windows in the eastern wall and from four in the western; on the first floor, the two windows at the entrance to the hall give a panoramic view of the city of Perugia. Total or partial darkening is controlled electrically from the dais or from the projection room. Enclosed in the wall near the dais there is hot and cold water. Behind the table is a large blackboard with indirect lighting. Microphone and loud-speaker equipment, as well as simultaneous translation equipment, have been installed, and the hall is centrally heated. The screen is lowered electrically from the ceiling just in front of the dais area (a solution which is not entirely satisfactory, but is dictated by considerations of projection). Good acoustics are assured by a ceiling consisting of differently inclined planes, lined with sound-absorbing material.

The lecture hall is not used for dissection, which is undertaken in special rooms and in another small tutorial room. Corpses are brought from all departments to the central area of the basement by means of an underground passage. In the western part is the post-mortem section.

The building is a concrete-cased steel-framed structure, faced with grey-coloured plaster. However, the two lateral corridors are built of vertical metal frames so as to permit a continuous series of windows. The roof is umbrella-shaped, with beams converging towards the retaining wall; particular care was required in its construction because of the thrust of the beams from above on the opposite wall of the building.

The foundations were laid on piles because the soil on the site consists of light clay with an admixture of sand. The total surface covered by the building is about 550 m².

This new Lecture Hall of Pathology is the largest, the most modern and the best-equipped of its kind in Italy. The building was designed by Prof. G. Nicolosi, Rome, and the construction was carried out by Fioroni Brothers, Perugia, under the direction of Dr. G. Tosti, Perugia.

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INSTABILITY OF SOME OF THE PRODUCTS FORMED BY THE ACTION OF *N*-METHYL-*N*-NITROSOURETHANE ON CYSTEINE *IN VITRO*. ROLE OF NEIGHBOURING GROUPS IN ENZYMATIC AND CARCINOGENIC ACTION

By Dr. R. SCHOENTAL

Toxicology Research Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey

IT has been suspected for more than a quarter of a century that sulphydryl-groups play a part in the carcinogenic process. Though much work has been devoted to their estimation in conjunction with the treatment with carcinogenic agents, and to other aspects of this problem, no clear picture emerged in this connexion. Recently, however, new developments in the field of carcinogenesis once more focused attention on the role of sulphydryls, mainly the fact that certain newly discovered carcinogens interact *in vitro* with free thiol groups without the need of enzymes. This is the case of 4-nitroquinoline-*N*-oxide[1], β-propiolactone[2], *N*-hydroxyurethane[3] and *N*-alkyl-*N*-nitrosourethanes[4].

Free sulphydryl-groups are known to be very reactive; their competition factor has been reported to be 1,000 times higher than that of terminal amino- or hydroxy-groups[5]. They can interact with carbonyl-groups, the more readily when these are activated, for example, by the presence of *N*-alkyl-*N*-nitroso-groups, as is the case with *N*-alkyl-*N*-nitrosourethanes and related carcinogens.

Detailed investigations of the reaction between cysteine and *N*-methyl-*N*-nitrosourethane (MNU)[6] disclosed that the reaction proceeds readily at room temperature with evolution of nitrogen and complicating mixtures of products are formed. The reaction appears to proceed according to Scheme 1. At *p*H 6·5 the main products were *S*-ethoxycarbonylcysteine and its methylester and *S*-methylcysteine and its methylester. The esters are unstable and hydrolyse on standing even at neutral *p*H. *S*-ethoxycarbonylcysteine itself is very sensitive to alkali. Dilute ammonia or phosphate buffer *p*H 8·0 at

room temperature transforms it rapidly into *N*-ethoxycarbonylcysteine, with the restoration of a free sulphydryl group. The latter is then capable of interaction either with a second molecule of MNU (to form *S,N*-diethoxycarbonylcysteine), or with the 'active methylene' released on the disruption of the MNU molecule (to form *N*-ethoxycarbonyl-*S*-methyl-cysteine) or with any other sulphydryl reagents, such as ethylmaleimide. The isomerization of the *S*-ethoxycarbonylcysteine to the *N*-derivative proceeds even in almost neutral, aqueous solutions (*p*H 7·2) when these are left at room temperature for a few days.

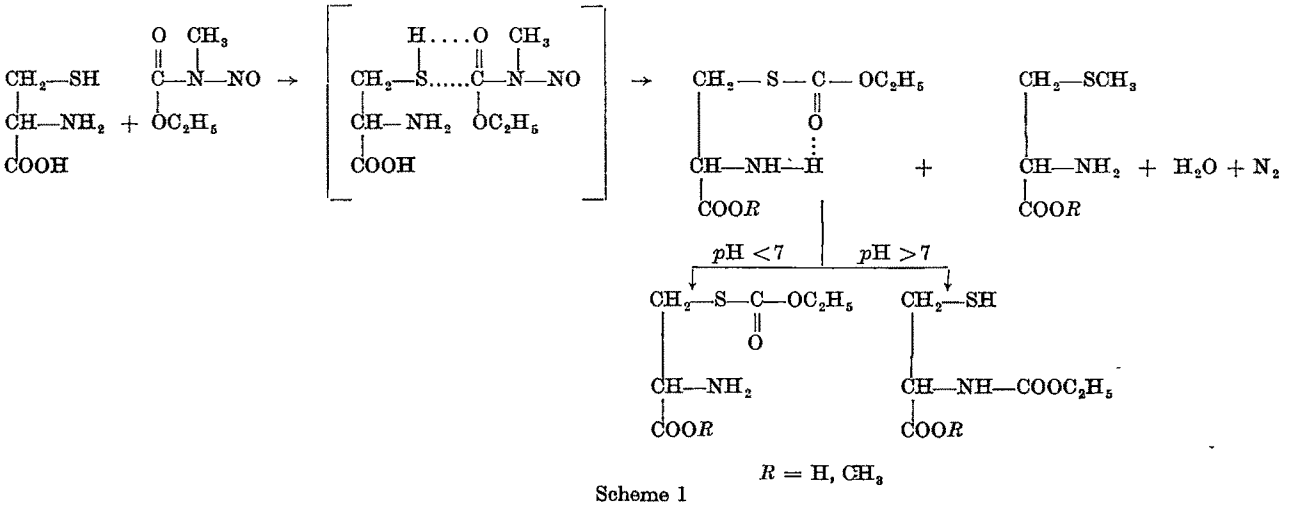
In cysteine, the sulphydryl- and the amino-groups are in close proximity and the isomerization is probably facilitated by a hydrogen-bonded cyclic intermediate.

Isomerization by alkali of *S*-substituted cysteines to their *N*-isomers have been observed in the case of the *S*-(2,4-dinitrophenyl)-derivative[7] and of the *S*-carbobenzoxy-derivative[8]. However, *S*-ethoxycarbonylcysteine isomerizes even more readily, even at the physiological range of *p*H, and its behaviour may serve to illustrate the action of sulphydryl enzymes.

Several thiol groups are usually present, as cysteine residues, in almost any molecule of a protein and enzyme. Sulphydryl groups exert several roles in cellular functions; they regulate the oxido-reduction potential, determine the folding and the tertiary configuration of proteins and only very few are actually involved in the catalytic activity of enzymes. For the latter role, the presence of appropriate neighbouring groups appears to be essential, such that would enable them to form cyclic intermediates with the substrate.

Sulphydryl groups, owing to their great reactivity, will interact more readily than other groups with certain substrates, but in order to be able to do so repeatedly, as is required for catalytic action, they must be able to hand over the substrate to other neighbouring groups and be restored for further action. *S*-ethoxycarbonylcysteine can be considered a prototype of such behaviour; its ethoxycarbonyl group migrates readily from the sulphur to the adjacent amino group with which it could not have reacted directly. It is possible that this isomerization proceeds by way of a hydrogen-bonded cyclic intermediate such as shown in Scheme 1. The restored thiol is then free for further action.

Among the products of the reaction between MNU and cysteine *in vitro* *S*-ethoxycarbonylcysteine methyl ester and *S*-methylcysteine methyl ester were present[6]. As mentioned already, these esters were unstable and hydrolysed readily even at neutral *p*H and at room temperature, probably due to the intra-molecular proximity of the amino- to the ester-group. Neighbouring groups are known sometimes to exert striking effects on the rate of hydrolysis of esters; for example, the β-benzyl ester of *N*-carbobenzyloxy-L-aspartyl-L-serylamide has been shown to hydrolyse 10^7 times faster than benzyl-propionate[16]. The authors suggested that this might be due to the ease of formation of a cyclic imide intermediate in the former. Other neighbouring groups, such as hydroxy-[17] or carbonyl-[18], increase also the rate of hydrolysis of esters. In some esterases and certain hydrolytic enzymes the 'active site' is known to be associated with the hydroxyl of serine residues, possibly in conjunction with histidyl-imidazoles[19]. Among the enzymes, in which the amino-



Scheme 1

In the case of the sulphydryl enzyme, creatine phosphokinase, Rabin and Watts[9] observed that the rate of the reaction of iodoacetate or iodoacetamide with the 'active sulphydryl' was independent of *p*H in the range 6·0–10·0. They suggested that this might be due to hydrogen bonding between the thiol and a basic group in its proximity, possibly the imidazole of a histidine residue. The reaction rate of these alkylating agents with thiol groups of several other enzymes, namely, of phosphoglucomutase[10], arginine kinase[11] and yeast alcohol dehydrogenase[12–14], has since then been reported to be independent of *p*H changes in a wide range.

In yeast alcohol dehydrogenase a histidine residue is actually adjacent to the cysteine bearing the 'active thiol' (according to Harris—quoted by Thomson *et al.*[15]), but in some of the other –SH enzymes this is not the case. As pointed out by Rabin, the basic group does not necessarily need to be present on the amino-acid bound by a peptide bond to the cysteine residue; it could be brought into close proximity in the tri-dimensional structure of the enzyme by appropriate folding of the protein.

acid sequence of the 'action site' has been established, a histidine-residue has been found to be adjacent to the 'active' serine-residue on the same peptide chain in phosphoglucomutase[20]; but in others, for example chymotrypsin or trypsin[21,22], histidine-residues are present only in remote parts of the peptide chain, yet by appropriate folding of the protein they might be brought into close proximity to the serinyl-hydroxyl. On denaturation, when the peptide chains unfold, the reactivity of the 'active' serinyl-hydroxyl is usually lost[19].

During physiological activity the attachment of a substrate to, and the release of the product from, the active sites of enzyme proceed continuously, but when a foreign chemical is mistaken for a normal substrate it might remain irreversibly attached to the active or its neighbouring group and inactivate the enzyme.

The typical sulphydryl reagents, such as ethylmaleimide, block probably all accessible thiols—hence their high toxicity and lethal effects on cells. Chemical carcinogens, however, appear capable of inducing more specific changes in the cell, such as are compatible with its survival, and

lead to progressively more pronounced changes until a tumour erupts months or years later.

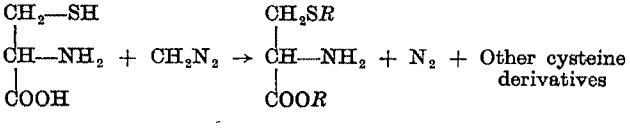
Many of the known chemical carcinogens have the following characteristics: (*a*) they can interact with sulphydryls without the need of enzymes, and (*b*) they are, or become, through metabolic transformation, bi- (or multi-) functional agents.

This is *par excellence* the case of *N*-methyl-*N*-nitrosourethane, one of the most effective carcinogens and capable of inducing cancer even with a single dose. This efficacy may be related to its acting as a multi-functional agent. When it reacts with sulphydryls it yields both an ethoxycarbonyl-group and an 'active methylene'; hence it is capable of concurrent substitution of several reactive groups. This is of obvious importance in cellular reactions, which mainly take place on surfaces. The probability of concerted multi-point substitution by mono-functional agents is obviously much smaller.

The sulphydryls which react with MNU and form *S*-ethoxy-carbonyl-derivatives will not necessarily remain substituted; as mentioned already, the ethoxycarbonyl-group can be shifted readily to a vicinal amino-group when the *p*H changes only slightly and a free thiol is restored. However, when the 'active alkylene', released *in statu nascendi* from the decomposing MNU, reacts with thiols to form thioethers or alkylene bridges, such derivatives will be more stable. If such derivatives were formed *in vivo*, especially on the structural proteins or enzymes, they could profoundly alter their tertiary conformations and the oxido-reductive or catalytic activity The part which such alkylations may play in the carcinogenic process is still not clear.

Many alkylating agents show no, or only marginal, carcinogenic activity, notably the alkyl halides and dialkylsulphates (Dr. P. N. Magee, personal communication). Physiological methylations whether of *C*-, *N*-, *O*-, or *S*- are essential biochemical processes in the cell. The difference between the various 'alkylating' agents may depend on the 'site' which is alkylated. Bulky 'active methionine' (*S*-adenosyl-methionine) or alkylating agents requiring enzymes will alkylate easily accessible reactive groups, while small 'active alkylenes', like those derived from the carcinogenic *N*-alkyl-*N*-nitrosourethanes or diazoalkanes, may be able to reach some of the more sterically hindered or 'buried' sulphydryls and other reactive groups. Indeed, the size of the 'active alkylene' may be related to the efficacy of the parent compound. It has been reported that MNU is more toxic and more mutagenic than ENU for the fungus, *Colletotrichum coccodes*[23]. In rats, given single doses of these two homologues by stomach tube, MNU proved to be about six times more acutely toxic than ENU (Schoental, unpublished results). Both compounds induced stomach tumours, but it is too early to evaluate their relative efficacy in this respect. However, an additional factor must also be considered: ENU reacts with thiol compounds more sluggishly than MNU, and the rate of evolution of the 'active ethylene' would be slower.

It could be argued that the role of *S*-ethoxycarbonyl-substitution should not be disregarded in the carcinogenic action of alkylnitrosourethanes in view of the carcinogenic activity shown by urethane itself. However, even in the case of urethane it has been reported[3] that *S*-ethyl-cysteine, as well as *S*-ethoxycarbonylcysteine, was present among the products of hydrolysis of a mercapturic acid-fraction obtained from the urine of rats dosed with this



R = H, CH_3

Scheme 2

compound. This suggests that ethyl-groups may be released from urethane. In experiments *in vitro* using cysteine (or glutathione), *S*-methyl-derivatives and their methyl esters, were the common products formed with both carcinogens, diazomethane (Scheme 2) and *N*-methyl-*N*-nitrosourethane (Scheme 1), and this would suggest that attention should be focused on products formed by the action of 'active alkylenes' with cell constituents, when considering the mechanism of carcinogenesis.

It is conceivable that in the intact cell some of the guanine-imidazoles of nucleic acids are in close proximity to certain thiols of structural proteins. It may be significant that isolated nucleic acids persistently retain small amounts of proteinaceous material, and that in certain cases it would appear that sulphur is involved in the links between the nucleic acids and the residual proteins[24]. Thus, it has been reported[25]: "The requirement of the reducing agent (β-mercaptoethanol) used for the isolation of DNA from mammalian sperm suggests the presence of a special sulphur-containing linkage between DNA (or a component attached thereto) in the sperm nucleus and the protein coat, cleavage of which is necessary before the nucleic acid can be released". It is also known that it is exceedingly difficult to free ribonucleic acids isolated from various viruses from residual proteinaceous materials[26]. In the case of the infective RNA isolated from ^{35}S-labelled tobacco-mosaic virus (TMV), the RNA retained ^{35}S-radioactivity, connected presumably with the residual protein, which resisted various attempts at its removal[27]. Eventually, treatment of the TMV–RNA with bentonite reduced the protein content to trace quantities[28]. The difficulties of obtaining nucleic acids free from small amounts of proteins (or peptides) would indicate the existence of, perhaps co-valent, bonds between proteins and nucleic acids. In this connexion, the fact that the majority of co-enzymes are either nucleotides or have structures analogous to nucleotides is of particular interest. Whether the sites where a protein-thiol is linked or close to a nucleic acid-imidazole represent the 'sensitive centres' involved in the carcinogenic action of alkyl-nitrosourethanes and of some other chemical carcinogens requires further investigation.

The carcinogenic *N*-methyl-*N*-nitrosourethane reacts *in vitro*, at room temperature, with thiol groups, with the evolution of nitrogen, and effects multi-point substitutions. With cysteine, at *p*H 6·0–7·0, *S*-ethoxycarbonylcysteine and *S*-methylcysteine and their respective methyl esters represent the main reaction products. At *p*H higher than 7·0, *S*-ethoxycarbonylcysteine isomerizes readily to *N*-ethoxycarbonylcysteine, and a free thiol is restored; the methyl esters also hydrolyse rapidly. This instability of the primary reaction products to slight shifts of *p*H is probably due to neighbouring groups. In cysteine, the thio-, the amino-, and the carboxy-groups are in close proximity. If the reaction *in vitro* occurs in the presence in the solution of nucleic acids, the latter become methylated mainly at the position 7 of guanine.

As the reaction between MNU and thio-compounds does not require enzymes and proceeds at the physiological range of *p*H, it is suggested that similar events could occur *in vivo*. Concurrent substitution of several reactive groups could occur, possibly in some structural elements which are brought into close proximity to a thiol-group, in the tri-dimensional framework of the cell. The thiol itself may not retain the substituent; estimation of free sulphydryls would then not detect the fact that these groups were primarily involved in the reaction.

The irreversible consequences and cancer which can follow a single dose of MNU[29] may be related to its ability to interact with specific 'sensitive centres' in the cell, possibly such as the sulphur links, which appear to exist between nucleic acids and some residual proteinaceous material. The well-known difficulties of removal of the residual small amount of proteinaceous materials from

isolated nucleic acids, whether from mammalian tissues or viruses, might indicate a specific biological role of these sites.

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PHOTOSENSITIZATION AND LYSOSOMAL DAMAGE

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ONE type of photosensitization results from the accumulation in the blood and tissues of substances normally eliminated in the bile; it is the accumulation of such substances which provokes the characteristic reaction of the skin to light. In several such instances with livestock the major factor in the photosensitization has been demonstrated to be the chlorophyll degradation product, phylloerythrin[1,2]. This porphyrin is but one of many such photosensitizers which have been experimentally shown to lead to abnormal reactions of the skin to light following their parenteral administration (for reviews see Clare[3], Blum[4]). For example, see the early work of Jodlbauer and Busck[5] with fluorescein dyes, and the experiments of Hausmann[6] and of Solomon[7] using haematoporphyrin. The latter experiments are of interest in relation to the well-known photosensitization associated with certain types of porphyria. Many photosensitizers are coloured substances and can exhibit their deleterious action in conjunction with light of the visible region. This is a useful, though not infallible, feature distinguishing porphyria from sunburn, which is commonly taken to be dependent on ultra-violet radiation for its development.

The absorption of light energy by a photosensitizer can be taken to result in the formation of an excited state. In some instances the full development of the photosensitization reactions has been shown to require oxygen[8] and to be essentially independent of temperature[9]. These findings suggest that an essential part of the process (at least in the particular instances investigated) is the formation of oxygen-containing free radicals of the peroxy-type. Such radicals are readily formed by the interaction of molecular oxygen and many other free radical systems[10]. If this view is correct, such an event would be harmful to the cells in which the reaction occurred (for example, epidermal cells). Lipid peroxidation, a process involving such radicals, is known to have a powerfully damaging action on lipid rich structures, for example, the many types of membrane system present in the cells[11]. The intracellular formation of significant quantities of such destructively reactive radicals would result in damage to mitochondria, the endoplasmic reticulum, lysosomes, and the external cell wall itself. The actual extent and intracellular localization of the damage would depend on many factors. For example: (*a*) the site or sites of production of the radical; free radicals are known[12] to have an effective sphere of influence in biological material of about 30 Å; (*b*) the concentration and nature of the radicals produced; and (*c*) the intracellular localization of free radical chain terminators (for example, vitamin E). Tappel and colleagues have clearly shown[13] that free radicals induced by a variety of methods (ultra-violet irradiation; cobalt-60 irradiation; hydrogen peroxide decomposition) caused rupture of a purified preparation of rat liver lysosomes. Swelling of mitochondria was also demonstrated in similar experiments[14], although high concentrations of radicals were required for both these purposes.

The possibility therefore arises that the action of photosensitizers can be placed on a biochemical basis by considering that they function as initiators of free radical (chain) reactions, the free radicals thus produced then causing disruption of intracellular membrane systems with consequent damage to, and eventually even the death of, the cells concerned. In this communication we present evidence for the validity of this argument, based on studies of one type of membrane-limited intracellular particle—the lysosome.

For experiments involving the use of liver homogenates, adult female albino rats were used. They were killed by cervical dislocation and the liver quickly removed, weighed and homogenized in 9 volumes ice-cold 0·25 M sucrose. The homogenate was centrifuged for 10 min at 600*g* to remove nuclei, red cells and debris; the supernatant was then centrifuged for 15 min at 15,000*g* to sediment a large particle fraction. This was re-suspended in ice-cold 0·70 M or 0·25 M sucrose.

Acid phosphatase in this fraction was estimated by the method of Berthet and de Duve[15], incubation time 10 min at 37° C, *p*H 5·0. β-Glucuronidase was estimated as described by Gianetto and de Duve[16]. Acid sulphatase was measured by the method of Roy[17]. Free and total activities of each enzyme were determined in the absence or presence of 'Triton *X*-100' respectively[18].

The effect of phylloerythrin and/or irradiation on the latency[19] of these acid hydrolases was determined in the following manner. A sample of untreated suspension and another containing phylloerythrin were irradiated in a cold-room at 2° C using an ordinary household electric lamp (60 W, distance 15 in.) for 30 min–1 h; stirring was

achieved using a magnetic stirrer. A pair of identical samples was also placed on top of the magnetic stirrer but was completely protected from light by enclosure in a thick cardboard box. In other experiments, irradiation was performed using a mercury vapour lamp under similar conditions.

For experiments involving skin sections, adult female albino rats were killed and the tail skin dissected off and frozen in carboxy-methyl cellulose at −20° C. Cryostat sections 7μ thick were cut and fixed for 10 min in ice-cold buffered formol–calcium (*p*H 7·2) washed in distilled water and treated for 10 min at room temperature with the following reagents: barbitone/HCl buffer (*p*H 7·2), buffered phylloerythrin solutions of differing dilution, buffered hydrocortisone sodium succinate, and buffered *d*-α-tocopheryl polyethylene glycol 1,000 succinate. The sections were irradiated in fluid-filled watch-glasses with light from a mercury vapour lamp (H.B.O. 200) for periods between 10 sec and 30 min, and subsequently washed in distilled water and incubated to demonstrate acid phosphatase activity. This was determined by a variant of Gomori's method[20]; sections were incubated for 1 h at 37° C.

Liver experiments. In this communication only the results found for acid phosphatase activity will be discussed in any detail. Irradiation alone (either visible or ultra-violet) produced a small decrease in the bound/free activity ratio for acid phosphatase (Table 1). This ratio is a measure of the intactness of the lysosomal membrane. Phylloerythrin alone produced a small rise in the bound/free activity ratio; the total activity was not significantly altered. However, the application of irradiation (either ultra-violet or visible) to the suspension containing phylloerythrin produced a considerable decrease in the bound/free activity ratio and in this case the total activity was also sometimes decreased. Thus, so far as acid phosphatase is concerned, phylloerythrin stimulated an irradiation-induced rupture of the large particles.

Table 1

Treatment	Phylloerythrin concn. (μM)	Total activity (% control)	Bound/Free Activity
(*a*) —	—	100	1·89
Irradiated	—	122	1·62
Phylloerythrin	100	116	2·25
Phylloerythrin + irradiation	100	130	1·42
(*b*) —	—	100	1·72
Phylloerythrin	60	94	2·50
Phylloerythrin + irradiation	60	77	1·44
(*c*) —	—	100	3·97
Irradiation	—	96	3·40
Phylloerythrin	70	98	4·80
Phylloerythrin + irradiation	70	84	3·10

Effect of phylloerythrin and of irradiation (60 min at +2° C) on rat liver acid phosphatase activity in large-particle suspensions prepared by differential centrifuging of sucrose homogenates. Three typical experiments are shown involving (*a*) and (*b*), ultra-violet irradiation of particles suspended in 0·7 M sucrose; (*c*) visible light irradiation of particles suspended in 0·25 M sucrose. Total enzyme activity was measured after bursting lysosomes with 0·1 per cent 'Triton-*X*-100' and values are expressed as a percentage of the control value. The bound/free activity ratio reflects the integrity of the lysosomal membrane at the end of the treatment period. Other details are in the text.

This pattern was not constant with the other enzymes studied. For example, irradiation of phylloerythrin-containing samples did not decrease the total β-glucuronidase activity and similar treatment did not cause significant alteration in the bound/free activity ratio of acid sulphatase.

Skin experiments. Buffer-treated sections showed no significant loss of punctate acid phosphatase activity until they had been irradiated for between 20 and 30 min (Fig. 1). Unirradiated sections treated with phylloerythrin showed marked punctate staining in the epidermis, indistinguishable from controls. Punctate staining was completely abolished in sections treated with phylloerythrin (final concentration 78–390 μM) and subsequently irradiated in buffer for 10 sec. A clear reduction in punctate staining was obtained in sections treated with very high dilutions of phylloerythrin (final concentration 7·8 mμM) and irradiated for 10 sec as compared with buffer-treated controls. Sections pre-incubated in phylloerythrin (390 μM) and placed in hydrocortisone before irradiation (10 sec) retained this punctate staining. Sections pre-incubated in phylloerythrin (78 μM) and placed in *d*-α-tocopheryl polyethylene glycol 1,000 succinate before 10-sec irradiation retained some particulate activity which was greatest in those treated with a dilute solution of the protector (0·005 mg/ml.) rather than a strong solution (0·5 mg/ml.).

The results outlined here show that phylloerythrin greatly potentiates the irradiation-induced rupture of particles rich in acid phosphatase activity in rat liver and rat tail epidermis. In this respect phylloerythrin is effective at a low final concentration and can utilize light energy of the visible region.

It is clear, however, that the effect of phylloerythrin on liver lysosomes is less marked than that on acid phosphatase-containing particles in rat tail epidermis. Liver lysosomes seem in this relative sense to be comparatively stable, a finding consistent with earlier results that high doses of free radicals were necessary for their rupture[13]. Liver lysosomes are also little affected during the early stages of acute liver injury[21] whereas they are rapidly affected by ischaemic anoxia[22].

It is our experience in using histochemical methods to demonstrate lysosomal particles that widely differing

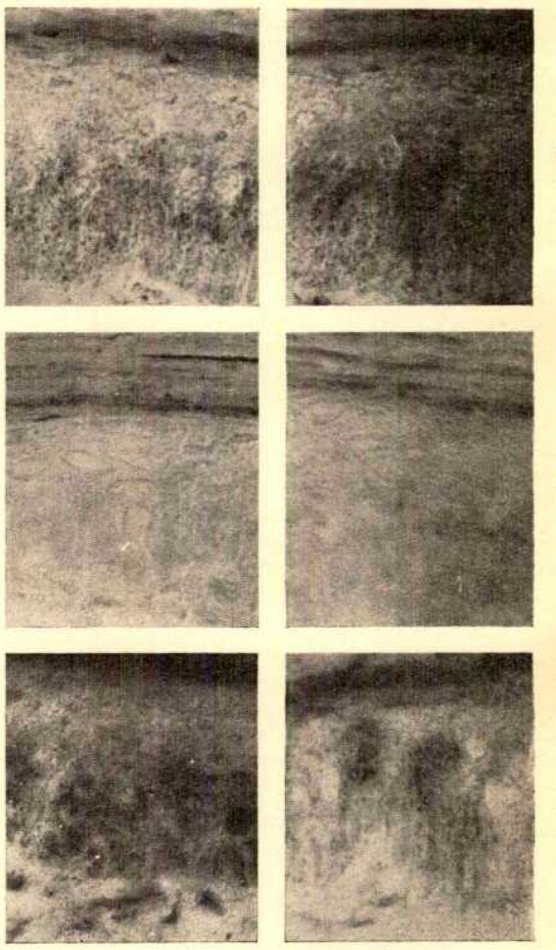


Fig. 1. Lysosomal acid phosphatase activity in rat tail epidermis. (*a*) Section treated with phylloerythrin (78 μM) but not irradiated prior to incubation. (*b*) Untreated control. (*c*) Section treated with phylloerythrin (78 μM) and irradiated for 10 sec prior to incubation. (*d*) Section treated with phylloerythrin (7·8 mμM) and irradiated for 10 sec prior to incubation. (*e*) Section treated with phylloerythrin (390 μM) and protected by hydrocortisone (1 per cent) during irradiation (10 sec). (*f*) Section treated with phylloerythrin (78 μM) and protected by tocopherol (0·0005 per cent) during irradiation (10 sec)

fragilities of these organelles occur in different tissues which demand appropriate adaptations in technique. These different fragilities may represent variations in reaction to the adverse conditions present during incubation as well as possible differences in the lysosomal structures themselves. Species differences also exist; for example, in human and in mouse tail epidermis in which lysosomal acid phosphatase activity has been demonstrated, the incubation times giving optimum punctate staining are 40 min and 75 min respectively. Rat tail epidermis gives good results after 60 min incubation, and by virtue of the relative simplicity of the technique and the ease with which effects on lysosomal staining can be produced, and observed, this material would appear advantageous as a useful tissue for an *in vitro* screening test for potential photosensitizers and protectors.

We have little direct evidence as to the detailed mechanism by which phylloerythrin induces lysosomal rupture on irradiation. However, the demonstration by Magnus, Porter and Rimington[23] that the action spectrum for the oedematous component in the photosensitization produced by porphyrins coincided closely with the positions of their Soret bands leaves little doubt that it is an excited form of the photosensitizer which is effective in this respect (that is, the Grotthuss–Draper law applies to these systems). It is possible that a free radical form of the photosensitizer is a consequence of this excitation stage. Mauzerall and Feher[24] have demonstrated the existence of porphyrin free radicals; a further example for this suggestion is the related pentaphenyl-pyrrolium ion radical which is stable[25]. (A short discussion on the occurrence of triplet states in porphyrin molecules following excitation is given by Reid[26].) Of more direct relevance, Norins[27] has shown a measurable increase in free radicals in skin following ultra-violet irradiation. The formation of significant intracellular quantities of extraneous free radicals can be expected to be injurious to the cells concerned. In particular, in the presence of oxygen, radicals of the peroxy type can be expected to be produced. In the presence of lipid-rich membranes such a production of peroxy-radicals would lead to the formation of lipid peroxides with consequent disorganization of the specific structural features of these membrane systems. Lipid peroxidation has been implicated in previous reports dealing with various types of cell damage[11].

The dependence of photosensitization on the level of oxygen[8] and the virtual independence of the disturbance to temperature variation[9] suggests a similarity to numerous examples of photodynamic action previously reported. In the latter events, the reactive species appears to be a triplet state (that is, a free radical structure)[28] of the sensitizer molecule which subsequently interacts with oxygen. It is interesting to note that powerful photodynamic effects in a series of dyes have been correlated with their fluorescence[26], and that an approximate correlation between photodynamic action and carcinogenicity has been reported by Alexander[28].

In view of the foregoing speculative remarks concerning the role of free radicals in phylloerythrin-induced photosensitization, we tested two substances which can function as chain terminators: vitamin E (as the soluble succinate salt) and hydrocortisone. Vitamin E is a well-known antioxidant and also inhibits reactions proceeding by way of a radical step[29]. Hydrocortisone has been shown to stabilize lysosomal preparations *in vitro* against various types of damage including irradiation[30]. Both vitamin E and hydrocortisone protected skin lysosomes against the combined influence of phylloerythrin and irradiation. These findings are consistent with the hypothesis suggested here.

However, despite the demonstration that low concentrations of phylloerythrin can enhance lysosomal lysis following irradiation, this certainly cannot be taken to imply that such a reaction is the sole and/or major cause of epidermal cell injury in cases of photosensitization induced by phylloerythrin. Other types of intracellular structure, for example mitochondria and endoplasmic reticulum, are known to be damaged by free radical-producing reactions. It is quite probable also that the cell membrane itself would suffer damage in the course of such reactions. If the energy system maintaining the sodium pump[31] were affected, then an increased water content of the cells would follow. A further intracellular organelle likely to suffer damage by lipid peroxidation and/or free radical interaction is the mast cell granule[32]. This could be of relevance to the erythematous component of the photosensitization. On the other hand, lysosomes possess properties which make them unique candidates for increasing the range and extent of cell damage resulting from absorbed radiation. By such a free-radical mechanism it is possible to conceive how irradiation of a small area of cytoplasm can cause a wide area of damage throughout the cell. In this connexion the speculations raised here come very close to the conclusions of Bacq and Alexander[33] on the mechanism of cell necrosis following irradiation. These authors proposed that the primary lesion was an alteration in membrane permeability resulting in the release of catabolic enzymes.

The mechanism proposed here for lysosomal rupture based on a free radical reaction does not necessarily entail uptake of the photosensitizer by the lysosome. Formation of significant numbers of radicals in the immediate neighbourhood of lysosomes should be quite sufficient for the production of lysis. However, adsorption of the reactive species (for example, phylloerythrin) in the lysosomal membrane prior to excitation would presumably be even more effective. The uptake of dyes and fluorescent materials by lysosomes from various tissues (for example, liver, kidney, HeLa cells) has been studied by many investigators[34]. It is possible that this uptake is primarily a reflexion of the pinocytotic activities of the cells concerned. If so, then considerable variations must be expected from tissue to tissue in this respect and, for instance, autofluorescence of epidermal lysosomes has never been observed by us even in sections from rats with ligated bile ducts, dosed systemically 24 h previously with phylloerythrin.

Finally, we think it worth speculating on the implications of such an uptake in terms of a physiological lysosomal function. Lipofuchsin accumulation is thought to represent an end product of one form of lysosomal deterioration[35]; the pigment is believed to arise from peroxidation of various types of intracellular lipid engulfed by the original lysosome[36]. If true, this process would be an example of a naturally occurring lipid peroxidation taking place within the lysosome. It is possible that the sequestration of toxic fluorescent material by lysosomes[37] in several tissues represents a defence mechanism rather than primarily an injurious event. That is, an attempt to remove potential chain initiators from the oxygen-rich cytoplasm, where peroxidation of sensitive lipoprotein membranes would seriously endanger the cell, into the lysosomes where peroxidation could be diverted into a process akin to lipofuchsin formation. Failure of such an uptake, leading to the initiation of cytoplasmic peroxidative chain reactions and wholesale rupture of lysosomes as shown here, could account for the isolated necrosis observed with such fluorescent materials, as has been pointed out recently[37].

A physiological process which, perhaps, provides an example of the speculation raised here is the enveloping of melanin granules by phospholipid membranes[38]. The protective function of melanin, involving absorption of radiant energy, is considered to involve free radical formation[39] which, on the basis of work presented here, would be potentially damaging to the epidermal cells unless confined and dissipated in a controlled manner.

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METABOLISM OF AFLATOXIN IN SHEEP: EXCRETION OF THE 'MILK TOXIN'

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COWS fed groundnut meal containing aflatoxin B_1 excrete in their milk a toxic factor which causes liver changes in ducklings indistinguishable from those produced by aflatoxin[1]. de Iongh *et al.*[2] showed that the toxicity of this milk was determined by the presence of a blue-violet (BV) fluorescent component, called the 'milk toxin', which had an R_F value well below that of pure aflatoxin B_1. They also demonstrated that the lactating rat was able to convert aflatoxin B_1 into this 'milk toxin'. Butler and Clifford[3] have shown the presence of this component as well as aflatoxin B_1 in the livers of rats given pure aflatoxin B_1 either orally or intraperitoneally, indicating that at least some aflatoxin B_1 is converted in the liver to the 'milk toxin'.

Sheep appear to be resistant to aflatoxin[4]. This is a peculiarity not shared by other species so far tested. In view of this resistance, the occurrence of aflatoxin or its metabolites in tissues and urine of sheep given a single dose was investigated.

Three yearling sheep were each given a dose (1 mg/kg) of mixed aflatoxins (B_1 36 per cent; G_1 52 per cent; B_2 3 per cent; G_2 2 per cent) dissolved in 2 ml. dimethylformamide, two by stomach tube and one by intraperitoneal injection. All were killed 2 h later; liver and kidney were removed and portions extracted for examination by thin-layer chromatography by the method described by de Iongh *et al.*[5], using 2 per cent methanol in chloroform as developing solvent. Urine samples were taken before dosing, 1 h after, and at death; a 60 per cent methanol–urine mixture was repeatedly extracted with chloroform and these extracts were concentrated and spotted on to chromatoplates similar to those used for the tissue extracts.

Fig. 1 shows the fluorescent spots seen when viewed in ultra-violet light. The liver extracts of all sheep showed the BV and blue-green (BG) spots at R_F values corresponding to the standard mixed aflatoxins B_1 and G_1 and also a slow running BV spot at R_F 0·2, the 'milk toxin' spot (M_2). This was less intense than the B_1 and G_1 spots. The kidney extracts showed fluorescent spots similar to those seen in the liver extracts, but the intensity of the 'milk toxin' spot at R_F 0·2 was much greater, whereas the B_1 and G_1 spots were less intense.

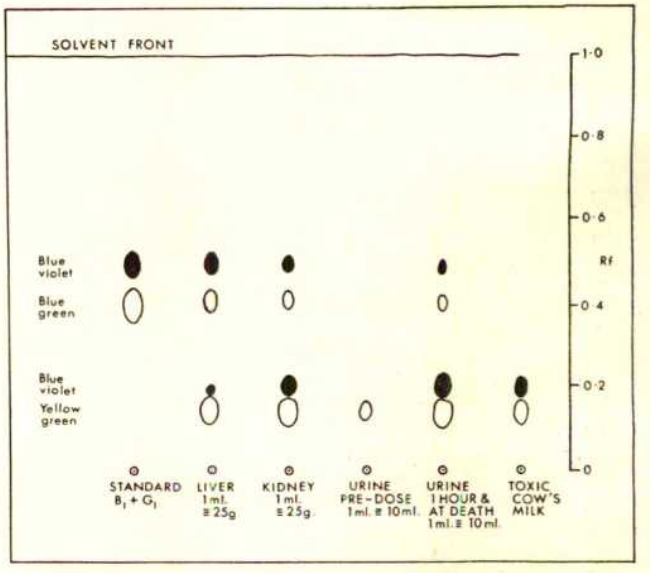


Fig. 1. Thin-layer chromatography of extracts of mixed aflatoxins B_1 and G_1; liver and kidney of sheep dosed with aflatoxins B_1 and G_1; urine from sheep before and after dosing; toxic cows' milk

The urine samples taken from the sheep before dosing showed no fluorescent spots except a yellow one at R_F 0·1 (U_3) which appears to be present in extracts of all tissues. The urine samples taken 1 h after dosing and from the bladder at death showed very intense *BV* 'milk toxin' spots (M_2), faint B_1 spots and somewhat stronger G_1 spots.

The R_F of the 'milk toxin' spot varied with the polarity of the solvent and the activity of the plate, so that a reference spot of authentic 'milk toxin' should be run on the plate if possible.

Although the findings reported by Butler and Clifford[3] indicated that the toxic component in milk was comparable to the slow-running *BV* spot obtained in extracts of rat liver and blood, it seemed desirable to investigate the identity of these compounds more rigorously. Since the amount of this component present in the urine of sheep dosed with aflatoxin was greater and more easily extracted than that present in the liver, it was decided to purify and compare the milk and urine components.

Several 500-g portions of dried toxic cows' milk were extracted for 6 h with light petroleum (40–60° C) in a Soxhlet extractor, air dried and re-extracted with methanol for 6 h. (Further extraction with methanol or chloroform yielded no more milk toxin.) The toxic fraction of this extract was concentrated as described by de Iongh *et al.*[2].

The combined residues from several extractions were then fractionated on a column of silica gel (20 g per 500 g of dried milk). The column was observed in ultra-violet light, a weak blue fluorescent band was eluted by chloroform (M_1), and a stronger *BV* fluorescent band by chloroform containing 2 per cent methanol (M_2, the milk toxin).

Further purification of M_1 and M_2 was achieved by repeated thin-layer chromatography on 'Kieselgel *G*' chromatoplates (thickness 750μ), portions of solutions of the column fractions being applied in a line across the bottom of the plate and using chloroform containing 3 per cent methanol as developing solvent. The fluorescent bands observed when the plate was viewed in ultra-violet light were similar in pattern to the spots described by de Iongh[2]. In each case the silica gel containing the blue fluorescent band was scraped from the chromatoplate, and eluted with cold methanol. The residue from the evaporation of the methanol filtrate was rechromatographed; this process was repeated until the material gave a single, well-defined spot on thin-layer chromatography.

The chloroform extract of urine from one of the sheep which had been dosed with mixed aflatoxins was also subjected to similar treatment. The plates showed three main fluorescent bands, *BG* (U_1), *BV* (U_2) and yellow (U_3) at R_F 0·45, 0·4 and 0·35, respectively. Purification by thin-layer chromatography was carried out as already described here.

These purified materials were then investigated chromatographically and spectroscopically.

The very small amount of M_1 had the same R_F as aflatoxin B_1, and thin-layer chromatography of a mixture of these two, using chloroform containing 2 per cent methanol for development, yielded only a single spot. It would appear probable that M_1 is aflatoxin B_1. Similarly, a mixture of U_1 and aflatoxin G_1 showed only a single spot and it is likely that these two compounds are identical.

Both M_2 and U_2 showed a long wave-length absorption maximum at 355 mμ in the ultra-violet, and the fluorescence spectra of both showed an emission peak at 425 mμ when excited at the maximum of 355 mμ. A mixture of M_2 and U_2 was subjected to thin-layer chromatography as shown in Table 1.

Table 1

Developing solvent	R_F	No. of spots
Trichloroethylene, chloroform, methanol (8 : 1 : 1)	0·25	1
Chloroform, methanol (97 : 3)	0·4	1
Ethyl acetate, methanol (9 : 1)	0·5	1
Trichloroethylene, acetone (1 : 9)	0·8	1

It would appear very probable that M_2 and U_2 are identical. The close resemblance of the spectral data of this substance to that of the aflatoxins[6] would make it appear possible that the main chromophoric system is similar.

If it is assumed that the extinction coefficient of the peak at 356 mμ in the 'milk toxin' is similar to that at 363 mμ in B_1, and that the molecular weights of the two compounds are also similar, then the amount of toxin present can be estimated spectrophotometrically as described previously for aflatoxin B_1 (ref. 7). (The ε values of the 363 mμ peak of the four aflatoxins are all about 20,000 (ref. 6).) On this basis the dried toxic cows' milk investigated averaged 10 μg toxin/kg.

Samples of urine can usually be obtained easily, and since extraction of up to 120 ml. of urine can be completed in about 6 h and the concentrate, without further treatment, can be spotted and run on a thin-layer chromatoplate, the presence of aflatoxin or 'milk toxin' spots on this may provide a useful field test for checking the ingestion by man or animals of aflatoxin-containing foods in those countries where *A. flavus* is ubiquitous.

Since the compound now referred to as the 'milk toxin' appears to be present in the liver, kidney and urine as well as in the milk of animals ingesting aflatoxin, it would seem no longer useful to retain the name 'milk toxin', and we suggest giving this compound the trivial name 'aflatoxin *M*', which indicates the original derivation from milk but is of more general applicability.

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MOLECULAR WEIGHTS OF PROLACTINS AND PITUITARY GROWTH HORMONES ESTIMATED BY GEL FILTRATION

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PRESENT knowledge of the molecular weights of prolactins and growth hormones is based largely on ultracentrifuge data, but reversible and irreversible aggregation of the hormones has sometimes rendered measurement difficult[1]. The fact that most of the reported molecular weights of growth hormones[2–7] were determined at about *p*H 2·3 or *p*H 10 also raises some doubt as to their molecular weights at physiological *p*H. As preliminary gel filtration experiments[8] indicated that the molecular weights of ox, pig and sheep growth hormones at *p*H 7·5 are considerably less than the presently accepted values, the subject has been investigated further. As a background to the use of gel filtration in this connexion, Andrews[9,10] has shown that the gel filtration method

gives useful estimates of the molecular weights of carbohydrate-free globular proteins over a wide range of molecular weights. In addition, the method seems to have special application to the estimation of the monomer molecular weights of proteins which show concentration-dependent association–dissociation behaviour[9–12].

Sheep prolactin (lots No. NIH-*P-S3* and *-P-S4*) and human (NIH-*GH-HS*269), monkey (NIH-*GH-M*655*A*), ox (NIH-*GH-B*2, *-GH-B*4 and *-GH-B*5), pig (NIH-*GH-P*476*C*) and sheep (NIH-*GH-S*646*A*) growth hormones were kindly provided by Dr. A. E. Wilhelmi and the Endocrinology Study Section, U.S. National Institutes of Health, ox prolactin by Dr. K. A. Ferguson, and human growth hormone, prepared by the Raben method[13], by Drs. A. Korner and M. S. Raben. A sample of human growth hormone monomer purified by gel filtration (Forsyth, Isabel A., and Andrews, P., unpublished work) was also used.

'Sephadex *G*-100' gel filtration columns (50 cm × 2·4 cm diam.) were used for molecular weight estimation as described by Andrews[9]. The columns were equilibrated with 0·05 M H_3PO_4 containing 0·1 M KCl and sufficient NaOH to bring the *p*H to 1·9, 2·5, 3·2, 4·0, 5·0 or 6·2, with 0·05 M *tris*-HCl (*p*H 7·5) plus 0·1 M KCl, or with 0·05 M H_3BO_3 containing 0·1 M KCl and sufficient NaOH to bring the *p*H to 8·5, 9·5, 9·9 or 10·3. Although the equilibration solutions had little or no buffering action at some *p*H's, no deviation from the required *p*H during column runs was detected. All experiments were performed at 2–5° C.

The columns were calibrated for molecular weight estimation at *p*H 7·5 and higher *p*H's with cytochrome *c*, ribonuclease, α-lactalbumin, myoglobin, soya bean trypsin inhibitor, ovalbumin and bovine serum albumin (see Andrews[9]). Calibration at *p*H's below 7·5 was based on ribonuclease, α-lactalbumin, ovalbumin and bovine serum albumin, which showed similar behaviour relative to each other over the whole *p*H-range investigated.

Samples were generally dissolved in 2 ml. of the equilibration solution for application to the columns, but at some *p*H's dissolution of the hormones was difficult. In such cases they were first dissolved in 0·2 ml. of 0·02 N NaOH, 1·6 ml. of equilibration solution was added, and the *p*H was adjusted with 0·02 N HCl. Serum albumin, ovalbumin or cytochrome *c* was included in each column run as a standard, the choice depending on the gel filtration behaviour of the hormone. Column effluents were collected in 3 ml. fractions, and protein concentration in the effluents was determined by extinction at 210, 215, 230, 280 or 410 mμ, depending on the nature and quantity of protein involved.

Sheep and ox prolactins. Elution diagrams (cf. ref. 14) indicated the presence of monomer (72 per cent and 82 per cent of the total protein, based on extinction measurements, in NIH-*P-S3* and *-P-S4*, respectively) and aggregated material in both sheep prolactin preparations. Estimations on 3 mg of either preparation indicated a monomer molecular weight of 23,500–24,000 at *p*H 7·5, but experiments with smaller amounts showed a concentration-dependent association of the monomer (Fig. 1*a*). Extrapolation to infinite dilution gave 20,000 as the monomer molecular weight. Greater aggregation of the monomer occurred in the range of *p*H 2–5 (Fig. 1*b*), but the very low solubility of the hormone in this region precluded further investigation.

Molecular weight estimates of 20,900 and 22,400 on 0·3 mg and 1·3 mg samples, respectively, of ox prolactin at *p*H 7·5 indicated a monomer molecular weight at infinite dilution of 20,500. An estimation at *p*H 2·5 (31,200) showed aggregation at low *p*H similar to that of sheep prolactin.

Human growth hormone. Elution diagrams indicated that the human growth hormone samples prepared by the Raben method contained only ~ 60 per cent monomer compared with 80 per cent in the NIH preparation and > 95 per cent in the monomer preparation. In every case, however, monomer molecular weights estimated at *p*H 7·5 were 21,000–22,500 when portions containing 0·5 mg of monomer were used, and further measurements extrapolated to infinite dilution (Fig. 1*c*) gave 20,500. Slight aggregation at higher and lower *p*H's was detected (Fig. 1*d*). The Raben preparations appeared to contain at least 20 per cent of dimer (mol. wt. 44,700).

Monkey growth hormone. The main component, presumably monomer (70 per cent of the total protein), of a 0·8 mg sample had a molecular weight of 21,000 at *p*H 7·5.

Ox, pig and sheep growth hormones. All the preparations contained 75 per cent or more of monomer. Molecular weights estimated on 1·0–1·2 mg samples at *p*H 7·5 were in the range 27,000–30,000, but allowance for concentration-dependent association (Fig. 1,*e,g*) indicated monomer molecular weights of 26,000, 22,500 and 24,000 for the ox, pig and sheep hormones, respectively. Aggregation of the ox hormone increased markedly at low *p*H, reaching a maximum at about *p*H 2·3 with a molecular weight indicative of dimer formation, but increased aggregation in alkaline solution was not observed (Fig. 1*f*). Experiments with 8 mg and 23 mg samples of ox growth hormone showed some concentration-dependence in its gel filtration behaviour at *p*H 10·3. Aggregation of the pig and sheep hormones also increased at low *p*H, since their molecular weights estimated at *p*H 2·5 (1 mg samples) were 36,700 and 39,000, respectively, but values at *p*H 9·5 and 10·3 were similar to those at *p*H 7·5.

Effect of storage on hormones in solution. All the foregoing molecular weight estimations were performed on freshly prepared solutions. To check for hormone degradation[15–17] during the time required for column runs (6–12 h), measurements at *p*H 7·5 were repeated on hormone solutions which had been stored at these *p*H's for at least 24 h in the cold. The estimated molecular weight of one ox growth hormone preparation (NIH-*GH-B*2) decreased by 20 per cent after storage for 24 h, but thereafter it remained constant. Otherwise no significant changes were detected.

The good agreement between molecular weights determined in the ultracentrifuge for sheep prolactin[14,18]

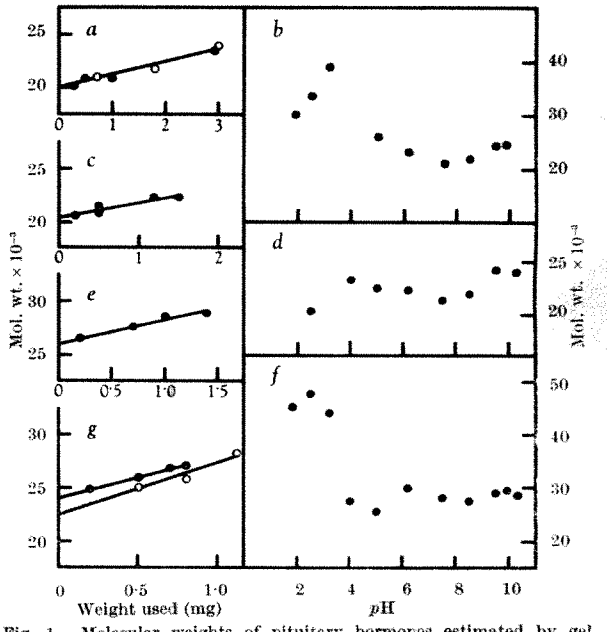


Fig. 1. Molecular weights of pituitary hormones estimated by gel filtration. *a*, Concentration-dependence, and *b*, *p*H-dependence (1-mg samples), for sheep prolactin NIH-*P-S3* (○) and NIH-*P-S4* (●). *c*, Concentration-dependence, and *d*, *p*H-dependence (0·5-mg samples), for human growth hormone NIH-*GH-HS*269. *e*, Concentration-dependence, and *f*, *p*H-dependence (1·2-mg samples), for ox growth hormone NIH-*GH-B*4. *g*, Concentration-dependence for pig growth hormone NIH-*GH-P*476*C* (○) and sheep growth hormone NIH-*GH-S*646*A* (●)

and human growth hormone[3] monomers and the molecular weights estimated by gel filtration for these hormones indicates that the gel filtration method is applicable to this type of protein. Aggregation of sheep prolactin in acid solution is evident both in the ultracentrifuge[14] and from its gel filtration behaviour (Fig. 1*b*). Sheep and ox prolactins appear to have about the same molecular weight and for ox prolactin the value is somewhat lower than the one originally proposed[19].

The gel filtration results clearly favour 21,500 (ref. 3) rather than 29,000 (ref. 1) as the molecular weight of human growth hormone, and they indicate that human and monkey growth hormones are very similar in size. On this account, a revision of the value of 25,400 for the monkey hormone[4] may be required. The higher literature value[1] for the molecular weight of human growth hormone presumably resulted from the presence of dimer and other aggregated material in the sample used for measurement, since the lower value[3] was determined on the purified monomer. The same reason may in part account for the differences between molecular weights determined in the ultracentrifuge at *p*H 9·9 for ox[5] (45,000), pig[3] (41,600) and sheep[7] (47,400) growth hormones and those estimated by gel filtration, for the latter method reveals no marked aggregation of these hormones at high *p*H. On the other hand, aggregation of ox growth hormone equivalent approximately to dimer formation at *p*H 2·3 is indicated by gel filtration, so that at this *p*H the ultracentrifuge[5] and gel filtration results are in agreement. Pig and sheep growth hormones also appear to aggregate at low *p*H. However, the molecular weight of 25,000 for pig growth hormone given by Wilhelmi[20] does accord with its gel filtration behaviour at physiological *p*H.

Except in one case, storage of the hormones in solution resulted in no change in their gel filtration behaviour, indicating that the low molecular weights estimated by gel filtration are not a result of hormone degradation[15–17] during the experiments. The exceptional case was the ox growth hormone preparation for which earlier gel filtration experiments[8] gave a molecular weight of 20,000. Whatever its significance, this change in gel filtration behaviour accounts for the differences between the earlier and the present (26,000) estimations of ox growth hormone molecular weight. In the circumstances, the latter value is the preferred one.

The gel filtration results suggest that the pituitary growth hormones of various species are much more alike in molecular weight than is at present believed[2], and moreover that they are similar to the prolactins in molecular weight. One consequence of the indicated uniformity in growth hormone size is that the tentative structure of ox growth hormone proposed by Li[19] needs revision. The similarity between the growth hormones and prolactins is of particular interest in view of the possibility that human growth hormone and human prolactin are identical[21].

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DIFFERENCES IN PATTERNS OF AGGREGATION OF MALIGNANT AND NON-MALIGNANT MAMMALIAN CELLS

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THE propensity of dissociated cells from a variety of embryonic avian and mammalian tissues to aggregate and to form organized multicellular 'aggregens' has been recently described[1–3].

This article reports the fact that definite differences have been observed in aggregation patterns between malignant and non-malignant cells. Emphasis is placed on the fact that the comparison was carried out on cells derived from the same primitive strains and cultured under identical conditions[4].

Two different strains of cells have so far been examined in this respect: (1) The cell line isolated by Rothschild and Febvre[5] from a Wistar rat embryo and cultured since 1963. These cells are devoid of malignancy when re-inoculated into the rat; they are designated here as the ER_1 line. However, when cultured in certain conditions[4], in the presence of Rous sarcoma virus (Bryan strain), these cells acquire highly malignant potentialities for the homologous animal species. The high cancer cell line is referred to here as CT_4. (2) The cell strains isolated by Sanford *et al.*[6] from a C_3H mouse embryo in 1954. Three different clones originating from the primitive line have been compared from the point of view of their aggregation patterns.

The clone N_2 is devoid of malignant potentialities. None of the 35 C_3H mice inoculated with 2 million cells developed tumours. The modal chromosome number of this line of cells is 62 (ref. 7). The clone N_1, another permanent clone derived from the primitive strain, became spontaneously highly malignant. All animals inoculated with 1 million cells died in less than 50 days, and autopsy showed rapidly growing tumours. The modal chromosome number is 55–56, all being telocentric. (In a recent communication, Scaletta and Ephrussi[8] give for the same strain a modal chromosome number of 49 and a variation range of 46–51.)

Clone M_6 results from hybridation of the two preceding strains. It has been shown that the modal chromosome number of the hybrid cells is of 100–115, and that the clone contains the karyotypes of both 'parental' cells. All lines of the hybrid cells were highly malignant when inoculated into C_3H mice[6]. Fig. 1 shows the morphological aspect of the three cell clones.

The various cell lines were grown in Petri dishes containing Eagle's medium enriched with amino-acids and vitamins, to which had been added tryptose phosphate (10 parts per 100) and horse decomplemented serum (10 parts per 100). Before use, the cells were treated for

3–5 min with a solution of trypsin 'Difco' (0·05 parts per 100) in Moscona's buffer[2] and centrifuged. They were then placed into Erlenmeyer vials containing 3 ml. of culture medium, the *p*H of which was adjusted to 7·4 in an atmosphere of 95 parts per 100 of oxygen + 5 parts per 100 of carbon dioxide. The stoppered vials were placed on the platform of a stirrer model *G* 76-10-25 (New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.) rotating at 60–70 revs./min. The results observed were similar to those for the two strains of cells used here.

The cells originating either from the ER_1 or from the N_2 clones formed minute clumps of a few cells in the culture; these were readily disrupted by tapping (Fig. 2). Moreover, only a small percentage of the cells tended to agglutinate, and the majority of them remained isolated and dispersed in the culture medium for the duration of the culture.

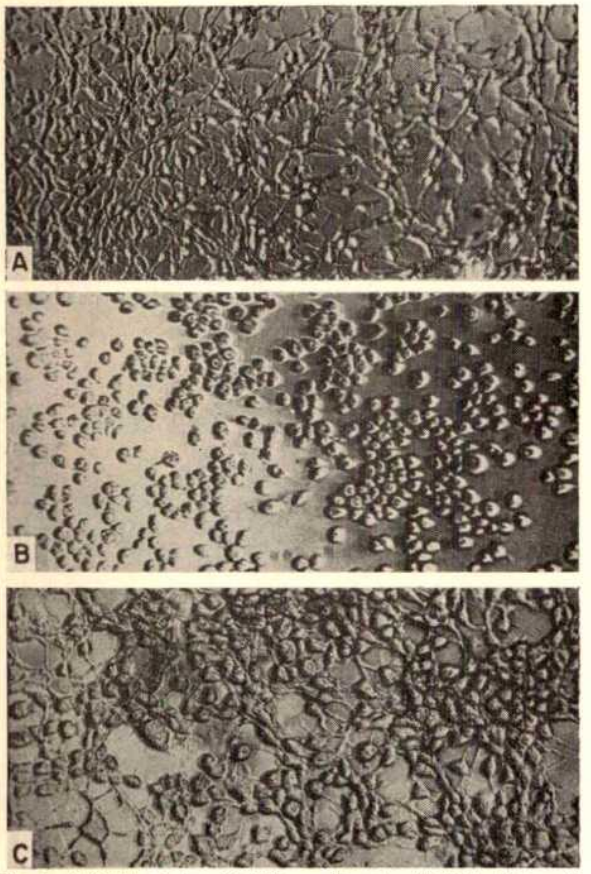


Fig. 1. (*A*) Cells of clone N_1 highly malignant. (*B*) Cells of clone N_2 non-malignant. (*C*) Hybrid cells of the two preceding clones, M_6, highly malignant. (× *c.* 320)

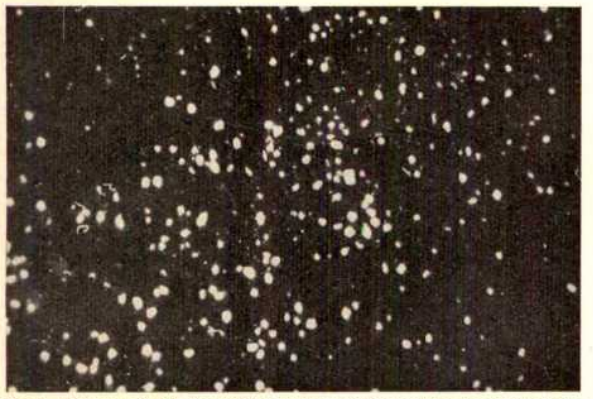


Fig. 2. Clumps of cells formed by the non-malignant N_2 clone cells after 6 days of culture. The majority of the cells are dispersed and isolated. (× *c.* 140)

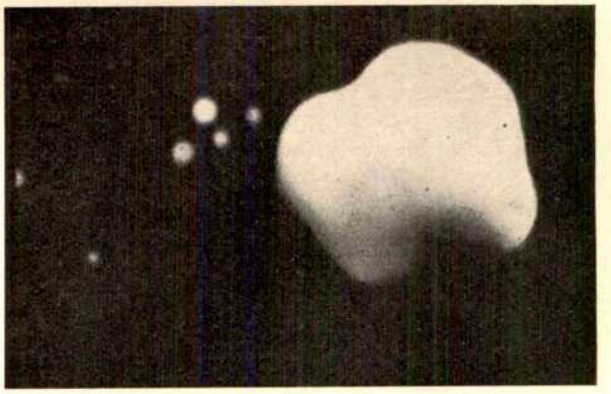


Fig. 3. Aggregates formed by the malignant N_1 clone cells cultured in identical conditions. The size of the *in vitro* formed tumours varies from 0·2 mm to 3 mm and more. The smaller aggregates tend to associate and to develop bigger structures. Fourth day of culture. (× *c.* 8)

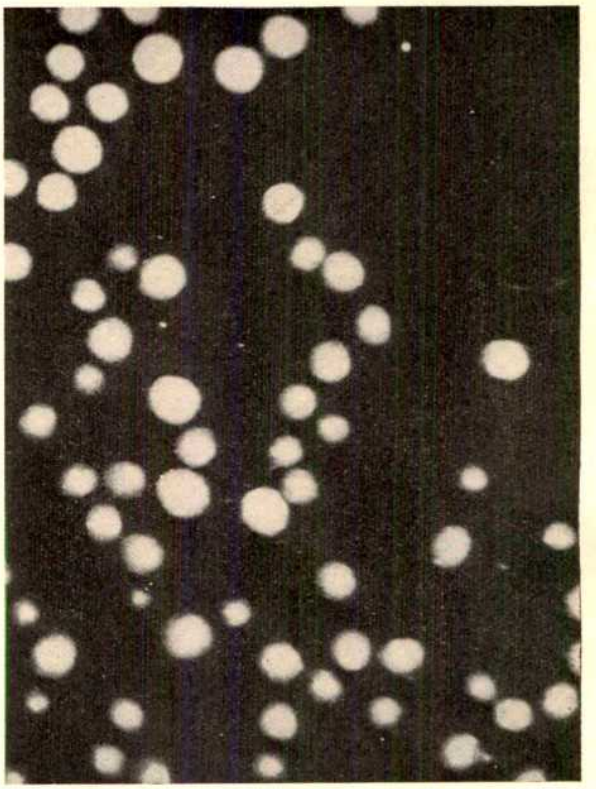


Fig. 4. Aggregates formed by the malignant hybrid M_6 clone cells. Spheroid aggregates of about 0·25 mm diameter. 48 h of culture. No isolated cells in the culture medium. (× *c.* 16)

The behaviour of the malignant cells was quite different. They readily stuck to each other, forming dense aggregates which rapidly increased in size by cell multiplication, and also by the adhering of smaller aggregates to their surface.

In the case of the N_1 clone, the aggregates formed roughly spherical or ovoid structures of 0·5–3 mm diameter within 3–5 days (Fig. 3). Practically no isolated cells were present in the medium. The aggregates were compact, cohesive and showed no tendency to dissociate even when vigorously tapped.

Although the same general patterns of aggregation were observed with all the malignant clones investigated, there seemed to be slight differences in the form and the size of the 'aggregens', depending on the cell strains and the age of the culture. The cells derived from clone CT_4 and from the hybrid M_6 clone formed smaller and roughly spherical aggregates of 0·2–0·4 mm diameter (Fig. 4).

Differences were also observed in the patterns of the migration of the cells from the culture-formed aggregates



Fig. 5. Stationary culture obtained from the clumps of N_2 cells. The cells tend to spread as a monocellular layer. (× *c.* 80)

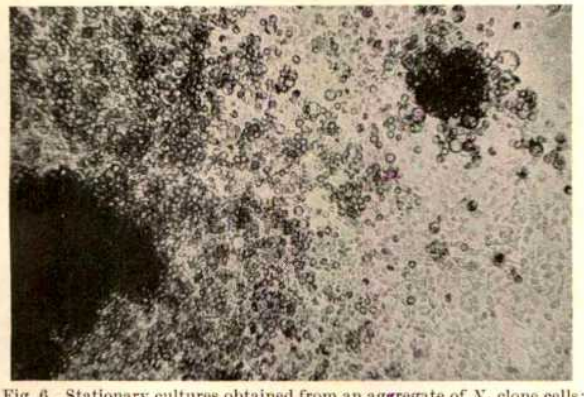


Fig. 6. Stationary cultures obtained from an aggregate of N_1 clone cells: the central part remains compact. The migrating cells tend to form satellite aggregates. (× *c.* 80)

when they were later grown in stationary cultures. The non-malignant cells rapidly spread over the medium and tended to form a regular monocellular layer, reproducing the aspect of the original culture (Fig. 5).

In contrast, the aggregates formed by malignant cells remained compact; the cells migrating from the periphery formed an irregular rim composed mainly of clumped cells which tended to develop satellite aggregates (Fig. 6).

The data reported here provide evidence that a definite difference exists in the aggregation behaviour between malignant and non-malignant cells. The discrepancies were observed with two different strains of cells, and the question arises whether the phenomenon is of a general nature. The non-malignant cells showed no tendency to aggregate when grown in agitated culture. In contrast, the cancerous cells rapidly developed voluminous multicellular tumours. The tumours were formed of cells which had developed an ability to adhere to one another, and which continued to multiply at least as long as the cells could receive nourishment. The cellular cohesion of these tumoral aggregates was strong. Their dimension and their shape depended on the type of cells and on the age of the culture.

In our experimental conditions, the highly malignant N_1 clone cells formed aggregates which were fairly large compared with those formed by the cells originating from M_6 hybrid clones, although the malignancy of both is of the same order. Rat cells rendered malignant by the Rous sarcoma virus behaved like the M_6 clone cells.

The main interest of our observation is in the fact that in both groups of experiments, the comparison was made between malignant and non-malignant cells originating from the same stem strain. The age and the rhythm of multiplication of the malignant and non-malignant clones were as identical as possible. Such a comparative investigation has not, to our knowledge, been carried out before.

In the Sanford strain the malignant clone appeared spontaneously and no explanation is yet available for this mutation, although the possibility of an accidental contamination by viruses cannot be excluded.

In the rat strain obtained from Rothschild and Febvre[5] the malignancy was induced by infecting the culture with Rous sarcoma virus. How far is it possible on the basis of the reported data to assert that there exists a definite correlation between the potential of malignancy and the ability of the cells to form aggregates? In the present state of our knowledge, such a hypothesis can only be conjectural. The aggregation phenomenon is likely to be conditioned by membrane changes which may be of a chemical and/or of a physical nature. The investigation at present in progress, using electron microscopy, indicates some puzzling differences in the membrane structures.

Whatever the real mechanism is, the observations reported here indicate that the malignancy of certain strains of cells whether spontaneous or induced result in an alteration of a biological genetically transmitted property, particularized by the aptitude of the cell to form aggregates. If this phenomenon proves to be a general one, its importance does not need emphasis. The research at present under way aims to provide additional data for the significance and the mechanism of the observed phenomenon.

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PRESENCE IN COLIC GLANDULAR CELLS OF VARIOUS MAMMALIAN SPECIES OF AN ANTIGEN CROSS-REACTING WITH HUMAN BLOOD GROUP A SUBSTANCE

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IT has been known for a long time that antigens similar to human blood group A, and sometimes B substances, exist in the upper gastro-intestinal tract of horse, hog and cow[1–8], and more recently of dog[9] and monkey[10]. This type of antigen seems to be absent in erythrocytes. The presence or absence of these tissue antigens may be important in establishing different groups within the same animal species[1].

Very little, if anything, is known about the presence of this type of antigen in the distal part of the bowel. Such information is much needed, considering the recent extension of investigations on the auto-immune origin of ulcerative colitis in man by means of the immunofluorescent method.

The data reported here indicate that an antigen identical or closely similar to the human blood group A

agglutinogen is present in colic glands of several mammalian species. They also suggest that this antigen is of tissue origin and not a bacterial contaminant.

An indirect immunofluorescent technique (Coons's 'sandwich' technique[11]) was used in all the investigations. 250 normal human sera were tested on alcohol-fixed cryostat sections of fresh, rapidly frozen animal colon and rectum. The following animals were investigated: 32 mongrel dogs, 16 rats (Wistar, Fischer and germ-free rats), 6 Macacus rhesus monkeys. Fluorescent rabbit anti-human γ-globulins were prepared by the method of Goldstein *et al.*[12].

The results obtained with rat colons are shown in Table 1. A bright homogeneous fluorescence restricted to the goblet cells, which indicates a positive reaction, was observed with 81 sera. With all other sera no staining was observed except for a very inconstant and moderate staining of the connective tissue and outer rim of glandular cells. Such a pattern could sometimes be obtained with the fluorescent reagent alone and was not considered to be indicative of a positive reaction. All 16 rats and 6 monkeys reacted in the same way with all positive sera. The picture was different in the dogs, and 13 dog colons only, out of the 32 tested, reacted with the same sera as rats and monkeys ('positive dogs') (Figs. 1 and 2). The other 19 dogs were negative with all sera ('negative dogs').

Table 1. Evidence for the Presence in the Rat Colic Glandular Cells of an Antigen related to Human Blood Group Substance by using the Indirect Immunofluorescent Technique

Blood group	No. of human tested sera	No. of positive sera
A	59	0
B	38	15
AB	20	0
O	133	66

Table 2. Correlation between the Anti-A Agglutinin Titre in Various Human Sera and the Positivity of Immunofluorescent Staining of Rat Glandular Cells

Blood group A erythrocytes agglutination titre of blood group O and B tested sera	No. of tested sera (by immunofluorescence)	Positive staining	Negative staining
1/16	13	5	8
1/32	14	9	5
1/64	17	13	4
1/128	11	10	1
1/256	7	7	0

The positive staining of colic mucosa glandular cells of all three species was abolished by previous absorption of sera with a dry tissue powder prepared from 'positive dog' colic mucosa. Staining was not affected by absorption with a similar powder prepared from 'negative dog' colic mucosa. These results seemed to indicate a specific immunological reaction.

The fact that positive staining was also observed with colons from pre-born and germ-free rats rules out the possible implication of a bacterial antigen.

The close immunological similarity of this tissue colic antigen to human blood group A substance is suggested by the restriction of positive results to sera from O and B groups. The incidence of negative staining with some

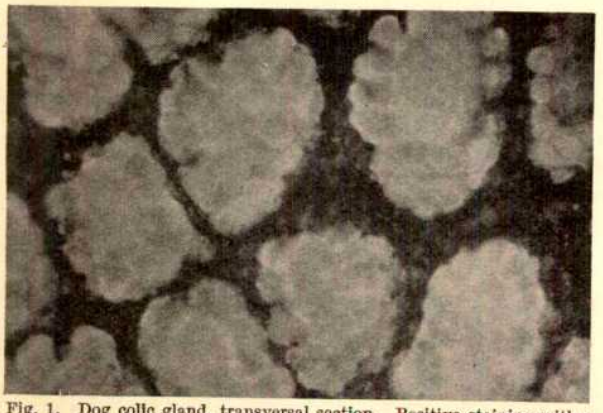


Fig. 1. Dog colic gland, transversal section. Positive staining with a blood group O serum (× *c.* 300)

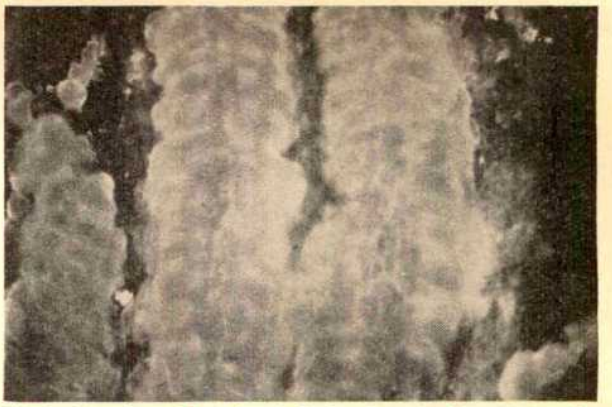


Fig. 2. Same dog as Fig. 1, longitudinal section. Positive staining with same serum (× *c.* 300)

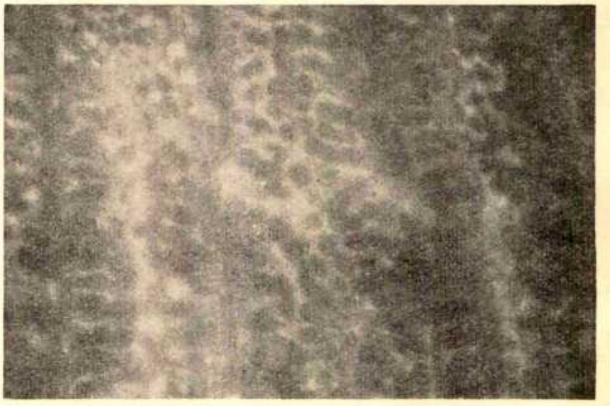


Fig. 3. Same dog as Fig. 1. Same serum after absorption on human blood group A erythrocytes. Negative staining. (× *c.* 300)

of these sera could be explained by a low titre of anti-A agglutinins, as all O and B sera with a high titre of anti-A agglutinins were constantly positive (Table 2). This similarity was confirmed by absorption investigations. The staining of animal colic glandular cells could always be abolished by previous absorption with human blood group A erythrocytes (Fig. 3), while it remained unaltered after absorption on B and O erythrocytes. On the other hand, absorption of O sera with colic tissue powder from 'positive dogs' prevented the agglutination of blood group A erythrocytes, but did not affect the agglutination of blood group B erythrocytes. Absorption with such powder from 'negative dogs' had no effect. Persistence of positive staining after absorption of sera on guinea-pig kidney powder excludes the similarity with Forssman antigen.

The antigen seems to be absent from dog erythrocytes since the absorption of sera with erythrocytes from 'positive dogs' did not prevent staining of colic glands nor agglutination of human blood group A erythrocytes. It appeared to be absent from gastric mucosa of all but one of the 13 dogs, and of all the rats and monkeys investigated. It could not be found in the salivary glands, kidney, spleen, liver or lungs of the rat.

This antigen probably belongs to the insoluble mucus fraction. Fluorescent staining coincides with periodic acid–Schiff staining of the next serial section. The antigen is left in the insoluble residue after prolonged extraction with solutions of increasing ionic strength including *C*1Na, Ca*TC* (ref. 13) and *C*12Ca, as shown by absorption investigations. The antigen could not be found in supernatant, using the technique of haemagglutination with tanned red cells[16]. Similar treatment of unfixed sections for 18 h did not prevent the fluorescent positive staining.

The data reported here indicate the presence, in colic glandular cells of rat, dog and monkey, of an antigen

cross-reacting with human blood group A substance. It is likely to exist in other animal species which were not investigated. Furthermore, our results demonstrate the absence of this antigen in the salivary glands and in all but one of the stomachs of the same animals. The fact that this antigen was found in pre-born and germ-free rats confirms the assumption that the substance is secreted by the colic glandular cells and is not a bacterial contaminant. Since blood group substances are extractable with phenol water solvents[14,15], the presence of this A-like tissue antigen should be taken into consideration in any attempt to detect specific antibodies in the sera of patients with ulcerative colitis and in any interpretation of positive results obtained with animal tissues[17].

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BEND PROPAGATION ALONG FLAGELLA

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THE planar undulatory waves which are the most familiar type of flagellar movement are composed of curved regions forming circular arcs, separated by shorter unbent regions[1,2]. These waves can be generated by the propagation along the flagellum of rather abrupt transitions between straight and bent states of the flagellum. This article is concerned with the mechanism for propagating such transitions, rather than with their generation.

The most thoroughly developed model for wave propagation along flagella is that proposed by Machin[3,4], which depends on the passive wave propagating properties of the flagellum. According to this model, active bending in one region of a flagellum will cause passive bending of a more distal region of the flagellum in the opposite direction. The passive bending will be caused by viscous bending moments generated as the flagellum moves against the resistance of its surrounding medium, and will be resisted by the stiffness, or elastic bending resistance, of the flagellum. Machin suggests that the active bending elements of the flagellum can be turned on by passive bending, so that the passively propagated bend is amplified to the value characteristic of the active wave, and can then stimulate the next region in the same way. The necessary mathematical relationships between passive and active bending have been formulated in some detail, but only for small-amplitude sinusoidal waves.

It seems difficult to apply Machin's model to the non-sinusoidal waves actually found on flagella, which show no obvious passive bending in inactive regions. A more serious objection is the difficulty of applying this model to the propagation of unilateral bending waves, which involve bending in only one direction. Unilateral bending is found on flagella under some abnormal conditions[2] and appears to be the typical form of propagated bending found on cilia[5].

The activation of bending locally by passively induced bending is a most attractive feature of Machin's model, because of its simplicity. I wish to propose a new propagation mechanism which preserves this feature, while avoiding the objections raised in the preceding paragraph. It is based on the local passive propagation of bending just ahead of a transition point, and is therefore much more closely comparable with other examples of wave propagation in non-linear systems, such as the nerve axon and explosive chemical reactions.

To illustrate this proposal a rather simple and naïve model for generating flagellar bends will be adopted. It consists of a pair of longitudinal fibrils with regular cross-connexions (Fig. 1*a*). The longitudinal fibrils can contract when activated, and can resist extension and compression equally in both the contracted and elongated states. The assumption that bending results from contraction of longitudinal fibrils is popular[3,5,6] but unproved. Recent work has suggested that the conspicuous peripheral filaments seen in electron micrographs of flagella and cilia do not change their lengths during movement[2,7], but a reasonable alternative has not yet been developed. The principles involved in the proposed propagation mechanism can be generalized to other bending models, but exposition is easier with reference to a concrete model for bending.

The presence of the type of passive propagation required for this mechanism can be demonstrated roughly by the following arguments. Consider the result of activation of fibrillar contraction on one side of the segment identified by the arrow in Fig. 1*a*. If the cross-connexions are completely rigid, bending will occur as shown in Fig. 1*b*. If the cross-connexions are flexible, instead of bending there will be shear displacement within the flagellum, as shown in Fig. 1*c*. The shear resistance of the cross-connexions must actually be intermediate between these two extremes. Since the force causing shear results from

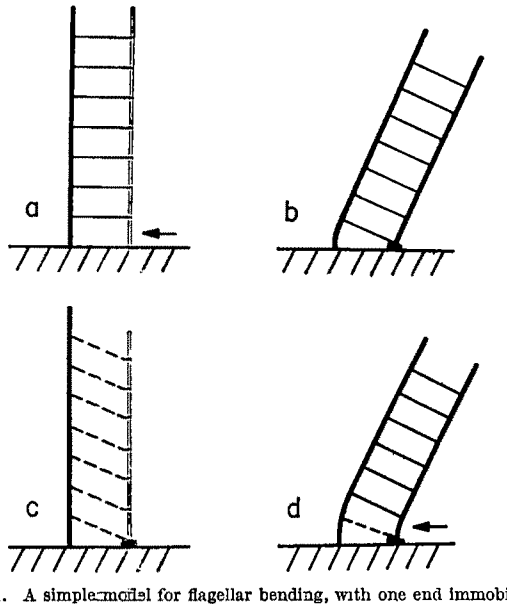


Fig 1. A simple model for flagellar bending, with one end immobilized

the difference between the longitudinal forces on either side of a cross-connexion, the force causing shear will be greatest, and therefore the greatest shear will occur, near the transition between active and inactive segments. An extreme case of such a gradient of shear is illustrated in Fig. 1*d*, where the lowermost cross-connexion is allowed to shear, while the other cross-connexions do not. With these conditions, the first two segments will bend as a unit, as shown in Fig. 1*d*, even though active contraction only occurs in the first segment. In general, then, unless the cross-connexions are completely rigid, shear will occur near the transition region, and bending will spread ahead of the region of active contraction. If bending the fibrillar segment identified by the arrow in Fig. 1*d* activates its contraction, the transition will be self-propagating.

In the following more rigorous development, the effects of the elastic forces within the flagellum will be developed first, and the viscous forces acting on the flagellum will be considered later. In a region of the flagellum which is bent to a radius of curvature, ρ, by contraction of the 'inside' longitudinal contractile fibril, there will be an elastic bending moment, M_e, equal to $2\alpha_0/\rho$, where α_0 represents the stiffness (bending resistance) of each half of the flagellum, or one of the longitudinal fibrils. M_e is equivalent to rF, where F represents a force tending to compress the 'outside' longitudinal fibril and extend the 'inside' longitudinal fibril and r corresponds to the distance between the two longitudinal fibrils.

The shear stress on a cross-connexion results from the difference between the values of F in adjacent segments along the flagellum and is therefore equal to $(\mathrm{d}F/\mathrm{d}s)\Delta s$, where s represents a measure of length along the flagellum and Δs is the distance between cross-connexions. The shear strain, S, at a cross-connexion is then given by:

$$S = \frac{1}{\alpha_2}\frac{\mathrm{d}F}{\mathrm{d}s} = \frac{2\alpha_0}{r\alpha_2}\frac{\mathrm{d}\left(\frac{1}{\rho}\right)}{\mathrm{d}s} \qquad (1)$$

where α_2 represents the stiffness (shear resistance) per unit length along the flagellum, thereby eliminating the need to include Δs explicitly.

The curvature of a segment of length Δs depends on the amount of active contraction, ΔL, of the inside longitudinal fibril in that segment; the difference, ΔS, between the shear strains at each end of the segment; and the relative compression and extension, $2\Delta C$, of the longitudinal fibrils by the force F:

$$\frac{1}{\rho} = \frac{1}{r}\frac{(\Delta L + \Delta S - 2\Delta C)}{\Delta s} \qquad (2)$$

Assuming that the cross-connexions are closely spaced, so that a treatment in terms of continuous functions is justified, this becomes:

$$\frac{1}{\rho} = \frac{1}{r}\frac{\Delta L}{\Delta s} + \frac{1}{r}\frac{\mathrm{d}S}{\mathrm{d}s} - \frac{rF}{\alpha_1} \qquad (3)$$

where α_1 represents the stiffness (bending resistance) of the whole flagellum, resulting from the resistance of the longitudinal fibrils to extension and compression. Then, from equation (1):

$$\frac{1}{\rho} = \frac{1}{r}\frac{\Delta L}{\Delta s} + \frac{2\alpha_0}{r^2\alpha_2}\frac{\mathrm{d}^2\left(\frac{1}{\rho}\right)}{\mathrm{d}s^2} - \frac{2\alpha_0}{\alpha_1\rho} \qquad (4)$$

which, after rearrangement, becomes:

$$\frac{1}{\rho} = \frac{1}{\rho_0} + \frac{1}{\alpha^2}\frac{\mathrm{d}^2\left(\frac{1}{\rho}\right)}{\mathrm{d}s^2} \qquad (5)$$

where:

$$\frac{1}{\rho_0} = \frac{\frac{1}{r}\frac{\Delta L}{\Delta s}}{1 + \frac{2\alpha_0}{\alpha_1}} \qquad (6)$$

and

$$\frac{1}{\alpha^2} = \frac{2\alpha_0}{r^2\alpha_2\left(1 + \frac{2\alpha_0}{\alpha_1}\right)} \qquad (7)$$

$1/\rho_0$ is the curvature which will result from contraction, in the absence of shear. Bending will be inefficient unless $2\alpha_0/\alpha_1$ is small compared with 1. The boundary conditions required for equation (5) are $1/\rho_0 = 1/\rho_0$ for $s < 0$ and $1/\rho_0 = 0$ for $s > 0$, and $1/\rho$ approaches $1/\rho_0$ for very large positive and negative values of s. A solution is:

$$\frac{1}{\rho} = \frac{1}{\rho_0}\left(1 - \frac{1}{2}\,\mathrm{e}^{\alpha s}\right), \qquad \text{for } s \leqslant 0$$
$$\frac{1}{\rho} = \frac{1}{2\rho_0}\,\mathrm{e}^{-\alpha s}, \qquad \text{for } s \geqslant 0 \qquad (8)$$

$1/\alpha$ represents a length constant for the exponential change of bending with distance from the point of activation at $s = 0$. This solution is illustrated in Fig. 2.

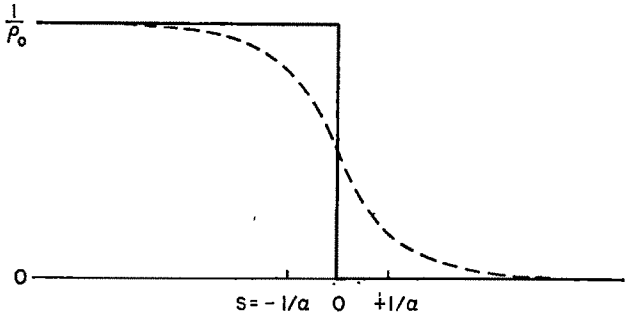


Fig. 2. Variation in curvature, $1/\rho$ (dashed line) along the flagellum near a transition point when contraction is abrupt (solid line)

The basic conclusion, that bending will spread ahead of a point of activation, does not depend on the simplification introduced by using continuous functions, as long as $1/\alpha$ is large compared with Δs. The bend will be self-propagating if contraction can be activated by bending less than $1/2\rho_0$. Since contraction at any point also tends to increase bending at that point, the system may have positive feed-back properties which increase the abruptness of the transition between unbent and bent states. However, contraction cannot be instantaneous, so the actual course of bending along the flagellum will not necessarily resemble the curve in Fig. 2.

Unbending might be activated in a similar manner if the bent elements adapt to the bent state and become sensitive to the passive unbending which will locally precede the transitions which reverse contraction. However, speculation about unbending is premature, since it is not yet known whether it is an active process similar to bending or a partially passive process which makes use of energy stored in elastic bending of part of the flagellum.

It remains to show that the bending resulting from shear, which will precede the point of activation, is significantly greater than the bending induced by viscous bending moments. The viscous bending moment, M_v, will enter by adding to the force F, so that:

$$rF = M_e + M_v \qquad (9)$$

Two additional terms must then be added to equation (4):

$$\frac{1}{r^2\alpha_2}\frac{\mathrm{d}^2M_v}{\mathrm{d}s^2} - \frac{M_v}{\alpha_1}$$

Since M_v varies almost sinusoidally along the length of the flagellum[2], with the periodicity given by the length L, its second derivative can be approximated by:

$$-\left(\frac{2\pi}{L}\right)^2 M_v$$

Using equation (7), the contribution from M_v then becomes:

$$-\left[\frac{\left(1+\frac{2\alpha_0}{\alpha_1}\right)}{2\alpha^2\alpha_0}\left(\frac{2\pi}{L}\right)^2+\frac{1}{\alpha_1}\right]M_v$$

For sea-urchin spermatozoa, L is approximately 9π μ (ref. 2), and the length constant, $1/\alpha$, is 1μ or less. With these values, the contribution from M_v becomes:

$$-\left[\frac{1+\frac{\alpha_1}{2\alpha_0}}{20}+1\right]\frac{M_v}{\alpha_1}$$

Since α_0 represents the stiffness of half the flagellum and α_1 represents the stiffness of the whole flagellum, their relative values depend on the relationships of structures in the cross-section of the flagellum. If the distribution of stiffness in a flagellum resembled that of a homogeneous cylindrical rod, α_1/α_0 would be about 7. Other distributions could increase this ratio, but it is unlikely to exceed 20. The contribution from M_v will therefore be no greater than $\frac{3}{2}\frac{M_v}{\alpha_1}$ and will be closer to M_v/α_1 if the length constant, $1/\alpha$, is small.

As pointed out previously[2,8], the constancy of circular curvature in the bent regions of a flagellum and the very slight amount of noticeable passive bending in the straight regions of a cilium or flagellum indicate that M_v/α_1 is small compared to $1/\rho_0$. The passive bending induced by viscous forces will therefore be significantly smaller than that resulting from shear strain near the transition regions. It may not, however, be completely negligible, in which case the properties of Machin's model might also be involved in controlling wave propagation.

One of the main arguments offered in support of Machin's model is the occurrence of synchronization between flagella which come into close proximity[4]. However, this implies no more than that wave propagation is sensitive to the resistance to movement offered by the environment, which is also shown under conditions such as increased viscosity[2,9]. This sensitivity can lie either in the passive disturbance which is responsible for activation or in the time-course of contraction following activation, since both will be involved in determining the propagation velocity. Since the mechanism proposed here still allows environmental factors to influence the time-course of contraction following activation, and therefore the propagation velocity, it can be adapted to fit observations of synchrony and the effects of increased viscosity.

In addition to the points raised at the beginning of this paper, there are several possible experiments which may discriminate between Machin's model and a model involving local spread of bending such as the one proposed here. Some of these are under way in our laboratory. It will be more difficult to distinguish experimentally between the model proposed here and models involving other types of locally spreading signals. However, the preservation of wave propagation in ATP-reactivated, glycerinated flagella which have lost their normal membrane properties[10,11] and the sensitivity of propagation velocity to viscosity and other mechanical factors, argues in favour of a very simple type of locally spreading signal, such as the one proposed in this article.

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IMMUNOLOGICAL TOLERANCE OF NEONATALLY INFECTED MICE TO THE MOLONEY LEUKAEMIA VIRUS

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TISSUE preparations containing the Moloney virus are capable of inducing a state of specific host resistance against the transplantation of small Moloney lymphoma isografts[1,2]. Concomitantly, serum antibodies appear which react specifically with Moloney lymphoma target cells in the cytotoxic and the fluorescent antibody tests and can also neutralize the leukaemogenic and immunogenic properties of the virus[2,3]. Transplantation resistance and serum antibodies can be readily induced even by highly diluted preparations of virus-containing material and, once attained, high antibody titres persist throughout the lifetime of the recipient. Mainly for this reason, we have postulated[3] that multiplication of the inoculated virus is mostly responsible for host immunization. It could not be decided, however, whether the rejection response and the concomitantly developing anti-target-cell antibodies are due to the antiviral response as such, or are mediated via new 'cellular' antigens, induced by the virus but different in specificity from the antigens of the mature virion, as in the polyoma and the Rous systems[4,5]. This distinction could only be made if lymphoma lines could be isolated which do not release any infectious virus.

With one notable exception, inoculation of leukaemia virus containing materials to adult mice has led to transplantation resistance and antibody formation regularly in all virus-host-tumour systems examined. The exception is represented by the high-leukaemia strain *AKR*, known to transmit the Gross virus vertically from parent to offspring. Lymphomas developing in this strain carry the same specific antigen(s) as Gross virus induced lymphomas of other strains, but neither *AKR* nor *AKR* × *C3H* F_1 mice respond to active immunization, and they fail to develop humoral (cytotoxic) antibodies or transplantation resistance after attempts at active immunization[6–8]. This suggests that they are tolerant to the agent, and, if it is a different entity, also to the 'cellular' antigen(s) induced by it. This interpretation is confirmed experimentally by the finding that *C3H* mice infected with the Gross virus neonatally could not be made resistant against the isotransplantation of Gross lymphoma cells, even if re-infected at an adult age, in contrast to *C3H* mice infected as adults[6]. Closely similar findings have been obtained with the Moloney agent. Adult mice infected with this virus regularly attain a state of specific transplantation resistance which is much stronger[2], as a rule (that is, protects from a large number of grafted neoplastic cells), than what can be obtained in analogous experiments with the Gross system[9]. Humoral antibodies are also regularly present in higher titres and can be demonstrated not only by the cytotoxic but also by the fluorescent antibody method. In contrast, mice inoculated as new-born

either entirely fail to react or show a deficient or delayed antibody response[10].

The work recorded here represents a further attempt to understand this tolerant state. It was tested whether neonatally infected mice which lack demonstrable antibodies can respond immunologically to a second virus challenge as adults and whether their non-responsiveness against cellular targets as revealed by the fluorescence test is paralleled by a similar deficiency in virus neutralizing ability. In addition, whether they were capable of rejecting small isografts from established Moloney lymphomas, known to be susceptible to the rejection reaction of virus inoculated adult mice, was investigated.

Antibodies directed against cellular targets were detected by the immunofluorescence test as applied to living cells[2,11]. The *YLD* lymphoma, induced by the Moloney agent in a *C57L*eaden mouse, was used as the standard target cell. The sera were tested for virus neutralizing activity by adapting the mouse antibody production (MAP) assay for the virus[3]. 0·1 ml. 1 : 100 diluted standard virus preparation was inoculated into *2A* × *CBA* F_1 or *ABY* × *CBA* F_1 mice after incubation with 0·05 ml. undiluted test or control serum for 4 h at room temperature. The sera of the recipient mice were tested for antibodies by the immunofluorescence test against *YLD* at appropriate intervals.

Fig. 1 illustrates the results of one experiment. Eight new-born (less than 24 h old) *A* mice, derived from two litters, were inoculated with 0·05 ml. of a 1 : 10 diluted virus preparation. Three adult *A* mice were inoculated simultaneously. The mice were bled individually and their sera were tested against *YLD* target cells in the immunofluorescence test. While sera of all mice injected as adults were highly positive 30 days later, none of the neonatally infected animals had significant antibody levels. Six in the latter group developed leukaemia 103, 103, 117, 158, 165, and 178 days after inoculation. The four neonatally infected mice that were negative on day 160 were re-infected with 0·05 ml. 1 : 10 diluted virus preparation on that day. Five previously untreated *A* mice of the same age were infected at the same time. Two of the four mice of the former category died with leukaemia before their serum could be collected for test. The other two could be tested and were found to remain negative during the entire observation period, that is, 205–270 days after the first and 45–90 days after the second virus dose. One died with leukaemia at the age of 220 days. The control mice which were infected as adults produced antibodies

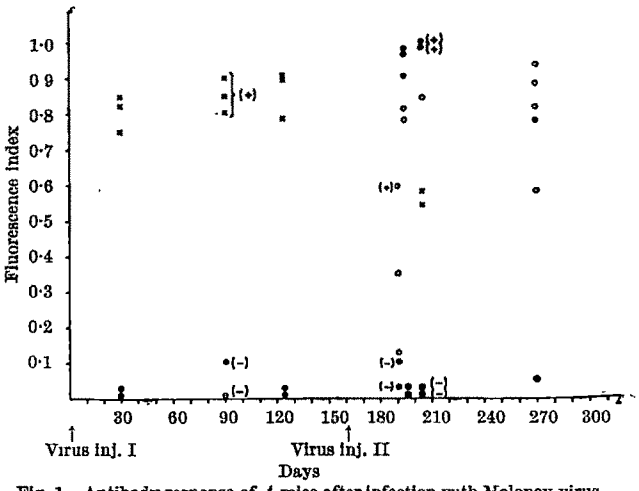


Fig. 1. Antibody response of *A* mice after infection with Moloney virus. ●, Two *A* mice (1♂ and 2♀) infected neonatally and re-infected on day 160; ×, three *A*♂ mice (3, 4 and 5) infected as adults on day 160; ○, five *A*♀ mice (including 6, 7 and 8) infected as adults on day 160. Antibodies were measured against *YLD* target cells by the immunofluorescence test and were expressed by the fluorescence index (+); and (−) denotes the results of virus neutralization tests performed with the same serum (cp. Table 2)

and maintained them in a high titre during the entire observation period of several months.

In order to test the responsiveness of the neonatally infected animals against an unrelated antigen, 0·1 ml. of a 25 per cent suspension of sheep red cells was injected at day 210 to the two non-reacting mice. Four days later the serum haemolysin titres were 1 : 320 and 1 : 640. Simultaneously injected controls gave 1 : 640; 1 : 1,280; 1 : 640 and 1 : 160, respectively. Thus the two non-responsive animals were capable of reacting against an unrelated antigen.

Table 1 shows further experiments of a similar design, performed with *A*, *C57L*eaden and *C3H* mice. Among the mice infected neonatally with the virus, all 5 *C3H*, all 15 *A* and 6 of 10 *C57L*eaden showed a deficient antibody response after 110–120 days. As in our previous work, the fluorescence index (FI) = 0·30 was taken as the threshold for a significantly positive reaction. Fourteen non-reactive mice were chosen for the experiments summarized in Table 1. When re-infected with a second virus dose at adult age, between 120 and 160 days, none of them responded with detectable antibody formation.

Table 1. Antibody Response of Mice inoculated neonatally with Moloney Virus and re-infected as Adults
(Neonatally infected mice shown in italics)

Mouse	No.	Litter	Virus inoculation on day 0*	Antibody-level on day 110–150		Virus inoculation on day 120–160†	Antibody-level					Notes
							Day 140		Day 150	Day 160–190		
				FI‡	VN§		FI	VN	FI	FI	VN	
C3H	1♂	506	+	*0·13*	*Neg*	+	*0·13*	*Neg*	*0·15*	*0·16*	*Neg*	Killed, no leukaemia, day 210
	2♂	506	+	*0 00*		+	*0·00*		*0 12*	*0·14*		Leukaemia, day 180
	3♂	492	+	*0·00*		+	*0·00*	*Neg*	*0·00*	*0·00*	*Neg*	Killed, no leukaemia, day 195
	4♂	492	+	*0·24*	*Neg*	+	*0·00*		*0·00*	*0·00*	*Neg*	Killed, no leukaemia, day 195
	5♂	492	+	*0·00*		+	*0 00*		*0·00*	*0 00*		Killed, no leukaemia, day 195
	6♂		−			+	0·11		0·14	0·41	Pos	
	7♂		−			+	0·16		0·34	0 84	Pos	
A ‖	9♂	1,604	+	*0 00*		+				*0·00*		Leukaemia, day 245
	10♀	1,409	+	*0·00*	*Neg*	+				*0·00*	*Neg*	Killed, no leukaemia, day 210
	11♀	1,409	+	*0·00*	*Neg*	+				*0·21*	*Neg*	Killed, no leukaemia, day 380
	12♀	1,410	+	*0·28*		+				*0·00*		Killed, no leukaemia, day 196
	13♀	1,410	+	*0·12*		+				*0·17*		Leukaemia, day 196
	14♀		−			+				0·88		
	15♂		−			+				0·76	Pos	
C57L¶	1♀	708	+	*0·00*		+				*0 10*		Leukaemia, day 210
	2♀	715	+	*0·17*		+				*0·04*		Leukaemia, day 172
	3♀	715	+	*0·10*		+				*0 21*		Leukaemia, day 120
	4♂	708	+	*0 19*		+				*0·06*		Leukaemia, day 245
	5♀		−			+				0·41		
	6♂		−			+				0·70		

* 0·1 ml. 1 : 5 diluted virus.
† 0·1 ml. 1 : 30 diluted virus.
‡ FI, fluorescence index of serum, tested against *YLD* target cells.
§ VN, virus neutralizing activity (for details see Table 3).
‖ FI of the serum of other neonatally injected *A* mice in the same experiment, bled at 120 days. 0 27, 0 30, 0·25, 0, 0·17, 0, 0·16, 0, 0, 0. 150 days. 0 14, 0 26, 0, 0·10, 0·32, 0·21.
¶ FI of the serum of other neonatally injected *C57L* mice in the same experiment bled at 120 days· 0 33, 0·25, 0 27, 0·32, 0·50, 0 40.

Table 2. EFFECT OF MOUSE SERA OBTAINED FROM INDIVIDUAL ANIMALS ON THE ANTIBODY-INDUCING CAPACITY OF A STANDARD VIRUS PREPARATION

(Neonatally infected mice shown in italics)

Source of admixed serum: Donor mouse (designated as in Table 1)	Collected on day of experiment	FI	Antibody-level of recipients inoculated with virus-serum mixture on day: 25 FI	33 FI	38 FI	45 FI
Exp. A						
1 *A.SW* ♀	*120*	*0·73*	*0·04*	*0 14*	*0 05*	*0·10*
2 *A.SW* ♂	*120*	*0·30*	*0 65*	*0 65*	*0·74*	
3 *A.SW* ♂	*120*	*0·62*	*0 23*	*0·34*	*0·37*	
10 *A*♀	*120*	*0·00*	*0·56*	*0·95*		
11 *A*♀	*120*	*0·00*	*0·42*	*0 54*	*0·50*	*0·78*
3960 standard Moloney antiserum pool		0·90	0 23	0 84	0·37	
Control serum			0 65			
—			0 81			
Exp. B			Day 35	42		
10 *A*♀	*192*	*0·00*		*0·86*		
11 *A*♀	*192*	*0·21*		*0 94*		
15 *A*♂	182	0·76		0 00	0·00	
2 *A*♀	*90*	*0·00*		*0·89*		
2 *A*♀	*205*	*0·10*		*0·86*		
1 *A*♂	*90*	*0·10*		*0·67*		
1 *A*♀	*205*	*0·00*		*0 91*		
6 *A*♀	205	1·00		0·20		
7 *A*♀	205	1·00		0·24		
Pool of 3 *A*♂ (3, 4, 5)	90	0·90		0·10		
1 *C3H*♂	*110*	*0·13*		*0·67*	*0·79*	
1 *C3H*♂	*160*	*0 16*		*0·89*		
3 *C3H*♂	*160*	*0·00*		*0·89*		
4 *C3H*♂	*110*	*0·24*		*0·77*		
4 *C3H*♂	*160*	*0·00*		*0 96*	*0·93*	
7 *C3H*♂	160	0·84	0	0 00	0·17	
5313 standard Moloney antiserum pool		0·90		0·00		
Control serum				0·73		
Exp. C			Day 22	30		
2 *A*♀	*190*	*0 12*		*0·81*		
1 *A*♂	*190*	*0 04*		*0·87*		
8 *A*♀	190	0 59	0 00	0·39		
1 *C3H*♂	*140*	*0·13*		*0·58*		
3 *C3H*♂	*140*	*0·00*		*0·79*		
Control serum			0·24	0 87		
—			0 18	0 82		

* 0·1 ml. 1 : 100 dil. standard virus preparation was inoculated to each mouse after incubation with 0·05 ml. undiluted test serum during 4 h at room temperature. Prior to inoculation 0·1 ml. balanced salt solution was added. Two adult *A* × *CBA* F_1 mice served as recipients in Exp. *A*, two *ABY* × *CBA* F_1 mice in Exp. *B*, and two *ABY* × *CBA* F_1 in Exp. *C*.

Some of the sera were also tested for virus neutralizing activity. On the basis of our previous experience[3], this was appraised by testing their ability to neutralize or reduce the antibody-inducing capacity of standard virus-containing homogenates. The results are given in Table 2. Three separate experiments were performed. They include two standard post-infectious sera of adult mice, collected 7–8 months after inoculation and with a positive known FI value. These served as positive controls for virus neutralization while normal mouse serum was used as a negative control. It appears that both standard Moloney antisera (pools 5313 and 3960) showed a clear neutralizing activity. The same was true for the sera of 5 mice infected as adults and for the sera of two neonatally infected *A.SW* mice that had high FI values. All 18 sera collected from 10 neonatally infected mice which gave a negative FI when tested directly against lymphoma target cells were also negative when assayed for virus neutralizing ability. Taken together, all tests thus show a clear inverse relationship between the FI of the serum admixed to the virus containing homogenate and the antibody level of the mice inoculated with the mixture and tested 33–45 days after inoculation.

Table 3 shows the results of the tests carried out to compare the ability of neonatally infected mice to reject small Moloney lymphoma isografts with animals infected as adults and untreated controls. The *YLD* lymphoma, previously found highly susceptible to the rejection response of syngeneic hosts infected as adults, was used for these tests as well. It appears that while all mice infected as adults rejected the grafts which grew regularly in untreated controls, mice infected as new-born were susceptible in 18 of 21 cases. Among the three resistant mice, two had positive antibody titres at the time of tumour cell challenge while all 18 susceptible animals were negative at the same time.

Table 3 TRANSPLANTATION OF SMALL *YLD* LYMPHOMA ISOGRAFTS TO SYNGENEIC MICE INOCULATED WITH MOLONEY VIRUS AS ADULTS OR AS NEW-BORN

Exp. No.	No of cells inoculated s.c.	Takes in syngeneic recipients 3 nights after virus inoculation: Injected with virus: Untreated	Neonatally	As adults	FI of neonatally infected mice at time of tumour cell challenge: Susceptible to tumour inoculation	Resistant to tumour inoculation
1	10^5		5/5	0/4	0, 0, 0, 0, 0	—
2	10^4	4/4	3/3	0/4	0, 0·14, 0·20	—
3	10^3	3/3	5/5	0/4	0 21, 0·13, 0·21, 0, 0	—
4	10^4	5/6	5/8	0/5	0, 0·24, 0, 0, 0 28	0 67, 0·28, 0·94

In conclusion, the non-responsiveness of neonatally infected mice after re-infection at adult age fulfils the operational criteria of immunological tolerance. The parallel presence or lack of the virus neutralizing and the anticellular activity of the various sera may be due to the identical specificity of the antibodies involved. This is particularly suggestive in view of the strictly parallel individual variation in the response of different neonatally infected mice in relation to the different test systems. Since tolerance may have been induced to more than one antigen at the same time, however, this evidence is merely suggestive but not conclusive.

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MAGNESIUM NUTRITION OF COTTON AS INFLUENCED BY SODIUM

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THE beneficial effects of sodium fertilization on the growth and yield of cotton have been reported by many investigators[1–5]. Several concluded that the best returns from sodium were obtained on soils deficient in potassium, and only in a few cases have physiological and nutritional reasons been advanced to explain the results. Mathews[5] attributed the yield increase in cotton to the effect of the sodium ion in partially substituting the potassium ion in some of its metabolic functions. Appling and Giddens[6] found that the contents of sodium and potassium in the various parts of the plant were directly proportional to the amounts of these elements applied as fertilizers, and that sodium accumulated in the upper leaves. They concluded that the preferential shift of potassium to the active meristematic regions indicated that sodium probably performed some of the functions of potassium in the

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Table 1. DRY WEIGHTS (g) OF 85-DAY-OLD COTTON PLANT FRACTIONS AS INFLUENCED BY MAGNESIUM AND SODIUM

Treatments	Young leaves	Old leaves	Young stems	Old stems	Roots	Squares	Bolls
1. +Mg +Na	13·17*a* *	29·54*a*	6·10*a*	36·19*a*	11 96*a*	8·22*a*	0·76
2. +Mg −Na	11·82*a*	31 94*a*	6·08*a*	39·89*a*	11·20*a*	8·37*a*	
3. −Mg +Na	3·17*b*	15·70*a*	0·91*b*	8·38*b*	3·30*b*	0 09*b*	
4. −Mg −Na	3·65*b*	12 74*a*	0·75*b*	7·34*b*	2 90*b*	0·12*b*	

* Duncan's multiple range test for the 5 per cent level of significance.

more mature tissue. Joham[7] established the ability of sodium to partially substitute for both potassium and calcium in their general cationic functions in the cotton plant. Sodium had a significant influence on 90-day-old cotton plants growing in nutrient solutions deficient in either calcium or potassium during the latter half of their growth.

While sodium was found to influence fruiting activity when either calcium or potassium was withheld, it also prevented wilting in plants grown in low calcium solutions. It was suggested that sodium probably mobilized stored calcium from the older tissue, but it had no influence on the movement of potassium. The results of further investigations suggested a role of sodium in maintaining a favourable protein status in plants deficient in potassium[8]. The level of leaf protein was related to the level of substrate potassium and sodium. Plants deficient in both cations had the lowest concentration of protein, while no significant differences were seen in those receiving either sodium or potassium.

The investigation recorded here was undertaken in the autumn and winter of 1962–63 as a logical extension of the problem of sodium substitution for the macronutrient cations in cotton nutrition.

Cotton seeds, variety 'Deltapine 15', were germinated in flats of sterilized perlite. Six days after emergence three uniform seedlings were washed free of perlite and transplanted into each of twenty plastic 6-litre pots containing the following nutrient solution: $Ca(NO_3)_2.4H_2O$, 5 mM; KNO_3, 3 mM; $(NH_4)_2SO_4$, 2 mM; K_2HPO_4, 11 mM; $MgCl_2.6H_2O$, 2 mM. The micronutrient elements supplemented to each pot consisted of 2 p.p.m. ferric iron, 0·5 p.p.m. each of manganese and boron, 0·05 p.p.m. zinc, 0·04 p.p.m. molybdenum and 0·03 p.p.m. copper. The solutions were aerated from a central source and the *p*H of the solutions was checked daily and adjusted if necessary by the addition of dilute nitric acid or ammonium hydroxide to maintain the range *p*H 5·0–6·0.

Four treatments were started thirty days after transplanting: (*a*) plus magnesium–plus sodium (+Mg +Na); (*b*) plus magnesium–minus sodium (+Mg −Na); (*c*) minus magnesium–plus sodium (−Mg +Na); (*d*) minus magnesium–minus sodium (−Mg−Na). $MgCl_2$ was eliminated from the −Mg series and NaCl (10 mM/l.) was added to the +Na series. The experimental design was a randomized block with five replications on the greenhouse bench.

Harvests were made 60 and 85 days after transplanting. At the 85-day harvest the leaves and stems were separated into old and new growth tissue, the latter being the growth made between 60 and 85 days. The tried, ground plant samples were ashed in a muffle furnace at 500° C and then dissolved in 5 per cent hydrochloric acid. Magnesium, sodium, potassium and calcium were determined using a Beckman *DU* spectrophotometer with a flame attachment.

Incipient magnesium deficiency symptoms were observed 12 days after the treatments were effected. Plants not receiving magnesium exhibited an interveinal chlorosis on the recently matured leaves. The interveinal areas gradually turned reddish brown in colour during the next 20–30 days, and the areas spread and coalesced. Stunted growth and shedding of flower buds were characteristic of the magnesium deficient plants

The addition of sodium to plus and minus magnesium solutions had little or no effect on the growth of cotton as indicated by dry weights (Table 1). However, in this experiment, sodium had a beneficial effect on the development of plants supplied with magnesium. Plants of the +Mg+Na treatment flowered earlier and produced more flowers than those of the +Mg−Na treatment.

It is apparent that substrate sodium had a marked influence on the distribution of magnesium in the cotton plant. Comparison of data in the +Mg+Na and +Mg −Na series at the 60-day harvest (Table 2) shows a decrease in magnesium content in the roots and stems with a corresponding increase in the leaves due to sodium. By the 85-day harvest sodium had little or no effect on stem and leaf magnesium in the +Mg plants although the trend observed in root magnesium at 60 days was still evident. In the −Mg series at the 60-day harvest differences in magnesium content in the tissue due to substrate sodium were not significant. However, there were significant magnesium–sodium interactions in all cases. By 85 days all the above-ground organs of the −Mg+Na plants contained higher tissue magnesium than corresponding parts of the −Mg−Na plants.

Thus it appears that sodium in the substrate may serve some function in the maintenance of magnesium. This was apparent in the +Mg plants at the early harvest and in the −Mg plants at the final harvest. An inverse relation was noted between root magnesium and substrate sodium at both harvests.

Marked diurnal variations of the magnesium content in the leaves of the cotton plant have been reported[9], and the mobilization of magnesium to the active growing points has been shown with other plants[10,11]. In this investigation the indications are that sodium in the substrate enhances this phenomenon, releasing the magnesium from the older tissue, thus enabling it to be re-utilized in the younger more active areas. If this is the case the possibility exists for the partial substitution of sodium for magnesium in the nutrition of the cotton plant. In this investigation the deficiency of magnesium was very severe and it may be that beneficial effects of sodium in the −Mg series were not evident at that level of magnesium nutrition. Support for this concept was evidenced in the flowering response of the +Mg plants to the additions of sodium. It may be that earlier flowering of the +Mg+Na plants was due to the greater movement of magnesium to the active growing tissues.

In stems and roots, tissue sodium was inversely related to substrate magnesium at the 60-day harvest. At 85 days substrate magnesium tended to decrease tissue sodium in roots and stems and increase sodium in the leaves (Table 3).

In the 60-day-old plants the distribution of potassium in the roots and stems was effected by significant magnesium–sodium interactions. In the −Mg series the +Na plants contained a significantly higher amount of potassium in the leaves than those of the −Na treatment (Table 4). The same trends were evident in the young leaf and young and old stem of the 85-day-old plants of

Table 2. EFFECT OF TREATMENTS ON THE MAGNESIUM CONTENT OF 60- AND 85-DAY-OLD COTTON PLANTS (M.equiv./100 g dry wt)

Age	60 Days			85 Days				
Treatment	Leaves	Stems	Roots	Young leaves	Old leaves	Young stems	Old stems	Roots
1. +Mg +Na	117·49*a* *	41·12*b*	15·37*b*	84 91*a*	86 06*a*	55 88*a*	33 60*a*	22·95*a*
2. +Mg −Na	66·58*b*	111 62*a*	33·74*a*	82 33*a*	85 08*a*	58·34*a*	34·42*a*	43·27*b*
3. −Mg +Na	41·41*c*	8·94*c*	11·14*b*	25·74*b*	27·37*b*	37 05*b*	24 42*a*	30·82*a*
4. −Mg −Na	15·66*c*	4 45*c*	8·33*b*	16 56*b*	13·75*c*	23·94*b*	10·32*b*	38·07*b*

* Duncan's multiple range test for the 5 per cent level of significance.

Table 3. Effect of Treatments on the Sodium Content of 60- and 85-day-old Cotton Plants (M.equiv./100 g dry wt.)

Age	60 Days			85 Days				
Treatment	Leaves	Stems	Roots	Young leaves	Old leaves	Young stems	Old stems	Roots
1. +Mg +Na	12·55*a* *	12·04*b*	12·28*b*	11·36*a*	75·73*a*	18·36*a*	14·31*b*	15·80*a*
2. +Mg −Na	7·72*b*	3·34*c*	3·02*c*	6·52*c*	32·60*c*	5·58*c*	4·37*c*	4·92*b*
3. −Mg +Na	11·33*a*	18·32*a*	33·26*a*	8·64*b*	50·82*b*	16·40*a*	20·25*a*	19·15*a*
4. −Mg −Na	9·12*b*	5·82*c*	5·15*c*	5·41*c*	30·82*c*	10·98*b*	6·65*c*	6·61*b*

* Duncan's multiple range test for the 5 per cent level of significance.

Table 4. Effect of Treatments on the Potassium Content of 60- and 85-day-old Cotton Plants (M.equiv./100 g dry wt.)

Age	60 Days			85 Days				
Treatment	Leaves	Stems	Roots	Young leaves	Old leaves	Young stems	Old stems	Roots
1. +Mg +Na	90·25*a* *	71·27*c*	158·52*a*	86·56*c*	78·27*a*	177·43*a*	53·63*c*	67·58*a*
2. +Mg −Na	86·66*b*	277·51*a*	190·76*a*	58·56*d*	98·43*d*	172·82*a*	69·64*b*	83·69*a*
3. −Mg +Na	95·89*a*	146·12*b*	131·73*a*	124·60*a*	126·80*b*	182·65*a*	89·11*a*	83·48*a*
4. −Mg −Na	85·64*b*	121·30*b*	93·46*a*	99·74*b*	113·33*b*	92·18*b*	56·92*c*	88·22*a*

* Duncan's multiple range test for the 5 per cent level of significance.

Table 5. Effect of Treatments on the Calcium Content of 60- and 85-day-old Cotton Plants (M.equiv./100 g dry wt.)

Age	60 Days			85 Days				
Treatment	Leaves	Stems	Roots	Young leaves	Old leaves	Young stems	Old stems	Roots
1. +Mg +Na	75·10*a* *	35·55*b*	37·86*b*	73·40*b*	94·80*a*	89·60*b*	62·60*b*	66·80*a*
2. +Mg −Na	74·00*a*	22·35*d*	14·55*c*	68·20*b*	73·00*c*	53·40*c*	38·20*c*	32·60*b*
3. −Mg +Na	80·60*a*	31·45*c*	150·80*a*	84·80*b*	84·60*b*	122·60*a*	91·20*a*	65·20*a*
4. −Mg −Na	80·30*a*	47·05*a*	28·58*b*	99·30*a*	95·90*a*	123·40*a*	60·90*b*	45·20*b*

* Duncan's multiple range test for the 5 per cent level of significance.

the −Mg series. These results indicate that plants growing in a nutrient medium deficient in magnesium absorbed more potassium in the presence of sodium, thus suggesting another beneficial effect of sodium under conditions of nutrient impoverishment.

In general, plants growing under conditions of magnesium deficiency contained more calcium in their tissue than those with an adequate magnesium supply (Table 5). This is in agreement with the concept of the cation equivalency in plants[12,13]. The distribution of calcium in some tissues was also subject to significant magnesium–sodium interactions. In the stems of the 60-day-old plants, the presence of sodium in the substrate increased the calcium content when magnesium was supplied, while the opposite effect was observed in the absence of substrate magnesium. In the roots of the 60-day-old plants substrate sodium was associated with an increase in tissue calcium regardless of magnesium treatment. In the −Mg treatments of the 85-day-old plants, substrate sodium depressed the calcium content in all tissue except the roots and old stems. The calcium contents in the old and young leaves of the −Mg−Na treatment were significantly less than those of the −Mg−Na treatment. In the presence of magnesium, however, sodium increased the calcium content in all tissue. While these findings are in agreement with those of Joham[7,14] they are contrary to those of Cooper *et al.*[2].

Thus it was found that under certain conditions sodium increased the magnesium, potassium and calcium contents in the cotton plant. In some cases the effect of sodium was even more pronounced under conditions of magnesium deficiency. In addition significant magnesium–sodium interactions were found to govern the uptake and accumulation of macronutrient cations. The results suggest that under conditions of magnesium deficiency, sodium enhanced the mobilization of magnesium from the older to the more actively growing tissues.

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CELL DISPERSING LIVER EXTRACT INFLUENCING BLOOD CLOTTING

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AFTER the addition of a cell-free liver extract to suspensions of thymus or lymph node cells in Earl's solution, the number of cells per mm³ was more than 200 per cent of the number of cells per mm³ after the addition of Earl's solution[1]. (This effect with the liver extract occurred immediately.) Experiments with pipettes of different diameters support the view that the liver factor contributes to the dissolution of cell aggregates in the suspension. It has been concluded that the addition of liver extract may be a useful method by which the

correct cell number in suspensions of lymphoid cells can be obtained, and the degree of aggregation in such suspensions can be estimated.

This communication reports experiments using similar liver extracts. Our first question was: Is liver extract more effective than extracts of other organs in preventing the formation of aggregates of thymus cells?

Homogenates of liver, spleen, kidneys, small intestines, lungs, muscles and testes (one part of the organ to two parts of Earl's solution) were prepared from guinea-pigs weighing approximately 200 g. The homogenates were centrifuged for 20 min at 2,500*g*; the supernatant fluids were the extracts used. They were kept at 4° C and used within 72 h of preparation. Serum was diluted with two parts of Earl's solution, stored and used in the same way as the extracts.

Thymus suspension was prepared as follows: the thymus from a guinea-pig was roughly freed from connective tissue, washed in Earl's solution, and stored in a moist chamber at 4° C for 4 h. The surface was then carefully freed from remaining connective tissue and the thymus washed once more. The organ was then cut up finely in 3 c.c. of Earl's solution, and the pieces stirred with a pair of forceps and pressed against the sides of the glass vessel. Another 3 c.c. of Earl's solution was added and the tissue mixture was then sieved through ordinary muslin. 0·025 c.c. portions of Earl's solution, of organ extract, and of diluted serum were placed in test-tubes. As soon as the cell suspension was prepared, 0·5 c.c. was quickly added to each tube with a 5 c.c. pipette. The mixtures were gently rocked for 1 h at room temperature, and the number of cells per mm³ counted. The cell counts were converted to relative numbers (the highest count per mm³ = 100). Five tests were performed.

The highest cell count was found in the mixture with liver extract in all experiments, and the lowest cell count was found in the mixture with Earl's solution (Table 1). This indicates that the liver extract was the most effective in preventing aggregate formation and that the aggregate-preventing factor or factors were present in all extracts and the serum.

Table 1. Relative No. of Cells per mm³ in Different Mixtures

	1	2	3	4	5	Mean
Susp. + Earl's	48·9	36·9	51·3	46·6	22·0	41·1
Susp. + serum	54·7	86·9	45·1	60·2	90·5	67·5
Susp. + liver	100	100	100	100	100	100
Susp. + spleen	77·4	62·0	64·8	85·0	56·8	69·2
Susp. + kidneys	78·0	91·2	68·3	75·9	76·7	78·0
Susp. + intestine	82·0	87·3	85·9	76·4	84·0	83·1
Susp. + lungs	51·9	50·0	49·5	76·1	39·2	53·3
Susp. + muscles	67·2	57·0	71·3	67·1	48·1	62·1
Susp. + testes	57·1	83·5	35·5	69·2	74·9	64·0
Susp. + Earl's	46·9	47·7	32·0	54·8	28·2	41·9

The earlier findings[1] and the results obtained as described here suggested that a factor is normally formed or stored in the liver which is necessary for the natural suspension of blood cells. Some preliminary experiments on thrombocytes in peripheral blood are reported here.

Homogenates of liver were prepared as already described, but from human liver. The supernatant after the first centrifugation was centrifuged for 1 h at 100,000*g*. After discarding the floating material the new supernatant was the extract. This was kept at 4° C, and used within 5 days following the first centrifugation.

The number of thrombocytes per mm³ were counted from: (*a*) heparinized blood 10 min after sampling; (*b*) the liver extract used; (*c*) a mixture of 0·5 c.c. heparinized blood and 0·5 c.c. liver extract after rocking for 1 h in room temperature; (*d*) a similar mixture of 0·5 c.c. heparinized blood and Earl's solution.

It is evident from Table 2 that the liver extract used was not free from particles, probably bacteria. With the method used, these would have been counted as thrombocytes. The dilution solution used for counting and the Earl's solution did not contain such particles. However, it is quite easy to make a correction for the presence of

Table 2. No. of Thrombocytes per mm³ in Different Mixtures

	Heparinized blood	Liver extract	Blood + liver extract	Blood + Earl's solution
(1)	244,000	31,600	214,600	63,600
(2)	254,000	31,600	184,000	94,800
(3)	210,200	31,600	153,200	67,200
(4)	182,000	31,600	180,800	36,400
(5)*	253,600	71,200	205,600	40,400
(6)*	258,800	71,200	206,000	46,000
(7)†	206,000	9,600	48,400	21,600
(8)†	220,000	9,600	98,400	35,200
(9)†	193,600	9,600	58,400	52,000
(10)	196,000	2,800	118,000	28,800
(11)	223,200	2,800	112,000	32,800

* The liver extract was stored at +4° C for 5 days.
† The liver was stored at +4° C for 2 days before the extract was prepared.

Table 3. Corrected No. of Thrombocytes in Different Mixtures

	Heparinized blood	Blood + liver extract	Blood + Earl's solution
(1)	244,000	396,000	127,200
(2)	254,000	288,000	189,600
(3)	201,200	274,400	134,400
(4)	182,000	329,600	72,800
(5)*	253,600	332,800	80,800
(6)*	258,800	333,600	92,000
(7)†	206,000	87,200	43,200
(8)†	220,000	187,200	70,400
(9)†	193,600	107,200	104,000
(10)	196,000	233,200	97,600
(11)	223,200	221,200	65,600

* The liver extract was stored at +4° C for 5 days.
† The liver was stored at +4° C for 2 days before the extract was prepared.

such foreign particles. Corrections were made, at the same time, for the dilution 1 : 2 in *c* and *d*: these are given in Table 3.

The correction formulae are: Corrected figure for the blood–liver extract mixture =

$$2 \times \left(\text{figure obtained} - \frac{(\text{figure for liver extract})}{2} \right)$$

Corrected figure for the blood–Earl's solution mixture =

$$2 \times \text{figure obtained}$$

The blood–Earl's solution mixture shows lower figures than the blood alone, which indicates the presence of aggregates, or loss of cells on the glass walls in this mixture. The blood–liver extract mixture shows higher figures than the blood alone. This indicates that there is a certain degree of aggregate formation or loss of cells on the glass walls already in the heparinized blood.

The ability of the liver extract to influence the suspension stability or the adhesiveness of thrombocytes suggested that it might be involved in the formation of blood clots. The following experiments were designed in order to test this possibility.

Whole blood of various species was mixed with equal parts of Earl's solution or with liver extract from different species. The liver extract was prepared as in the first experiment. The mixtures were gently rocked for 1 h at room temperature. Smears were prepared from the mixtures and examined histologically for the degree of

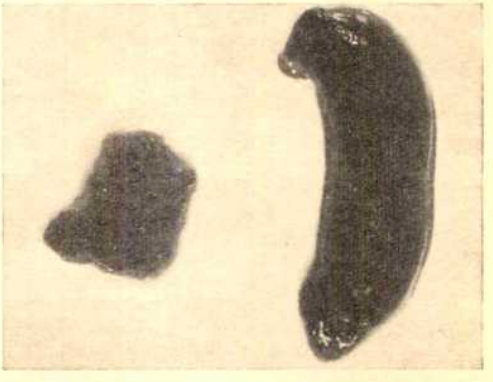
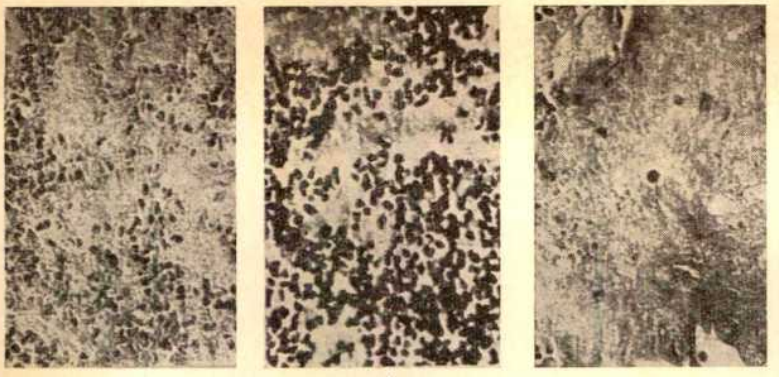


Fig. 1. Blood from guinea-pig. To the left, clot formed by rocking with guinea-pig liver extract. To the right, clot formed by rocking with Earl's solution



a *b* *c*

Fig. 2. Blood from National Institutes of Health New Zealand albino rabbit (× 140). (*a*) Clot formed on rocking with guinea-pig liver extract. (*b*) Clot formed on rocking with Earl's solution. (*c*) Clot formed on shaking with Earl's solution (defibrination)

erythrocyte aggregation. After rocking, the clots were observed and a few were embedded and sectioned for routine histological examination. The sections were stained according to Weigert (Romeis 1948, § 1424). In one case a blood–Earl's solution mixture was shaken vigorously in a relatively large vessel for defibrination.

The following combinations of blood and liver extract were tested: (1) blood from National Institutes of Health New Zealand albino rabbits with guinea-pig liver (5 tests); (2) blood and liver from guinea-pigs (7 tests); (3) blood from Swedish farmer's rabbits with guinea-pig liver (2 tests); (4) blood from National Institutes of Health New Zealand albino rabbits with human liver (1 test); (5) human blood with human liver (4 tests); (6) blood and liver from Swedish farmer's rabbits (2 tests).

In three of the combinations tested (1, 2 and 4) there was a clear-cut difference between the clots: the clot in the mixture with Earl's solution was an ordinary clot containing numerous red cells, but in the mixture with liver extract the clot was small and pale, containing relatively few red cells (Fig. 1). Histological examination revealed that the pale small clot obtained with liver extract in combination (1) was a fibrin clot resembling the clot at defibrination (Fig. 2). The smears from these combinations (1, 2 and 4) did not reveal differences in the size of red cell aggregates in the mixtures with liver extract or those with Earl's solution.

In other combinations performed (3, 5 and 6) there were no similar differences between the clots.

It is of limited value to discuss at this early stage the liver factor or liver factors. It will suffice to say that there may be something of great biological interest hidden here. The factor preventing the thrombocyte aggregation may also be of clinical interest. Without doubt, addition of liver extract can be recommended as a method for obtaining a correct cell number in artificially prepared suspensions. The liver extract has been shown to prevent the formation of aggregates in suspensions of bone marrow cells[2]. Furthermore, serum has been shown to split aggregates of sarcoma cells[3].

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CONTROL OF SELF-IRRADIATION DECOMPOSITION OF TRITIUM-LABELLED COMPOUNDS AT HIGH SPECIFIC ACTIVITY

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THE increasing use of tritium compounds as tracers at very high specific activity (some examples, refs. 1–3) has presented growing problems to both supplier and user in minimizing the rates of decomposition of these compounds on storage.

Since publication[4] of the first extensive examination of the decomposition of tritium compounds, several investigators have published their observations of the self-decomposition of groups of tritium compounds such as amino-acids[5,6], steroids[7] and fatty acid esters[8], or of specific tritium compounds such as thymidine[9,10], chimyl alcohol[11], *cyclo*hexane[12] and *n*-propanol[12]. Methods adopted to control decomposition rates have included, for example, storage in various solvents[4–6,12], dispersal on paper[5,6] and on various solids[12], such as charcoal, benzanthracene, silica gel, cellulose and ferric oxides, low-temperature storage (−196° C)[10], the use of radical scavengers[9,10] for compounds stored in aqueous solution, and the inclusion of tritium compounds as clathrates[13]. It is doubtful whether decomposition can be eliminated completely by any method. One answer to the problem is, of course, to use the compound immediately after purification, but this is not always practicable or economical, and better methods for reducing the rates of decomposition to a tolerable level are still needed.

This article presents preliminary results of the investigation of two important aspects of the control of the decomposition of tritiated organic compounds from self-irradiation on storage. These are: (1) use of radical scavengers for compounds stored in aqueous solution; (2) storage at very low temperatures (−196° C). Both these methods have been found to reduce substantially the rate of decomposition as illustrated by the examples given in Tables 2 and 3.

Use of Radical Scavengers

The protective action of benzyl alcohol[9] or cysteamine[10] against radiation decomposition for compounds in aqueous solution has previously been mentioned. To be effective in reducing the rate of self-decomposition of tritium compounds stored in aqueous solution, additives must effectively and efficiently 'scavenge' hydrogen and hydroxyl radicals, which arise by radiolysis of the water[14].

Three compounds were selected for detailed investigation:

(*a*) *Benzyl alcohol*, which has also the advantage of being a bacteriostat. It is not easily removed, however, and furthermore it can give rise to aldehydes, phenols and acids by interaction with H and OH radicals[15]. Although these radiolysis products should not be radioactive (assuming there is no hydrogen–tritium exchange and no interaction with labelled species), their presence may be undesirable for some investigations.

(*b*) *Ethyl alcohol*, which is easily removed and, at the low concentrations normally required (less than 5 per

cent), is unlikely to produce side-effects in research investigations. Disadvantages include the formation of aldehydes and glycols by reaction with free radicals[15], and the presence of alcohol may actually accelerate the rate of decomposition of the solute[16,22]. Under some storage conditions esterification of the solute can occur[5,6].

(c) *Sodium formate (or formic acid)*. The effects of radiation on aqueous solutions of formic acid are among the reactions of this type that have been subjected to most intensive investigation[15]. The main products from the interaction of formate with H or OH radicals are carbon dioxide and water:

$$\cdot H + HCOOH \rightarrow H_2 + \cdot COOH$$

$$\cdot OH + HCOOH \rightarrow H_2O + \cdot COOH$$

$$2 \cdot COOH \rightarrow HCOOH + CO_2$$

Net reaction

$$HCOOH \rightarrow H_2O + CO_2$$

Thus, during the course of its protective action, the formate concentration is likely to decrease giving water and carbon dioxide. There would seem no obvious disadvantages in the use of formate as a radical scavenger, except, of course, when the tritiated compound is to be used in a system which must be free of formate. For many classes of labelled compound it could be removed fairly easily.

Scavenging Efficiencies

To compare the scavenging efficiencies of benzyl alcohol, ethyl alcohol and formate, solutions of thymidine-2-^{14}C (specific activity 18·3 mc./mmole) at 10 μc./ml. containing the scavengers in concentrations ranging from 0·001 per cent to 1 per cent were irradiated with γ-rays to a total dose of 2·5 megarads. The degree of protection, as measured by the amount of thymidine-2-^{14}C surviving (determined chromatographically)[9], is shown in Table 1.

Table 1. The Effect of γ-irradiation* on Solutions of Thymidine-2-^{14}C containing Free Radical Scavengers

Scavenger	Concentration (per cent)	Degree of protection
None (controls)	—	Nil
Benzyl alcohol	1	90–95 per cent
Benzyl alcohol	0·001	Approximately 50 per cent
Ethyl alcohol	0·001–1	Nil
Sodium formate	0·001–1	Nil

* For these irradiations I thank the Irradiation Service, U.K. Atomic Energy Authority, Harwell, Berkshire.

These results indicate that neither ethyl alcohol nor sodium formate was effective in protecting the thymidine-2-^{14}C and only benzyl alcohol at 1 per cent concentration afforded a high degree of protection. The results are interpreted to suggest that both ethyl alcohol and formate react rapidly[20,21] with the free radicals produced by the γ-rays and that these compounds did not survive long enough to protect the thymidine. Benzyl alcohol, on the other hand, containing the aromatic nucleus is much more resistant to destruction[15] and afforded a better protection for the thymidine-2-^{14}C. Provided sufficient ethyl alcohol or formate were present in solutions of tritiated compounds, these two scavengers, which readily interact with the free radicals, should provide a high degree of protection and substantially reduce the rate of decomposition of the tritiated compound.

In Table 2 are shown results obtained on keeping selected tritiated nucleosides and amino-acids of high specific activity in solutions containing benzyl alcohol, ethyl alcohol or sodium formate as radical scavengers.

The results show that all three scavengers are effective in reducing the rate of self-irradiation decomposition of the tritium compounds, but the efficiency of formate, even at concentrations of 0·01 per cent, is particularly interesting. Compared with the controls the rate of decomposition is reduced almost by an order of magnitude; thus the $G(-M)$ value for tyrosine, labelled in the 3 and 5 positions of the benzene nucleus at a specific activity of 48 c./mmole, is reduced from 0·27 in the controls to 0·03 when stored in 0·1 per cent sodium formate solution. For the nucleoside uridine labelled specifically in the 5-position of the pyrimidine moiety, the $G(-M)$ value in the controls (0·67) is reduced to 0·17 in solutions containing 0·01 per cent sodium formate. In the examples investigated, ethyl alcohol appears only slightly more effective than benzyl alcohol.

It should be remembered that these additives are chemical impurities. The value of additives as radical scavengers for retarding the rate of self-irradiation decomposition for tritium compounds in solution should be balanced against their possible effects in any investigation. Although the scavengers are present in low chemical concentrations, this will often exceed the concentration of the tritiated compounds, especially for those compounds at very high specific activity.

The use of additives (free radical scavengers) to control decomposition promises to be a most useful, practical and convenient method for minimizing the decomposition of tritium compounds stored in aqueous solution at high specific activity.

It is interesting to note also that L-tyrosine-3,5-T racemizes on storage in solution, even in the presence of formate as radical scavenger. This effect, which has been observed also with aqueous solutions of other tritiated amino-acids (L-leucine-4,5-T; L-phenylalanine-4-T; and L-methionine-T(G)), is under further investigation[17], but preliminary results suggest that this phenomenon may be induced by β-radiation.

Storage of Tritium Compounds at −196° C

Storage of aqueous solutions of tritiated thymidine at −196° C was first examined by Apelgot *et al.*[10]. Their results indicated that the rate of decomposition was substantially reduced at this very low temperature. The rates of decomposition of a number of tritiated compounds stored at −196° C have now been examined and the results are shown in Table 3.

Storage of freeze-dried tritium compounds tends to give rather erratic results, the rate of decomposition depending on how the material is spread on the surface of the glass tube. Note, for example, the difference between a 0·25 mc. (containing 18·5 μg) and a 1-mc. (containing 74 μg) ampoule of folic acid-3′,5′-T. Although the rate of decomposition of tritium compounds stored in the solid state is reduced at −196° C, it is still, nevertheless, substantial and in general the use of a protecting solvent is necessary[4].

The rate of decomposition of tritium compounds in aqueous solution increases as the dose per unit time is increased, that is, as the activity concentration per ml. is increased (at constant specific activity). It is interesting to note, however, that at −196° C the decomposition rate for tetrasodium 2-methyl-1,4-naphthaquinol-6-T diphosphate in solution at 27 c./mmole is remarkably constant over quite a wide range of activity concentrations (see Table 3).

At −196° C the proportion of free radicals which have sufficient energy for interaction with the solute is expected to be quite small[18] and it is, in fact, observed (Table 3) that the presence of benzyl alcohol as a free radical scavenger has a much less pronounced useful effect at this temperature. This suggests that secondary radiation effects[4], that is, the interaction of the tritiated compound with reactive species (such as free radicals), are almost eliminated at −196° C.

Table 2. Storage of Tritiated Compounds in Aqueous Solution in the Presence of Free Radical Scavengers

Compound	Specific activity (c./mmole)	Activity concn. (mc./ml.)	Scavenger	Age (months)	Storage temp. (°C)	Dose (rads)	$G(-M)$	Decomposition (per cent)
Cytidine-T(G)	2·5	1	None	8	2°	$7·2 \times 10^4$	0·3	5
Cytidine-T(G)	2·3	1·2	1% benzyl alcohol	9	2°	$9·7 \times 10^4$	0·12	2
Cytidine-5-T	9·4	1	None	10·5	2°	$9·5 \times 10^4$	0·24	20
Cytidine-5-T	14·6	1·2	1% benzyl alcohol	5	2°	$5·4 \times 10^4$	0·03	2
L-Histidine-2,5-T	6·5	1	None	3	2°	$2·7 \times 10^4$	1·3	50
L-Histidine-2,5-T	6·5	4	1% benzyl alcohol	4	2°	$1·4 \times 10^5$	0·4	10
DL-Leucine-4,5-T	10·9	1	None	8	2°	$7·2 \times 10^4$	0·38	30
DL-Leucine-4,5-T hydrochloride	10·9	6·4	None	12	2°	$7·0 \times 10^5$	0·1	10
DL-Leucine-4,5-T hydrochloride	10·9	6·4	1% benzyl alcohol	12	2°	$7·0 \times 10^5$	0·1	10
DL-Leucine-4,5-T hydrochloride	10·9	1	80% ethyl alcohol	12	2°	$1·1 \times 10^5$	0·02	2
DL-Leucine-4,5-T hydrochloride	10·9	1	80% ethyl alcohol	18	2°	$1·6 \times 10^5$	0·02	4
DL-Leucine-4,5-T hydrochloride	10·9	1	1% benzyl alcohol	12	2°	$1·1 \times 10^5$	0·02	2
L-Leucine-4,5-T	7·1	1	None	9	2°	$8·1 \times 10^4$	0·5	30
L-Leucine-4,5-T	8·2	2	1% benzyl alcohol	21	2°	$3·8 \times 10^5$	0·07	11
L-Tyrosine-3,5-T	48·0	1	None	3	2°	$2·7 \times 10^4$	0·27	35
L-Tyrosine-3,5-T	48·0	1	None	4·5	2°	$4·1 \times 10^4$	0·25	50
L-Tyrosine-3,5-T	48·0	1	1% benzyl alcohol	3	2°	$2·7 \times 10^4$	0·08	11
L-Tyrosine-3,5-T	48·0	1	0·1% ethyl alcohol	3	2°	$2·7 \times 10^4$	0·07	9
L-Tyrosine-3,5-T	48·0	1	0·1% sodium formate	3	2°	$2·7 \times 10^4$	0·03	4
L-Tyrosine-3,5-T	48·0	1	0·1% sodium formate	4·5	2°	$4·1 \times 10^4$	0·02	4
Uridine-5-T	22·2	1	None	4·5	2°	$4·1 \times 10^4$	0·56	50
Uridine-5-T	22·2	4·1	None	4·5	2°	$1·7 \times 10^5$	0·67	60
Uridine-5-T	22·2	4·1	0·1% ethyl alcohol	4·5	2°	$1·7 \times 10^5$	0·33	30
Uridine-5-T	22·2	4·1	0·01% sodium formate	4·5	2°	$1·7 \times 10^5$	0·17	15
Uridine-5-T	11·1	6·1	None	9	2°	$4·9 \times 10^5$	0·3	25
Uridine-5-T	11·1	6·1	1% benzyl alcohol	2	2°	$1·1 \times 10^5$	0·3	6
Uridine-T(G)	2·44	2	None	8	2°	$1·4 \times 10^5$	2·0	35
Uridine-T(G)	2·44	2	1% benzyl alcohol	8	2°	$1·4 \times 10^5$	0·3	5
Uridine-T(G)	2·44	2	None	13	20°	$2·3 \times 10^5$	1·2	30
Uridine-T(G)	2·44	2	1% benzyl alcohol	13	20°	$2·3 \times 10^5$	0·3	8
Uridine-T(G)	2·44	6·9	None	13	20°	$8·1 \times 10^5$	1·3	35
Uridine-T(G)	2·44	6·9	1% benzyl alcohol	13	20°	$8·1 \times 10^5$	0·6	17

Table 3. Decomposition of Tritium Compounds at −196° C*

Compound	Specific activity (mc./mmole)	Solvent	Activity concn. (mc./ml.)	Age (months)	Temp. (° C)	Dose (rads)	$G(-M)$	Decomposition (per cent)
Benzyl-T penicillin (potassium salt)	112	None	—	9	−40°	$2·4 \times 10^7$	22	20
Benzyl-T penicillin (potassium salt)	128	None	—	17	−196°	$4·0 \times 10^7$	17	25
Benzyl-T penicillin (potassium salt)	128	Water	2	6	−196°	$1·1 \times 10^5$	9	6
Folic acid-3′,5′-T (potassium salt)	5,420	None	—	2	−40°	$2·2 \times 10^8$	1	10
Folic acid-3′,5′-T (potassium salt)	5,420	None	(0·25 mc. ampoule)	5	−196°	$5·5 \times 10^8$	0·4	10
Folic acid-3′,5′-T (potassium salt)	5,420	None	(1 mc. ampoule)	5	−196°	$5·5 \times 10^8$	0·8	20
L-Histidine-2,5-T	1,000	None	—	7	−40°	$3·9 \times 10^8$	1·6	10
L-Histidine-2,5-T	1,000	None	—	2·5	−196°	$1·4 \times 10^8$	0·9	2
L-Leucine-4,5-T	7,100	Water	1	6	+2°	$5·4 \times 10^4$	0·5	20
L-Leucine-4,5-T	7,100	Water	1	6	−196°	$5·4 \times 10^4$	0·1	5
L-Leucine-4,5-T	7,600	Water	4·8	5·5	−196°	$2·4 \times 10^5$	0·2	10
Methotrexate-3′,5′-T	9,170	None	—	2	−40°	$3·6 \times 10^8$	0·4	6
Methotrexate-3′,5′-T	9,170	None	—	4	−196°	$7·0 \times 10^8$	0·15	5
Tetrasodium 2-methyl-1,4-naphthaquinol-6-T diphosphate	27,000	Water	390	10	+2°	$3·5 \times 10^7$	0·3	75
	27,000	Water	240	6	−196°	$1·3 \times 10^7$	0·06	10
	27,000	Water	2	6	−196°	$1·1 \times 10^5$	0·06	10
Thymidine-methyl-T	4,200	Water	0·4	22	+2°	$7·9 \times 10^4$	0·5	40
Thymidine-methyl-T	4,200	Water	1·3	7	−196°	$8·2 \times 10^4$	0·04	2
Uridine-5-T	11,100	Water	6·1	9	+2°	$4·9 \times 10^5$	0·3	25
Uridine-5-T	13,000	Water	5	6	−196°	$2·7 \times 10^5$	0·07	5
Uridine-T(G)	2,440	None	—	7	0°	$7·2 \times 10^8$	2	30
Uridine-T(G)	2,440	None	—	9	−196°	$8·1 \times 10^8$	0·4	11
Uridine-T(G)	2,440	Water	2	8	+2°	$1·4 \times 10^5$	2	35
Uridine-T(G)	2,440	Water	2	9	−196°	$1·6 \times 10^5$	0·15	3
Uridine-T(G)	2,440	Water†	2	9	−196°	$1·6 \times 10^5$	0·26	5
Uridine-T(G)	2,440	Water	6·9	9	−196°	$5·6 \times 10^5$	0·4	8
Uridine-T(G)	2,440	Water†	6·9	9	−196°	$5·6 \times 10^5$	0·26	5

* Values at other temperatures are included for comparison.
† Containing 1 per cent benzyl alcohol.
G equals generally labelled in non-labile positions (ref. 19). The $G(-M)$ values recorded in Tables 2 and 3 are the number of molecules of the tritiated compound destroyed per 100 eV of absorbed energy.

It appears, therefore, that many tritium compounds, preferably dispersed in a solvent, have a much reduced rate of decomposition when stored at −196° C. It is perhaps only inconvenience which prevents a wider use of storage at −196° C.

There is obviously much more information to be obtained concerning even the two aspects of tritium compound decomposition briefly described in this article. Experiments are continuing at the Radiochemical Centre.

I thank Dr. J. R. Catch for his interest, and other colleagues for their assistance.

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AN ALTERNATIVE MULTI-TARGET EQUATION USEFUL FOR FITTING SURVIVAL CURVES OF X-RAY IRRADIATED PROTOZOA

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SYSTEMATIC attempts to explain the behaviour of irradiated biological materials have been collected into a group of concepts generally termed 'target theory'. Target theory has undergone constant modification, especially in its objectives; and even though there are objections to the implications of this approach it still provides a logical method and useful vocabulary for a systematic and quantitative investigation of radiation effects. Target theory has been most extensively developed for biological materials which show an exponential decay of activity with dose; this response has been called 'single hit' and implies that some sort of an all-or-nothing event has occurred which either inactivates the material or leaves it completely unharmed. Many biological materials do not show the 'single hit' type of inactivation, but show curves of dose versus activity which have shoulders indicating some damage must accumulate before the full inactivation is expressed; such a response curve is called sigmoidal.

The equation:

$$S = 1 - (1 - e^{-Kx})^N \qquad (1)$$

where S is the surviving fraction, x is the X-ray dose, and K and N are experimentally determined parameters, frequently called the multi-target equation of target theory, has been used as the mathematical model for the sigmoidal type of inactivation of micro-organisms and single cells[1]. This equation can be derived from two assumptions: (1) the inactivation of the targets is a 'single hit' type event; (2) there are N targets each of which must be inactivated to kill the organism. The parameter K is related to the sensitivity of the individual target, being the reciprocal of the mean lethal dose (37 per cent survival dose) for the targets considered individually.

Plots of the observed surviving fraction of various irradiated unicellular biological materials against X-ray dose have almost invariably produced dose–response (survival curves) of the sigmoidal type. In the case of single mammalian cells the observed survival seems to fail to fit the multi-target equation for several reasons. There appears to be a substantial part of the cell population inactivated by the 'single hit' mechanism, that is, N equals 1 for part but not all the population[2]. The 'single hit' component causes the survival at low doses to deviate from values of N and K determined at high doses, and the survival line shows a finite slope as it approaches zero dose instead of the zero slope predicted by the multi-target equation.

The growth phase of cells has also been shown to affect the response of irradiated cells in the cases where it has been investigated[3,4]. It has been shown that a non-homogeneous population could produce quite complex survival curves, depending on the composition of the population and the manner in which the dose–response curve varied in the various subcomponents of the population. When a population of mammalian cells, synchronized with respect to phase in the growth-cycle, was investigated for radiation response, it was found that the response at lower doses seemed sigmoidal with a slope depending on phase in the growth-cycle, but at higher doses a steeper decline of survival might occur.

Survival curves of Protozoa do not show the same deviations from the simple multi-target behaviour that mammalian cells show[5]. If there is a component of the population which is inactivated by 'single hit' kinetics, which would cause the survival curve to approach zero dose with a finite slope rather than zero slope, it must be an exceedingly small fraction. In the case of Protozoa the surviving fraction is 1·0 until quite large doses have been delivered, then a small increase in dose produces a large drop in survival.

The problems originating in the variation in sensitivity during the growth-cycle also seem to be minimal in Protozoa. The sharpness of the threshold would be impossible if there were large differences in the radiation response during the growth-cycle. In the case of *Paramecium aurelia* it has been shown, using animals synchronized by selection in the process of division, that the variations in radiation sensitivity during the growth-cycle are much less than the corresponding variations in mammalian cells, and the data are compatible with the hypothesis that there is no variation in radiation sensitivity during the growth-cycle[6].

Table 1

Eq. No.	Exp. No.	LD_{50}	N	K	χ^2	DF
1	26	6·43	275	0·93	7·33	4
2	26	6·56	9·12	0·06	4·83	4
1	27	7·66	255	0·77	5·35	4
2	27	7·7	27·9	0·06	3·96	4
1	28	6·97	1,544	1·1	2·98	4
2	28	7·1	56·6	0·09	1·62	4
1	29	Computer programme failed to converge				
2	29	6·65	126	0·09	1·59	3
1	30	6·34	10·7	0·44	1·01	4
2	30	6·65	1·83	0·03	0·88	4
1	31	6·79	565	0·99	1·88	4
2	31	6·87	15·9	0·07	1·62	4
1	32	6·19	2,035	1·29	1·31	3
2	32	6·26	67·02	0·12	1·14	3

Survival curve parameters as determined by the two equations. The dose units for the LD_{50} and parameter K are expressed in minutes at 45 krads/min.

Fig. 1 (dotted curve) and Table 1 present experimental data from a number of X-ray survival experiments on *Paramecium aurelia* and illustrate the ability of equation (1) to serve as a model for the inactivation of this protozoan. The data have been fitted using an electronic computer, IBM type 7040; the programme is an iterative type kindly prepared by Dr. Alan Ross and Mr. Stuart Riley of this institution. The values of χ^2 in the separate cases suggest that, within the limitations of the experimental data, equation (1) closely approximates the functional dose–response relation.

There are, however, reasons for seeking a different mathematical model to describe the dose–response relation for X-ray irradiated *Paramecium aurelia*. There is no easy technique for determining the survival of *Paramecium* at extremely low levels as there are for bacteria or tissue culture cells, so survival curves are seldom extended to surviving fractions below 10^{-3}. However, theoretical curves, computed from equation (1), best fitting the data at higher levels of survival, consistently overestimate the survival at the highest dose points instead of a random distribution about observed points at the higher dose-levels. Another reason for further consideration of the model equation is the extremely high values of N. It is difficult to imagine a cellular structure duplicated

thousands or tens of thousands of times, which, if only one N is undamaged, will permit the survival of the animal. The theoretical curves could be made to fit the points of low survival better by increasing the parameter N, but the foregoing objection would be intensified.

Neither objection can be regarded as sufficient reason to discard a model which fits the experimental data to the degree indicated by the χ^2 values; however, a slight modification of equation (1) produces an improved fit of the experimental data and considerably mitigates the two objections already cited.

Equation (1) has been modified in such a manner that the dose appears raised to the second power:

$$S = 1 - (1 - e^{-Kx^2})^N \qquad (2)$$

The symbols have the same meaning as before.

Table 1 illustrates the fact that equation (2) gives an improved fit to the data points and Fig. 1 shows that, although there is still a tendency to overestimate the survival at higher doses, equation (2) is close to the observed values. Table 1 also shows that the parameter N, although still a substantial number, is much less than the value found using equation (1). *Paramecium*, it should be noted, has a complex nuclear organization and a particular bit of genetic information might be duplicated up to 100 times.

Calkins has already reported[7] and Figs. 2 and 3 show another X-ray response of *Paramecium aurelia* in which the functional relation between exposure and response is of such a nature that it is the square of the dose which is significant. Fig. 2 shows the average growth of *Paramecium* following irradiation; since non-surviving animals die without division, the growth is determined entirely by survivors of the various doses. It is evident that there is a progressive depression of the resumption of growth by the increasing radiation doses. An electronic computer programme has been used to determine the delay of growth of the isolated irradiated individuals. Fig. 3 shows the average delay of growth of animals given various irradiation doses and the standard error of this delay plotted as a function of the square of the X-ray dose. The dose-squared dependence shown in Fig. 3 has been repeated in many other experiments.

The dependence of an effect on the square of the absorbed dose could originate in many different ways. One manner of producing such a functional relation could be an interaction between two radiation-produced events which are themselves linearly dependent on dose; the dose-squared relation between effect and dose of ionizing radiation is well known in certain types of chromosome aberrations and seems logically explained by the interaction of effects which show a linear dependence on dose[8].

If the correct functional relations between dose and response can be established for this animal then they will undoubtedly suggest further avenues of investigation in the effort to understand the complex and often bewildering manner in which irradiated organisms behave.

This work was supported by National Institutes of Health, U.S. Public Health Service grant *GM* 12030-01. I thank Dr. Alan Ross for the extensive development of computer programmes required for the analysis of my data, and his assistant Mr. Stuart Riley. I also thank Yvonne Gover for her assistance.

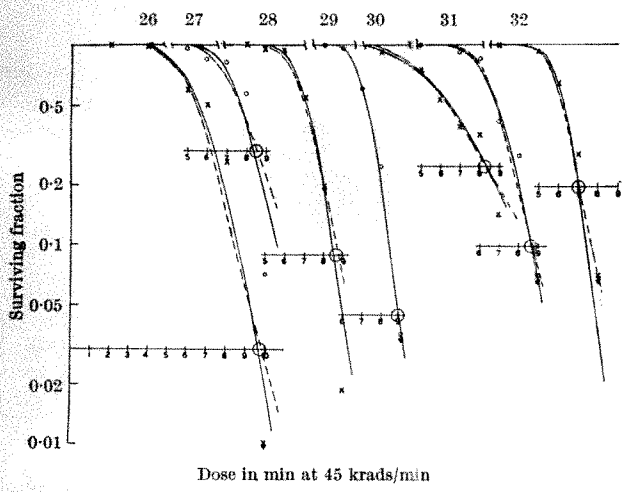


Fig. 1. Computer fit of observed survival of X-ray irradiated *Paramecium aurelia*. Dotted lines indicate fit by usual multitarget equation (equation 1); solid lines are fitted to the data points by the multitarget equation with dose-squared term (equation 2). Downward-pointing arrows indicate no animals survived in a group which would have produced a surviving fraction at the position of × or 0 if one animal survived

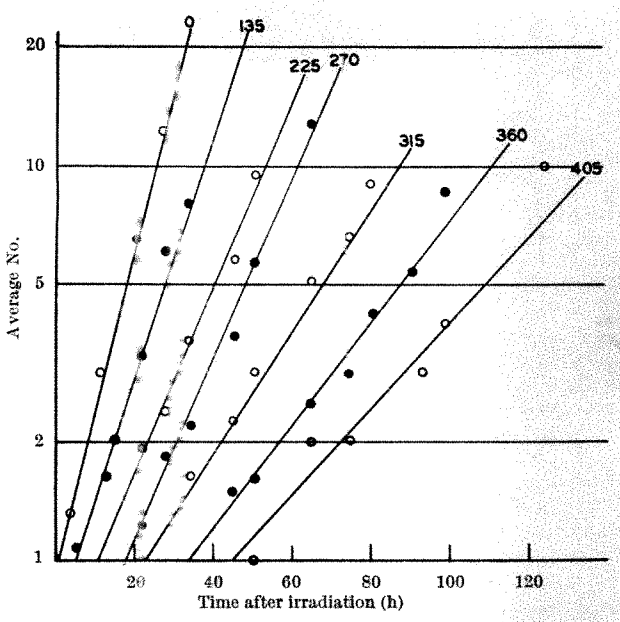


Fig. 2. A plot of the average post-irradiation growth of *Paramecium aurelia*. Lines are fitted to the data points by computer; the number at the top of each line indicates the absorbed dose in krads given the groups of irradiated animals

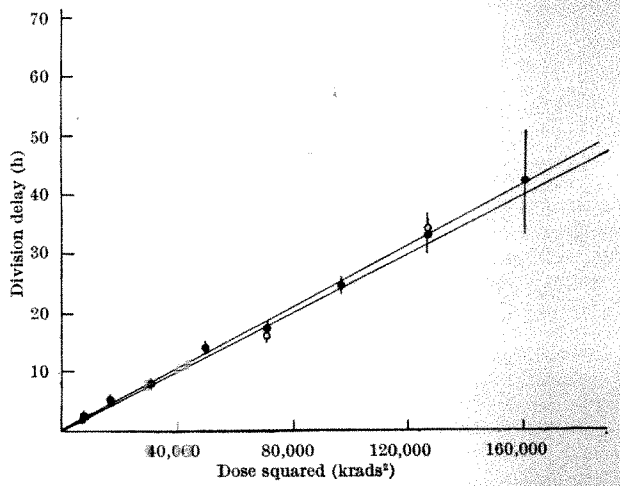


Fig. 3. The average delay of resumption of growth (division delay) of surviving X-ray irradiated *Paramecium aurelia* plotted as a function of X-ray dose squared. Bars indicate the standard error of the division delay points; lines are fitted to data points by computer

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NEAR ULTRA-VIOLET SPECTRUM OF WHITE POTATO MITOCHONDRIA

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CHANCE *et al.*[1,2] and Holton[3] observed a broad absorption peak at 315–320 mμ in the difference spectrum of animal mitochondria. This peak has been attributed to bound pyridine nucleotide[1,3] or to a modified form of ubiquinone[2]. During investigations of plant mitochondria, the presence of a similar peak has been detected in mitochondria isolated from white potato tuber, Jerusalem artichoke tuber and mung bean hypocotyls. This article concerns the characterization of this absorption peak in white potato mitochondria.

Mitochondria were prepared as described[4] and tested in a reaction medium consisting of 0·3 M mannitol, 10 mM phosphate buffer (*p*H 7·2), 10 mM potassium chloride and 5 mM magnesium chloride. All experiments described herein were performed with succinate as substrate.

In a difference spectrum of these mitochondria, anaerobic minus aerobic steady states (Fig. 1), an absorption peak is found at 315 mμ, and a shoulder at 340 mμ. The shoulder can be attributed to intramitochondrial pyridine nucleotide; extraction of these mitochondria showed a total pyridine nucleotide content (NAD + NADH) of 5·0 mμmoles/mg mitochondrial nitrogen. In agreement with published reports[5] neither NADP nor NADPH was present in detectable amounts.

Fig. 2 shows a series of experiments carried out to examine the participation of the 315 mμ compound in oxidative phosphorylation. Fig. 2*A* shows the pattern of oxygen uptake detected polarographically. The respiration is stimulated by ADP (state 3) and the rate decreased on exhaustion of added ADP (state 4), showing that the mitochondria have respiratory control[6]. Fig. 2*B* is a fluorescence trace obtained with an Eppendorf fluorometer[7]. Fluorescence is low during state 3. It increases in state 4 and decreases at anaerobiosis (state 5), suggesting that some quenching occurs as soon as anaerobiosis sets in. Figs. 2*C–E* illustrate typical traces obtained with an ultra-violet double-beam spectrophotometer[8] using 305, 320 and 350 mμ as measuring beams with 375 as reference wave-length. In later experiments 300 was used in preference to 305 as a measuring beam. The absorption changes at 350 are typical of energy-linked pyridine nucleotide reduction[9,10], while at 305 mμ the absorption gradually increases on addition of ADP, and on exhaustion of added ADP a first-order decrease of absorption occurs. It should be noted that no steady state was observed during state 3 or 4 or this wave-length. On anaerobiosis, however, the absorption increases to a steady level. The optical changes at 320 mμ are clearly intermediate between those at 305 and 350 mμ.

The results with the double-beam spectrophotometer suggested that it was possible to separate and define more clearly the two components suggested in Fig. 1. Measurements were therefore made over the 290–360 mμ range of wave-length. The degrees of optical changes after ADP addition were determined with reference to the time of ADP addition. At shorter wave-lengths this was determined by extrapolation. Fig. 3 shows the results where the rate of absorption increase immediately after ADP addition is also plotted. The spectrum for anaerobiosis is essentially the same as that shown in Fig. 1. While the 340 mμ absorbing compound is distinguished in the spectrum for state 4 (curve *B*), the 300 mμ species is clearly separated both in the spectrum for the rate of absorption increase and in the plot for the time of ADP exhaustion (curve *A*). These results demonstrate that the 315 mμ peak and the 340 mμ shoulder observed in Fig. 1 are resolved into 300 and 340 mμ absorbing components. The selection of 300 and 350 mμ in Fig. 2 was made to minimize overlap of the two components. In view of the evidence presented, the 315 mμ peak in Fig. 1 can be attributed to the simultaneous absorption of 300 and 340 mμ components at anaerobiosis.

In view of the fact that ADP increases absorption of the 300 mμ component and that the absorption decreases on

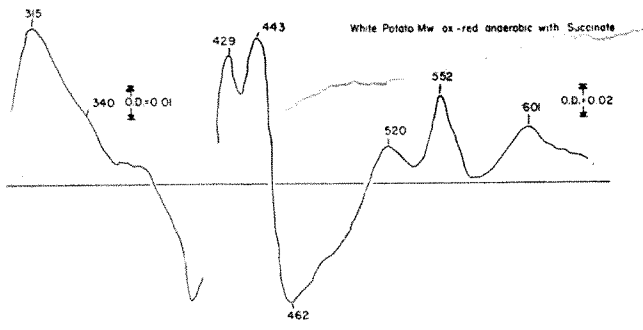


Fig. 1. Difference spectra, anaerobic steady state minus aerobic steady state

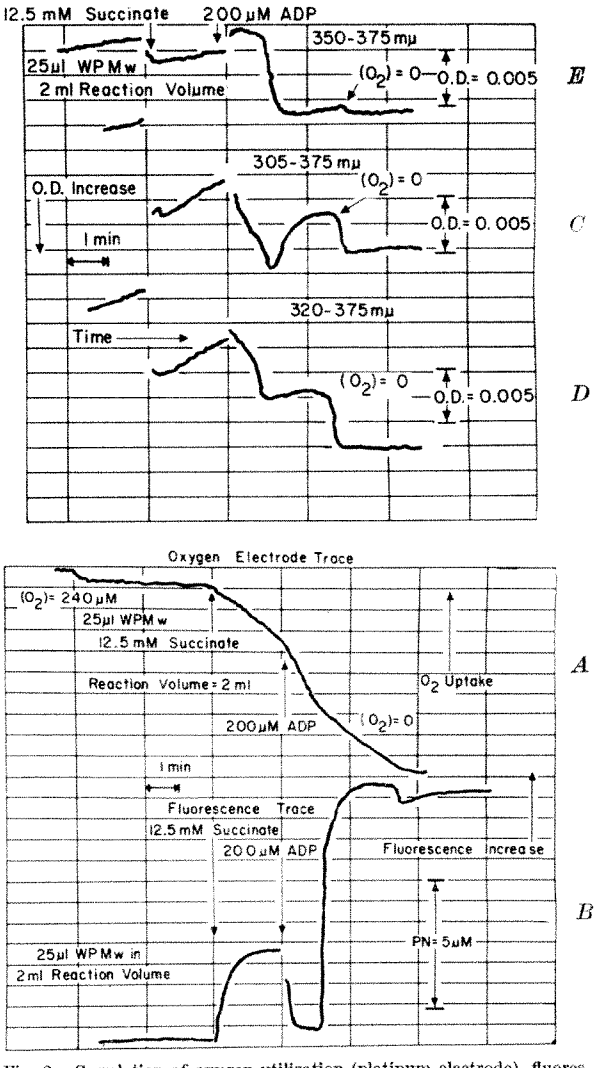


Fig. 2. Correlation of oxygen utilization (platinum electrode), fluorescence, and ultra-violet absorbing species in oxidative phosphorylation

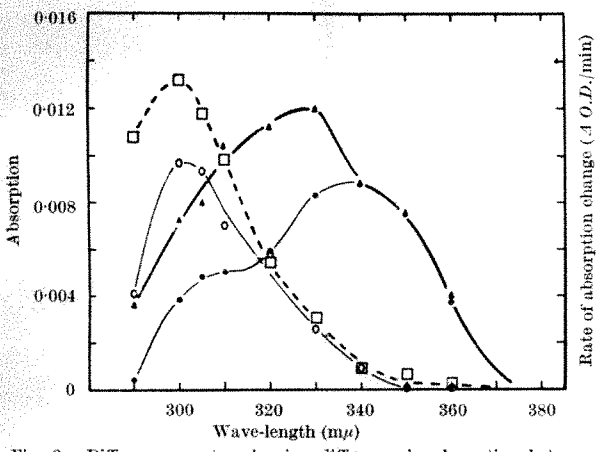


Fig. 3. Difference spectra showing difference in absorption between immediately after ADP addition and (*A*,—○—) at the end of state 3, (*B*,—●—) at the end of first-order change, or (*C*,—▲—) at anaerobiosis. (*D*,- -□- -), the rate of absorption change after ADP addition. Timing and addition sequence were the same as in Fig. 2 for all the runs

ADP exhaustion, two questions arise: (*a*) Does the ATP formed during state 3 inhibit the increase in absorption at 300 mμ ? (*b*) Does the presence of ADP inhibit the first-order decrease of absorption ? Fig. 4 shows the results of an experiment designed to answer these questions. Treatment of the mitochondria with ATP brings about the first-order decrease of absorption after succinate addition, and the kinetics of increase and subsequent decrease of absorption on ADP addition is essentially the same in the presence of ATP as in the absence of added ATP. A further examination of the questions was made by adding excess ADP to bring state 5 directly from state 3. In the presence of excess ADP, the first-order decrease does not occur while the increase in absorption is not affected. One may conclude, therefore, that the ATP formed in state 3 does not interfere with the appearance of the 300-mμ component and both the exhaustion of ADP and the presence of ATP are the necessary conditions to bring about the first-order change of absorption. Fig. 4*C* points out, furthermore, that the ATP-dependent decrease of absorption is indeed an oxygen-dependent process. This latter point is confirmed by the observation that addition of ATP in state 5 did not cause any first-order change.

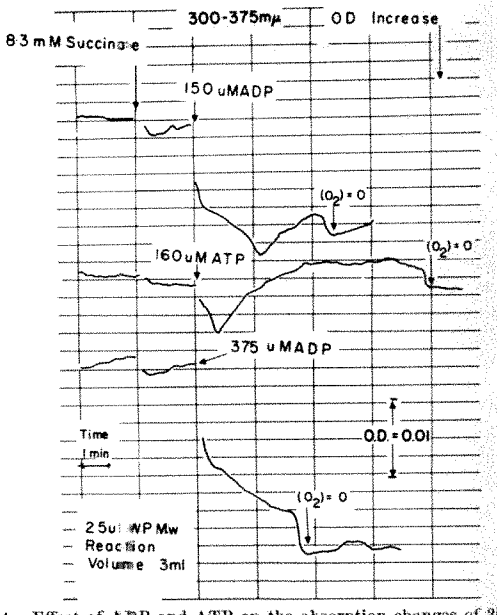


Fig. 4. Effect of ADP and ATP on the absorption changes of 300 mμ compound. Measurements taken with ultra-violet double-beam spectrophotometer. *O.D.* increase, downward deflexion

In conclusion, the 300 component behaves in a manner similar to a respiratory pigment; the response to ADP and oxygen suggest that it is involved in oxidative phosphorylation. The kinetics of oxidation and reduction of the 300 mμ component distinguish it from pyridine nucleotide and ubiquinone. The exact nature of this component is at present not known.

This work was supported by research grants from the National Science Foundation.

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REFLECTIVITY OF ATMOSPHERIC SHOCK WAVES

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A SHOCK front is a thin layer which separates two regions of different density in the propagating medium. Light incident on such a region will be partially reflected in accordance with Fresnel theory: the percentage reflected is related to the change in density and to the thickness of the transition region. The reflectivity of shock fronts has been studied theoretically by Cowan and Hornig[1], who also made experimental measurements which substantiated the theory. The theory predicts reflectivities of the order of 10^{-7} with shock waves of mach 5 or greater propagating in gases at normal temperature and pressure. For lower mach numbers the thickness of shock front becomes of the order of the optical wave-length and the reflexion coefficient falls exponentially. The reflexion is specular.

During laser ranging experiments at our laboratory, reflexions have been observed from locally turbulent regions in the atmosphere. It has been shown that Fresnel reflexions are not large enough to explain such observations[2]. Since it was very unlikely that the density variations in the turbulence observed were either as abrupt or as large as those which would be found at a shock front, it was felt that the reflectivity of atmospheric shock fronts might be higher than predicted by the theory. Therefore, tests on a shock wave propagating in the atmosphere were made. The tests yielded surprisingly high values of reflectivity, of the order of 5×10^{-3}, from the region of the shock front.

Experimental Determination of the Reflectivity of a Shock Front

The method used in the experiment was to measure with a laser range-finder[3] the range first to an explosive charge and second to a shock front propagating from the charge, while simultaneously monitoring both the transmitted energy and the reflected energy on a wide-bandwidth dual-beam oscilloscope. In the second measurement, the firing of the laser range-finder was delayed until after the detonation in order to allow the shock front time to propagate ahead of the fireball produced by the

explosion. High-speed shadowgraphic cinephotographs[4] were taken of the explosion to confirm that the reflexions measured were from the shock front region, and not from the fireball or extraneous matter present.

A photograph of the laser range-finder is shown in Fig. 1. The transmitter is a *Q*-switched ruby laser with an output wavelength of 6943 Å, a peak power of about 5×10^5 W, an output beam divergence angle of about 0·5 milliradian, and a pulse length of 30 nsec. The ranging accuracy is ±1 ft. To discriminate against the noise from the fireball and/or shock wave, a tuned multiple dielectric filter having a full-width-at-half-power of 5 Å and a peak transmission of 0·4 was used in the receiving optics. The receiver was gated by an S.T.C. circuit so that the receiver did not saturate from the optical noise power transmitted through the filter.

The explosive was suspended at a height of 10–15 ft. above ground. The detonator cap and all lines and cables were placed in such a position that none of them was blown forward by the explosion. The charge was 1–10 pounds of composition *C*, which yielded shock waves in the range of mach 1·25 to mach 1·6. The separation of the laser range-finder and the suspended charge was about 470 ft.

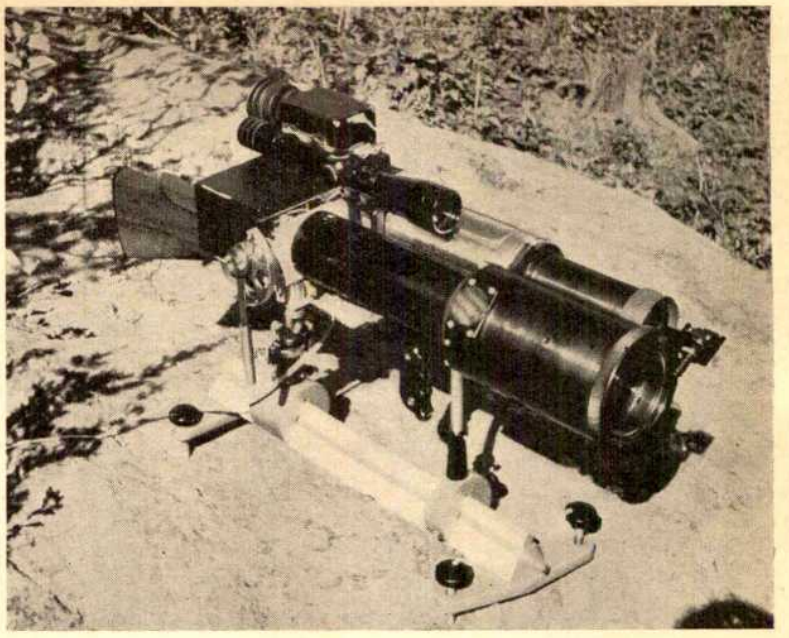


Fig. 1. Laser ranger

The experimental procedure begins with the start of the drive mechanism of the 'Fastax' camera. After the camera drive attains the proper speed, the charge is detonated and, after a delay of 1–4 msec, the laser range-finder is fired. Timing signals are recorded on one channel of the oscilloscope at the time the charge detonates and also when the laser fires. The detonation signals are obtained by means of a piezoelectric barium titanate disk buried in the charge. The laser transmitter emits a single pulse toward the approaching shock wave. A fixed fraction of the energy in the transmitted pulse is coupled directly into the receiver. This pulse starts the range counter and the second oscilloscope trace. When a reflected signal is received the counter is stopped. In addition, the output from the receiver is displayed on the second trace of the oscilloscope. The oscilloscope display generated prior to detonation is generated in exactly the same manner, except that the detonator is disconnected and the reflexion occurs from the suspended charge.

The range counter on the laser range-finder was used to determine differences in the range—this counter having a resolution of 1 ft. The scope traces were used to estimate the reflectivity of the shock front. The reflectivity values were estimated by comparing the signal reflected by the shock front with that reflected from the suspended charge prior to detonating. To make certain that variation in output power of the laser did not affect the results, both reflected signals were normalized by sampling the transmitted beam. It was necessary to estimate in some cases what portion of the laser beam was intercepted by the charge. This estimate was rather rough (within a factor of 2 or 3), so the values of reflectivity should only be treated as correct in order of magnitude.

Fig. 2 shows a portion of a typical film from the 'Fastax' camera. The frame rate was 3,750 frames per sec. The flash in the second frame indicates initiation of the explosion (the figure is a negative, so the flash appears as a darkening). The oscilloscope data give the delay times between detonation and ranging with the laser range-finder. It can, therefore, be determined which frame of the film (±1 frame) in Fig. 2 shows the positions of the shock front and fireball at the time of ranging.

In Fig. 2, the lower series of frames is a continuation of the upper with time increasing from left to right in both cases. The dark rectangular area is a 'Scotchlight' screen[4] mounted opposite the camera. The shock wave appears in Fig. 2 as a thin bright band travelling across the screen. In the frame at the left end in the lower series, the shock front is a horizontal line at the centre of the screen in line with the longest marker (a white strip also). In the following frame the shock front has moved upward a short distance. (The upward direction in Fig. 2 corresponds to the direction toward the laser range-finder.) The fireball appears in Fig. 2 as a dark mass at the bottom of each frame. The separation (along the laser line-of-sight) of suspended charge, shock front, and the foremost edge of the fireball could be determined by projecting distances scaled from Fig. 2 on to the laser line of sight.

Complementary data from the range counter, the oscilloscope record, and the high-speed film are available from four shots. These data are shown in Table 1. It should be noted that the separation between the shock front and the fireball was between 2·5 ft. and 5·5 ft. These distances are sufficient to ensure that the ranges obtained were actually occurring from the shock front and not from the fireball or from solid matter.

Assuming the explosive to have a (diffuse) reflectivity of 0·1, values of (diffuse) reflectivity for the shock front were calculated. The values obtained varied between 0·003 and 0·01 with a mean of 0·0065.

On one shot the laser was aimed below the charge during the explosion so that the laser beam was incident at an angle of about 0·3 radians—much greater than the transmitter beam divergence of about 0·5 milliradians. Within the limited accuracy of the reflectivity estimates, there is no reduction in reflectivity for this shot. Thus, the reflecting mechanism is not specular.

The experiment demonstrates that optical reflexions from shock fronts produced by explosions in the atmo-

Table 1

Shot No.	Range counter difference: charge to shock front (ft.)	Film and scope data: Charge to fireball edge (ft.)	Film and scope data: Charge to shock front (ft.)
1	7	3	5·5
2	8	4	9·5
3	9	5·5	10·7
4	9	7·5	10·5

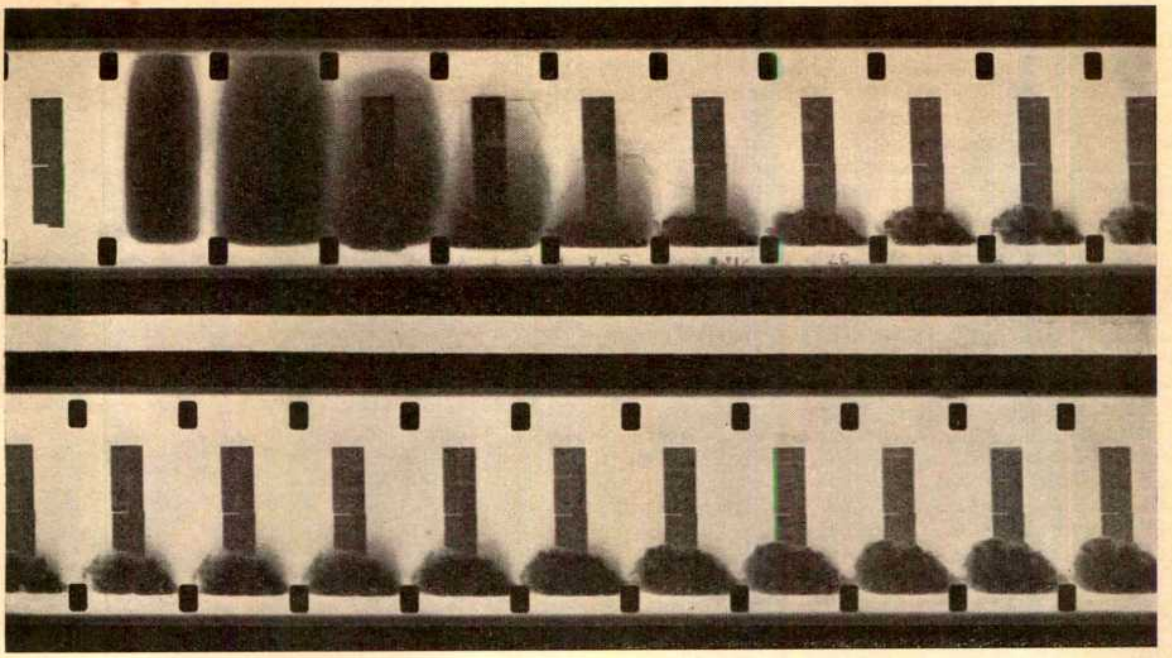


Fig. 2. Film strip from 'Fastax' photographs of the explosion

sphere can occur which are much larger than would be expected on the basis of Fresnel reflexions. The reflexions also appear to be non-specular.

The most likely explanation for the anomalously high reflectivity observed is that water droplets are condensed. This is suggested by the fact that previous results with (presumably) dry gases in shock tubes were adequately explained as Fresnel reflexions. The mechanism for any such condensation is not obvious. Other possibilities are: (1) that particles are swept along with the shock front, (2) that reflexions occur from an ionized plasma. The fact that the light source used in the experiment is much more coherent than those used in previous work may also need to be considered.

Investigations are being carried out to decide which, if any, of these explanations is the correct one.

This work was supported by a United States Air Force contract *AF*08(606)–6426. I thank I. N. Court and T. L. Sly for valuable assistance.

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BORON CONTENT IN RELATION TO ORGANIC CARBON AND TO PALAEOSALINITY IN CERTAIN BRITISH UPPER CARBONIFEROUS SEDIMENTS

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In sedimentary rocks the presence of fossil species of groups of life now characteristic of exclusively marine environments, such as brachiopods, cephalopods and certain families of lamellibranchs, indicate past marine conditions. Furthermore we have good reason, at least by Carboniferous times, to postulate that salinities were no different from those of seas to-day[1]. In the Upper Carboniferous, members of some other fossil groups, on the evidence of their association and relative position in short sequences of changing facies, appear to have ranged from marine to brackish water. Yet others, by the same kind of criteria, evidently characterized brackish water environments and may have ranged into fresh water[2]. The latter include the 'mussels' of the Coal Measures, lamellibranchs of the extinct Family Anthracosiidae and some members of the Myalinidae. These lamellibranchs, which have left prolific accumulations of shells at many horizons, have been cautiously described as non-marine. We can be sure only of the fact that palaeosalinities were in general lower in Carboniferous times during the prevalence of non-marine conditions, as is evidenced by life assemblages of 'mussels', than during the deposition of bands with marine fossils.

In attempts to find other independent criteria to differentiate marine sediments of all ages from those of lower salinity, including fresh water, much recent work has been directed towards the quantitative determination of minor elements, especially boron[3]. The use of total boron content and boron in illite[4] as indicators of palaeosalinity in British Upper Carboniferous rocks has been questioned independently by both of us[5,6]. For example, from an investigation of a sequence of fine-grained shales and shaly mudstones with non-marine faunas in the Lower Coal Measures of Worsborough, Yorkshire, Eagar concluded that evidence from total boron contents of 21 samples was at variance with all other evidence regarding palaeosalinity. In this investigation, however, the most striking relationship appeared to be an antipathetic one between total boron and organic carbon, the latter representing original organic matter in the sediments. The relation-

ship, it was concluded, might be direct or indirect. The possibility of there being a direct relationship between these elements was later noted by Curtis[7,8] from work on a slightly higher horizon in Yorkshire. Curtis directed attention to Bader's demonstration[9] that certain clay minerals, including illite, will quickly and strongly absorb up to 350 per cent of their own mass of soluble organic matter from solution; he pointed out that this same mechanism could inhibit boron sorption in the natural environment.

The purpose of this article is to supplement published data from the Worsborough section with checks on total boron contents and with determinations of the potassium contents of the samples, work carried out by Spears. These results are presented in order to investigate the nature of the carbon–boron relationship and its bearing on boron as a supposed index of palaeosalinity, not only at Worsborough but also in certain other Carboniferous successions.

Boron contents of the samples in the Worsborough section were originally determined by a microchemical technique in the laboratories of the North Rhine–Westphalian Geological Survey of Krefeld[10]. Ernst later pointed out that these figures were too high as a result of an error in standardization later discovered[11], but that the carbon–boron relationship was not affected by the revised figures[12]. A plot of the spectrographic results gives essentially the same graph as that produced by Eagar[5] from microchemical results. There is one difference, however, in that all organic carbon peaks now correspond precisely with boron troughs (Table 1), whereas in the first plot, when microchemical determinations were used, it was noted that in the case of three organic carbon peaks out of six the fall in boron slightly preceded the rise in organic carbon.

In the earlier work on Worsborough no potassium figures were available. The new analytical data have been plotted on Fig. 1, from which it will be seen that although samples with a high content of organic carbon have low boron values, potassium figures are also much lowered. The correlation between boron and potassium is good ($r = +0{\cdot}93$). The best fitted line, however, is the quadratic curve $y = 12{\cdot}71x - 5{\cdot}65x^2 - 2{\cdot}56$, where x represents boron in p.p.m. and y is the percentage of potassium oxide. The expression approximates to a straight line for the greater part of the recorded data but gives proportionately higher boron values when potassium oxide is very low and very high. There is some scatter of points about the lines on Fig. 1, which must be regarded as inevitable, but it is more convenient to discuss its possible causes later in the article when Fig. 3 is described.

Table 1

	Height above the coal ft.	in.	Organic carbon	Sulphur	Free SiO_2	K_2O	B in p.p.m.*	$B/K_2O \times 10^3$
(7)	61	6	19·45	0·62	38·55	1·93	46	2·38
	37	0	0·62	0·04	18·69	4·58	120	2·62
(6)	36	6	12·42	0·79	13·13	2·65	59	2·23
	36	0	1·65	0·10	16·45	4·16	81	1·95
	31	9	0·69	0·03	11·03	2·70	86	3·18
(5)	30	0	5·32	0·44	17·20	2·93	62	2·11
	29	6	1·01	0·04	17·78	4·53	92	2·03
	28	3	1·31	0·04	16·72	4·69	113	2·41
	26	0	1·85	0·14	20·00	4·30	93	2·16
(4)	25	6	21·32	0·95	6·20	1·36	34	2·50
	24	3	1·10	0·09	17·28	4·73	105	2·22
(3)	23	9	3·37	0·40	16·80	4·31	88	2·04
	23	6	1·49	0·45	18·10	4·54	115	2·53
	21	9	0·71	Nil	16·47	4·75	103	2·17
(2)	21	0	4·60	0·60	15·89	2·96	54	1·83
	20	3	0·94	0·12	20·98	4·46	94	2·11
	18	9	0·78	0·13	24·10	3·99	90	2·26
	10	0	1·19	Nil	18·73	4·48	97	2·16
	5	0	0·75	0·03	19·03	4·61	96	2·08
	4	3	0·64	0·01	19·73	4·40	83	1·88
	3	2	8·44	0·22	10·56	2·30	41	1·79
(1)	2	9	43·37	1·00	1·30	0·16	24	15·00
	2	3	41·26	1·08	0·72	0·18	24	13·33

Samples from the measures above the Flockton Thin Coal at Cold Bath Opencast, Worsborough, near Barnsley, Yorkshire. Determinations of organic carbon, sulphur and free silica by A. Parr-Burman and G. Y. Gillespie, United Analysts, Ltd., East Boldon; K_2O and boron by D. A. Spears. The samples at 61 ft. 6 in. and 3 ft. 2 in. above the coal are additional to those used by Eagar (ref. 5), Fig. 1. Dark organic-rich bands are numbered.

* Boron content average of triplicate determinations. Average coefficient of variation is 6·5 per cent.

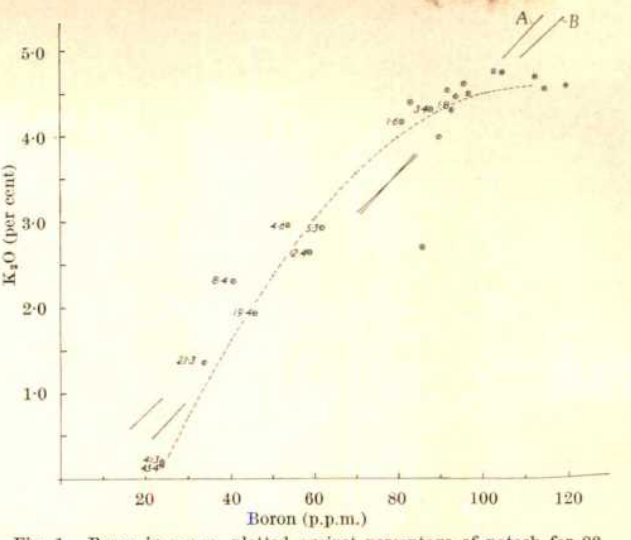


Fig. 1. Boron in p.p.m. plotted against percentage of potash for 23 samples of shales and shaly mudstones from the succession above the Flockton Thin Coal at Coal Bath Opencast, Worsborough, near Barnsley. Figures for organic carbon are inserted when they reach more than 1·5 per cent. Boron and potash determinations by D. A. Spears; organic carbon by A. Parr-Burman, United Analysts, Ltd., East Boldon. The curve which provides the best line for the data has been computed by S. M. Ali of the Statistical Laboratory, University of Manchester. *A*, B on K_2O; *B*, K_2O on B

We consider first the approximately linear relationship. If most of the boron and potassium is contained in the clay mineral illite[13], as seems reasonable from the evidence of numerous analogous investigations[3], then the sympathetic behaviour of these two elements appears to reflect merely abundance of illite. Samples with a relatively low content of both boron and potassium have high contents of organic materials. For these Worsborough sediments the organic material, which at a first approximation may be considered to contain negligible quantities of both boron and potassium, has simply acted as a diluent together with associated carbonates.

Boron and potassium are, however, both essential to biological growth and are known to be concentrated in many plants and animals. Therefore, it becomes of importance, in testing this hypothesis, to assess evidence bearing on the relative magnitudes of contributions to these elements made by clay minerals on one hand and organic matter on the other. Whenever cellular structure has permitted identification, organic carbon in British Coal Measures has proved to be almost exclusively derived from coal-forming land plants. By analogy with the conclusions of Breger and Brown[14], who investigated richly carbonaceous shales of late Devonian age in the eastern United States, and of Tourtelot[15], who worked on late Cretaceous sediments of the same country, the total organic fraction of both marine and non-marine sediments is also likely to be predominantly of the remains of land plants. This conclusion applies especially to samples with very high organic contents. Potassium may reach more than 3 per cent of the dry weight of fresh material from living land plants[16], but reductions in the content of potash have been found in vegetable litter[17] and a further decrease is apparent in humus. In raised bog peat, for example, proportions of potassium in dry matter are of the order of 0·05 per cent or less and concentration of this element in peat waters is correspondingly increased[18]. It is clear, therefore, that in carbonaceous shales the contribution of potassium from plant matter can probably be neglected, but that beds of swamp peat, the original material of Carboniferous and many other coals, might initially hold appreciable quantities[19]. Many living land plants, however, have boron concentrated in their leaves and whole-plant analyses range from 10 to more than 90 p.p.m. in different species[20]. These figures are of magnitudes comparable with those of vitrains in Tertiary[21] and

Carboniferous coals, including British seams. Six British vitrains, analysed 15–16 years ago[22], varied in their boron contents mainly between 2·5 and 8 p.p.m., but ranged up to 70 p.p.m. More recently Roga[23], working on Polish coals, after removing extrinsic boron compounds by density separation, found the remainder to be strongly associated with the vitrain in amounts ranging from 66 p.p.m. in lignites, through 19 to 1·6 p.p.m. in bituminous coals, ultimately to 1 p.p.m. in anthracites. It therefore appears that except in the richly carbonaceous shales of Worsborough (organic carbon more than 15 per cent) the contribution of boron from organic matter can be neglected—a conclusion in agreement with that of Tourtelot[24]. We must, therefore, conclude that for most of the range of the samples at Worsborough the antipathetic relationship noted between total boron and organic carbon contents results primarily from organic carbon acting as a diluent.

From the same survey, however, it is clear that boron in organic matter of plant origin might be expected to make a small but fairly rapidly increasing contribution to total boron as organic carbon increases to more than 15 per cent and as, at the same time, through a diluent effect, the clay mineral content of the sample decreases. An organic source of boron might, therefore, be a contributory cause of the very high ratios of B/K_2O observable in the samples at the base of the section at Worsborough, almost immediately overlying the Flockton Thin Coal (Table 1). On the other hand, the low potassium contents of these specimens could be due to the presence of an illite which has become highly degraded, the increased boron/potassium ratio being the result of the greater stability of boron in the illite lattice. It might also be argued that in these circumstances appreciable boron might be contributed by minerals other than illite. However, such minerals have not been detected. Moreover, an unsuccessful search for tourmaline was made by Spears[6] in comparable Coal Measure sediments, a result in support of the conclusions of Kneuper[25]. On this basis we consider the presence of significant amounts of tourmaline in the Worsborough sediments most unlikely. Both Walker and Price[26] and Spears[6] have noted a tendency for the boron/potassium ratio to increase as the potassium decreases in other sediments in the Carboniferous. The same phenomenon may possibly be connected with the very high B/K_2O ratios of the basal sediments at Worsborough, but if the remaining samples are plotted with these no significant or suggestive relationship between total boron and boron/potassium ratios is revealed. The final possibility, that these high ratios indicate greatly increased sorption of boron by illite, is better discussed after consideration of the significance of the curvature of the fitted line in Fig. 1 and of the data in Fig. 2, in which the increase in B/K_2O ratios at high levels of organic carbon is more clearly seen. There is a suggestion of a continuous series of points between medium-rich to extremely rich carbonaceous shales. Those samples with an organic carbon content of more than 40 per cent consist of cannelly shales little removed from high-ash low-carbon cannel and bituminous coals. If the increase in the boron/potassium ratio in this direction is due to an increasing contribution of boron from organic material of plant origin, or perhaps more strictly to an increasing B/K_2O ratio for the organic constituents of the sediments, then we might expect to find a series of points which continue into the realm of coals. Data from British coals appear to be lacking, but from the very detailed work of Headlee and others on sections within West Virginian coals[19] it seems clear that B/K_2O ratios for coal seams may reach 70 or more in the centres of seams, but that in general they decline towards the margins, so that values of 18–4 occur at the extreme tops of seams and in the richly carbonaceous roof shales. These values are entirely of the right order of magnitude to support our very tentative suggestion of continuity in boron/potassium ratios between carbonaceous sediments and coals. The view is further supported by a consideration of the theoretical curve drawn in Fig. 2 and discussed in the following paragraphs. Nevertheless, we must stress that regional variations in the boron contents of coals of similar ranks are by now becoming apparent. Moreover, the small amount of evidence at present available from coal surveys suggests that such factors as seam thickness and conditions of accumulation and burial may greatly influence the final boron content of the coal[27].

The statistically significant curvature of the fitted line in Fig. 1 is also of interest where organic carbon figures are lower; it suggests that the diluent effect alone is insufficient to explain the considerable decreases in the total boron contents of the dark shale bands. This fact may be simply tested in examples with 10–4 per cent organic carbon by comparing percentage decreases of total boron with those of illite as measured by potash determinations. The slightly larger percentage decreases of the total boron contents seems to imply that less boron was sorbed by the illite under conditions where organic matter was abundant. The B/K_2O ratios of samples having less than 10 per cent organic carbon, in fact, show an inverse correlation with carbon (Fig. 2, where $r = -0{\cdot}45$, a figure which is marginally significant at the 95 per cent level). Since we have concluded that the total boron content of these samples is not appreciably more than that of the clay minerals (essentially the illite content) and since the total potash content (reflecting illite) does not show any significant relationship with the B/K_2O ratio (boron in illite), then it seems likely that boron in illite bears a direct relationship with organic carbon in this range of the Worsborough sediments. If the increase in B/K_2O ratio with decreasing organic carbon reflects a small increase in the sorption of boron by illite, as we might reasonably suppose, then the relationship with organic carbon might perhaps have arisen through the inhibiting action of the original fraction of soluble organic matter in the water, a mechanism to which we have already made reference.

The configuration of the plotted points in Fig. 2 may be considered in the light of a model based on our present knowledge of the sources of boron and organic carbon in sediments and on certain assumptions which we can make about conditions of deposition during the formation of the Worsborough beds. Organic carbon (C, below) can be considered as composed originally of a detrital fraction, the debris of land plants and a soluble fraction, organic molecules in solution. Where organic carbon is high the detrital fraction will obviously predominate, whereas when the total organic carbon content is low the soluble fraction may well have been appreciable. The ultimate boron content of the illite must consist of the sum of the inherited boron (B_1, below) and the boron extracted from solution

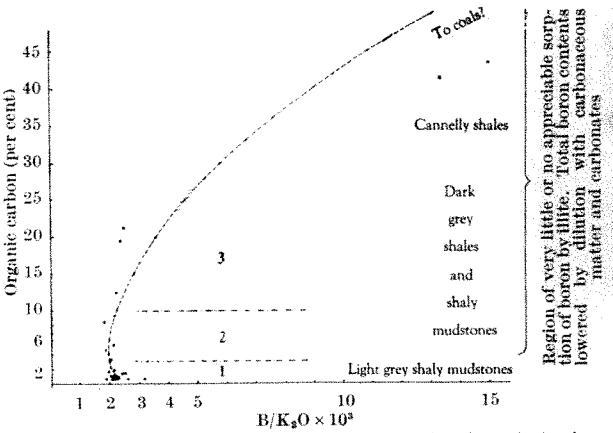


Fig. 2. Percentages of organic carbon plotted against boron/potassium ratios for 23 samples from Worsborough. The curve (see the text, equation (3)) has been computed by R. M. C. Eagar. 1, Region of small amount of boron sorption by illite; 2, total boron content mainly 'inherited' in the illite; 3, total boron content mainly 'inherited' in the illite, but with an increasing proportion of boron contributed by carbonaceous matter (land plants) as the total organic content of the samples increases

near and at the site of sedimentation (B_2, below). There is no reason to suppose any alteration in the source of the sediments deposited at Worsborough, so that we may presume that the inherited or detrital fraction of the boron has remained constant or approximately so. Let us assume also, for the moment, that palaeosalinity changes have been inappreciable, so that sorbed boron is also proportional to potash. Plant debris provides the third source of boron (B_3) and has already been discussed. We may regard B_3 as being a constant but very small fraction of vegetable debris, appreciable only in richly carbonaceous samples.

We have then:

$$\text{B (total boron)} = B_1 + B_2 + B_3 \qquad (1)$$

where

$$B_1 = a\ K_2O,$$

$$B_2 = \frac{b\ K_2O}{C}$$

$$B_3 = c\ C$$

and a, b and c are constants.

Substituting in equation (1) we have

$$B = a\ K_2O + \frac{b\ K_2O}{C} + c\ C \qquad (2)$$

We have noted, however, that K_2O is roughly inversely proportional to C through a dilution effect, so that $K_2O = d/C$ (as may be demonstrated by plotting).

Dividing equation (2) by K_2O and then substituting for K_2O in the third term we have

$$\frac{B}{K_2O} = a + \frac{b}{C} + e\ C^2 \qquad (3)$$

when the constant $e = \frac{c}{d}$

The curve of equation (3) has been plotted in Fig. 2 with the values of the constants determined by trial and error as $a = 1{\cdot}75$, $b = 0{\cdot}547$ and $e = 0{\cdot}0045$. It will be seen that the plotted path provides a good fit for the available data. The equation will account for the very high B/K_2O ratios when organic carbon contents of the samples approach those of low-grade coals and as the contents of potash are greatly reduced through diluency. It will further account for the broad minimum of these values in medium-rich carbonaceous shales, and finally for the small apparent increase in B/K_2O ratios which occurs at low levels of organic carbon and which may indicate appreciable sorption of boron from brackish water by illite under these conditions. We may, therefore, adduce a little more contributory evidence in support of our tentative hypothesis set out in Fig. 2. On the other hand, we do not know whether the presumably slower rates of sedimentation which prevailed during the deposition of most of the darker shale bands (but excluding the band 61 ft. 6 in. above the coal, Table 1) may have introduced other variable factors such as low E_h values which might have affected the result. (G. D. Nicholls has pointed out to us that when organic contents of the sediments were high, low E_h values might have affected the stability of the associated clays. In these conditions the formation of authigenic illite could have been hindered by the production of Fe^{++}-bearing clays, for example, chamositic minerals. Failure or reduction of authigenic illite would have slightly reduced the uptake of boron.) We point out merely that the data of Fig. 2 will at present bear the interpretation that a small amount of boron sorption could have occurred in conditions where soluble organic matter in the palaeo-environment was presumably low and that at medium and high levels of organic carbon, sorption has evidently been less or inappreciable.

Our new data from Worsborough considerably affect the use of boron analyses in the investigation of palaeosalinity in these and similarly constituted sediments. With the assumption that no appreciable boron or potassium is contributed by sources other than the illite, boron/potassium ratios may be multiplied by a constant to arrive at the boron content of the illite, which is the "adjusted boron" of Walker *et al.*[26]. In the case of the Worsborough sediments it will be seen from Table 1 and Fig. 1 that six of the organically rich bands (excluding the basal one, Band 1) have B/K_2O ratios which are no higher than those of the lighter-coloured grey mudstones. The mean boron/potassium ratio for the dark bands, 2·11, is actually lower but insignificantly different from the mean for the grey mudstones, 2·27, although faunal considerations carry the suggestion that the former were laid down under higher palaeosalinities than the latter[5]. The exceptional very high ratios for the two samples from the basal richly carbonaceous band or cannelly shale, overlying the coal, have already been discussed. Although the fauna of all seven dark shale bands are essentially similar, fish scales are more abundant at the lowest horizon, 2 ft. 2 in. above the coal, than elsewhere in the succession. Furthermore, it might possibly be inferred, mainly on geological considerations involved in the cyclic deposition of the Coal Measures, that palaeosalinities could have reached a maximum during the time immediately succeeding the submergence of a coal forest, that is, the peat of the Flockton Thin Coal, and therefore at the time when these beds were being laid down. Nevertheless, we have already produced suggestions and some evidence for two possible contributory causes to these high ratios—degradation of the illite and the presence of appreciable quantities of boron from the debris of land plants when illite contents were extremely low. We should, therefore, be very wary of accepting a third factor—much increased uptake of boron by illite in increased palaeosalinities—when we have already noted geochemical data which could be interpreted as suggestive of a decrease in boron uptake with increasing organic matter; and when, furthermore, we have obtained these results from bands of shale nearly all closely comparable in fauna with the critical bed under discussion. Finally, we note that the increases in boron sorption which must be postulated to account for the observed high ratios are wholly at variance with the model we have developed and seemingly irreconcilable with the available amount of illite as measured by potash analyses. The balance of the evidence is, therefore, most unfavourable to the use both of total boron and boron in, or supposedly in, illite as indicators of palaeosalinity in the Worsborough succession. In so far, however, as changes in palaeosalinity in the Worsborough succession may well have been small, it could be argued that the case is not conclusive. (M. A. Calver, at a meeting of the Yorkshire Geological Society in Leeds (January 18, 1964), when describing the palaeontology of recent cores obtained from the Clay Cross Marine Band in the eastern Pennine Coalfields, about 200 ft. above the Flockton Thin Coal, pointed to several areas in which stunted shells of *Anthracosia*, a non-marine lamellibranch, had been found within the strata of the Marine Band. Shells of this genus on this horizon may lie on the same bedding planes as unquestionable marine fossils. From these recent observations the inference may be tentatively drawn that faunas of *Anthracosia*, the shells of which occur in the carbon-poor sediments of Worsborough, where they are also rather small or stunted, could tolerate an appreciable degree of salinity.)

Further evidence bearing on the issue may fortunately be obtained by comparing the Worsborough analyses with others already published from the Yorkshire Coalfield, where there is evidence of unquestionable marine faunas. In the work of Curtis[3] two sections of the Mansfield Marine Band were investigated. The majority of his 39 samples are characterized by marine fossils, but three come from strata containing non-marine lamellibranchs. Another six results for shales and shaly mudstones yielding non-marine lamellibranchs may be found in the work of Walker and Price[26], so that with the present results from Worsborough the number of analyses for non-marine shales approaches that for marine ones. Since all these samples are located in the same general area of Yorkshire and its

southern border and are of closely comparable geological age they may be compared with some justification (Fig. 3). It will be seen at once that the correlation between boron and potassium for both marine and non-marine shales is again evident, although there is some scatter about the lines. The scatter is to some extent inevitable for several reasons such as experimental error, which is greater for boron than for potassium. Furthermore, illite is thought to be merely the major host for boron and potassium, and, therefore, it is possible that small quantities of these elements do occur independently of one another. The ratio of boron to potassium in illite presumably changes to some extent, because there is no reason to believe that the controls determining the amount of potassium of detrital origin to that of non-detrital origin will be exactly the same for boron. Small amounts of sorbed boron, which we admit may have been proportionately slightly greater in marine than in non-marine conditions, would also add to the dispersion. From a re-examination of Fig. 3 it will be seen that there is no apparent separation of samples into marine and non-marine categories. Although the range of potash contents is greater in the non-marine than in the marine samples and the range of organic carbon very considerably greater, with the result that the regression lines diverge more in the marine samples, the scatter is virtually the same for both. When the fitted lines are calculated by the method of the reduced major axis[29] there proves to be no significant difference in the slopes of the lines, nor is there significant displacement, towards the boron axis, of the marine sample. The means of the boron and potassium contents are greater for the marine than the non-marine samples (92 compared with 78 p.p.m. and 3·96 compared with 3.50 per cent respectively), but the mean B/K_2O ratios, 2·42 for marine and 3·11 for non-marine samples, are not significantly different. The latter results clearly indicate that the differences arise through the presence in the samples of constituents other than illite and by dilution effects, primarily through organic carbon. Statistically the marine and non-marine samples are indistinguishable with respect to their boron and potassium contents. Boron/potassium ratios do not statistically reflect differences in palaeosalinity in these 66 samples from the Yorkshire Coal Measures.

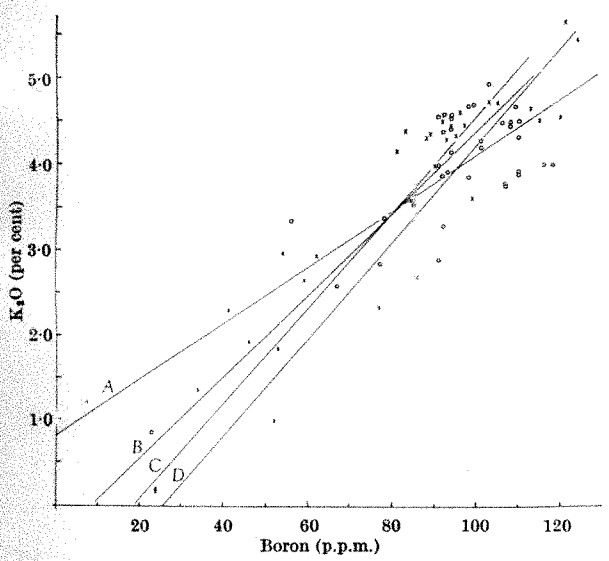


Fig. 3. A comparison of 66 samples from marine and non-marine sediments of the Coal Measures in the areas around Doncaster, Barnsley and Wakefield. Differences in the slopes and displacements of the regression lines are not statistically significant. The reduced major axes, which approximately bisect the angles formed by the regression lines, have been omitted to preserve clarity in the figure. ○, Marine shales: Mansfield Marine Band, Little Smeaton and Bilby boreholes. ×, Non-marine shales: Worsborough (ref. 23), Wakefield Westgate Brickworks (6 from ref. 26), and Little Smeaton borehole (ref. 3). *A*, K_2O on B (marine); *B*, K_2O on B (non-marine); *C*, B on K_2O (non-marine); *D*, B on K_2O (marine)

We therefore conclude that changes in palaeosalinity in the measures with which we are concerned are indicated neither by changes in total boron contents nor in boron/potassium ratios, and that this generalization applies both within single sections and also when averages are taken throughout the samples investigated. We consider that a limited amount of sorption of boron by illite may have occurred both in marine and non-marine conditions, where the water was often brackish, but stress that our evidence indicates that any fraction of boron taken up from solution must have been relatively small. Much the greater part of the boron was evidently carried into the sedimentary area in suspension, as the detrital or inherited fraction in illite, a conclusion which is in agreement with those of Nicholls[30], Hirst[31] and others, including Spears[6].

The antipathetic relationship previously noted by Eagar between total organic carbon and boron contents at Worsborough has proved to be indirect, in so far as it arises from dilution of the samples by other constituents, primarily organic carbon; but we have also found some evidence to suggest a direct inverse relationship between carbon and boron/potassium ratios. This relationship is apparent at levels of organic carbon below 10 per cent and may be connected with a process such as Curtis has suggested in which boron sorption can be inhibited by soluble organic molecules. When the organic carbon contents of the samples are very high (15 per cent or more) we have noted the possibility that an appreciable quantity of boron may be contributed by debris from land plants and boron/potassium ratios of the samples may be raised by this means. B/K_2O ratios may, in fact, form a continuous series increasing in value in samples ranging from carbonaceous shales to low-ash bituminous coals; but this tentative suggestion requires further investigation.

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[3] See Curtis, C. D., *Geochim. Cosmochim. Acta*, **28**, 1135 (1964).
[4] Harder, H., *Geochim. Cosmochim. Acta*, **21**, 123 (1961).
[5] Eagar, R. M. C., *Nature*, **196**, 428 (1962).
[6] Spears, D. A., *Geochim. Cosmochim. Acta*, **28**, 315 (1965).
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[8] Curtis, C. D., *Second Intern. Meet. Organic Geochemistry*—Paris, 1964 (Pergamon, in the press).
[9] Bader, R. G., *Occas. Pap. Grad. School Oceanog.*, No. 1 (1962).
[10] Ernst, W., Krecji-Graf, K., and Werner, H., *Geochim. Cosmochim. Acta*, **14**, 211 (1958).
[11] Ernst, W., in a communication to Eagar (July 6, 1964), has pointed out that a colouring agent used in the microchemical determination of boron at Krefeld during this time was later found itself to contain traces of boron.
[12] Ernst, W., *Fortschr. Geol. Rheinl. v. Westfal.*, **10**, 254, footnote (1963).
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LETTERS TO THE EDITOR

ASTRONOMY

Structure of the Crater Alphonsus

The photographs of the Crater Alphonsus obtained by *Ranger* IX show several features which may have structural significance from the point of view of the history of the lunar crust.

An examination of the northern side of Alphonsus (Fig. 1) indicates a horizontal offset along the central spine. The southern side, being irregular in outline, is more difficult to interpret. The general impression of the overall outline is, however, that the eastern (astronautical) side is displaced toward the south with respect to the western side.

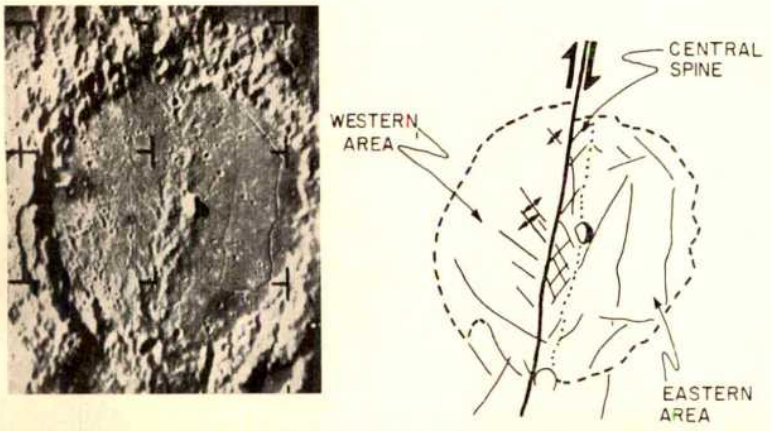


Fig. 1. *Ranger* IX photograph No. 0537 and schematic line drawing. The central spine area separates the western area from the eastern area. The dextral strike-slip fault is indicated by the offset of the northern 'shore' and by the orientation of the folds and lineaments. The central spine and central peak are probably volcanic

The presence of strike-slip faults on the Moon has been a matter of controversy for some time. Fielder[1,2] has described several of them on the basis of outlines, while MacDonald[3] and Baldwin[4] believe that there are no large-scale strike-slip faults. The presence of a strike-slip fault on the floor of Alphonsus is suggested not only by the offset of the northern 'shore', but also by the analysis of the lineament, as will be shown here.

Three areas can be defined on the floor of Alphonsus; the area west of the central spine, the area of the central spine with the central peak, and the area east of the central spine. The movement seems to have occurred on the border between the central spine and the western area, aligned with a lineament that goes from one of the sides of Ptolomaeus to Arzachel.

Three sets of lineaments are present on the floor of the crater: set *A* with azimuth approximately north-east–south-west, set *B* with azimuth approximately north-west–south-east, and set *C* approximately north–south. Few other lineaments of different azimuths are present, and they may be explained by distortions produced by the crater rim. The regularity of some of the lineaments along their extent, especially the rilles, is an indication of an extreme areal homogeneity in the material which constitutes the floor of the crater. Some of the lineaments in the western area, with azimuth of approximately north-west–south-east, appear under careful examination to be folds rather than depressions. Folds have not previously been noticed on the lunar surface.

Although the horizontal displacement could be apparent, rather than real, due to vertical motion of the inclined sides of the crater, the lack of a difference in elevation between the east and west rims and the character of the lineament is more consistent with an offset due to a dextral strike-slip fault. In particular, the fractures and folds oblique to the fault are characteristic of the dragging produced by strike-slip faults on overlying formations[5,6]. This can be easily verified by applying a couple with one's hands to a sheet of paper. The folds, oriented approximately north-west–south-east, match the proposed dextral movement of the strike-slip fault.

Rather than one single fault, it seems likely that a fault zone is present, extending the whole width of the central spine of Alphonsus.

It is possible that the central spine was formed by igneous eruptions which were localized by, and occurred along, the fault zone; and that the central peak could be similar to the famous *aiguille* of Pelée Mountain in Martinique, that is, a peak caused by the slow extrusion of a very viscous magma. The similarity between photographs of Pelée Mountain[7] and the central spine of Alphonsus is striking. The rest of the crater floor could be covered by ignimbritic material, which often accompanies Peléan volcanism. The lineaments on the central spine area indicate that the movement continued during and after the eruptions.

Regardless of the origin of the material forming the central spine and floor of the crater, it would seem from both the general outline of the crater and the presence of the folds that Alphonsus is a crater deformed by a strike-slip fault of dextral type.

I thank the National Aeronautics and Space Administration and the Jet Propulsion Laboratory for providing the *Ranger* photograph, and Dr. J. W. Salisbury for his advice.

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GEOPHYSICS

Explosive Phase Transitions in the Earth's Mantle

The Earth's crust and mantle deform viscoelastically. There has, therefore, been some question as to whether stress relaxation rates in the upper mantle and lower crust might not exceed any realistic rates of stress accumulation. If this is so, earthquakes could not be generated by rebound of elastic strain; that is, by either the type I or type II source of Honda[1]. It might, therefore, be worth-

while to explore the possibility of an earthquake mechanism in which stress is suddenly applied rather than suddenly released. One such mechanism is a phase transition, quasi-instantaneous over a finite volume and involving lattice condensation. Recent experiences with underground nuclear explosions[2] have shown that explosive strain may release an adequate portion of energy in the form of S waves, so that observed radiation patterns may well be accounted for by an explosive (or implosive) phase transition.

For a reaction to be quasi-instantaneous, free energy must decrease and some of the heat liberated must remain in the system, thereby increasing temperature and rate of reaction. If, therefore, phase transitions are a source of seismic energy, mineral phases in the mantle must become metastable before reaction. A mineral phase could become metastable in the process of moving to a new pressure–temperature (PT) environment. Vertical migration of mantle material appears to be the only mechanism which could substantially change the PT environment.

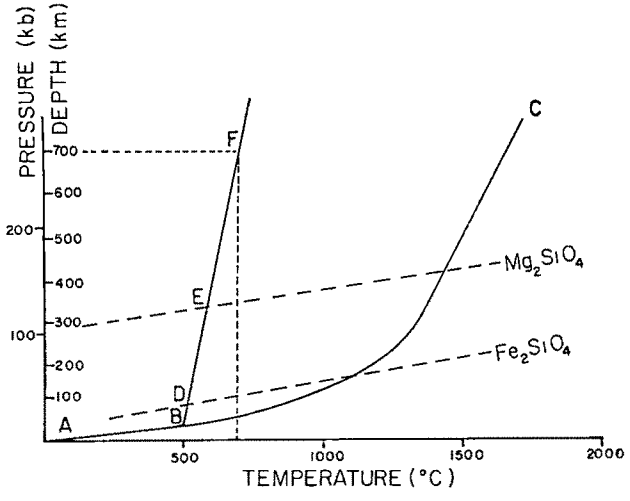


Fig 1. Pressure-temperature variation in the crust and upper mantle BF is the adiabatic dT/dP gradient[3]. ABC is the stable mantle dT/dP gradient[4]. Mg_2SiO_4 indicates the olivine-spinel transition curve for forsterite and Fe_2SiO_4 that for fayalite Therefore D and E indicate the depths in a descending current at which fayalite and forsterite would change to spinel or become metastable Point F indicates the depth on the adiabatic gradient corresponding to 700° C

Several processes might initiate vertical currents in the mantle. For example, a local increase in density near the top of the mantle would result in a gravity potential capable of initiating a downward current. Increase in density could be due to local cooling possibly caused by upward migration of radioactive isotopes and consequent impoverishment in heat sources during crustal differentiation. Downward segregation of mafic constituents during granite formation would also lead to local density changes in the upper mantle. Finally, rapid sedimentation and consequent isostatic adjustment could initiate a downward migration of mantle material. Now, pressure–volume changes in the mantle are usually considered to be adiabatic. Therefore, the adiabatic (dT/dP) gradient will determine temperature in a descending current initiated by any of the mechanisms already described. Since the adiabatic gradient[3] (Fig. 1, BEF) is less than the estimated dT/dP gradient in the stable mantle[4] (Fig. 1, ABC), the temperature in the sinking current would become progressively less than the temperature in the adjacent mantle. The downward driving potential for a mantle current therefore increases with depth so that a current, once initiated, should persist provided that heat exchange remains negligible.

The exact composition of the mantle is unknown, but olivine is generally considered to be a major constituent. Both forsterite and fayalite have high-pressure spinel polymorphs[5,6] and consequently might become metastable during downward migration in the mantle. Assuming a temperature before migration similar to that at the top of the stable mantle, transition from olivine to spinel would occur at a depth determined by the intersection of the olivine–spinel dT/dP transition curve and the adiabatic gradient; that is, point E in Fig. 1 for forsterite and point D for fayalite. The activation energy for reactions involving lattice rearrangement in the solid state is high. Consequently, the probability of transition at equilibrium could approach zero, so that olivine in the mantle would become increasingly metastable as it moved through and past equilibrium along the PT path determined by the adiabatic gradient BF (Fig. 1). However, with continued downward migration the temperature of the metastable olivine, and consequently the probability of transition, would increase and the spinel phase would eventually be nucleated. If, as is generally believed, mantle processes are essentially adiabatic, heat liberated during the reaction would accumulate within the system, thereby increasing nucleation and reaction rate until the transition became implosive.

We have suggested this mechanism[7] for generation of earthquakes by quasi-instantaneous phase transition from a metastable state as a possible basis for future investigation: a new investigation of the olivine–spinel transition in fayalite[6] suggests that the reaction is completely reversible at temperatures greater than 700° C. If this is so, the temperature in the upper mantle where downward migration might start must be considerably less than 700° C. Assuming the stable mantle dT/dP gradient proposed by Verhoogen[4] (Fig. 1) the temperature at the Mohorovičič discontinuity, that is at a depth of 40 km, would be about 500° C. If downward migration is initiated from such an environment, and temperature in the descending current is determined by the adiabatic dT/dP gradient[3] (Fig. 1), the temperature would reach 700° C at a depth of 700 km. On the basis of these data, then, earthquakes could not be generated below a depth of 700 km.

We suggest that more information on the kinetics of possible solid state reactions in the mantle be made available to support existing data. In particular, the response of powdered fayalite or its spinel polymorph to heat and pressure may not be comparable to the response of olivine and spinel crystals in the mantle. Also, atmospheric carbon dioxide trapped in mineral powders could take part in laboratory reactions and so act as a catalyst. Yet again, results obtained from fayalite may not be applicable to forsterite, believed to be the predominant component of mantle olivine. Nevertheless, this example shows that the temperature at any depth in a sinking current can be inferred once adequate data are available. Furthermore, the relation, if any, between earthquake foci and density discontinuities should be investigated because the depth and nature of phase transitions might be inferred from such information. Finally, first motion and energy data should be analysed without prior assumption of any specific pattern.

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PHYSICS

Laser Probing the Lower Atmosphere

Synge[1] first suggested in 1930 that density measurements could be made of the lower atmosphere by observing the light scattered from the beam of a search-light. Elterman[2] in 1950 and onwards has operated the method in New Mexico with many improvements of the techniques. Fiocco and Smullins[3] in 1963 substituted a laser beam for the search-light beam and achieved comparable results. The equipment used in the experiments described here was planned in 1962 and erected in Kingston, Jamaica, 18° N., 77° W.

The equipment used was an optical radar suitable for detecting the amount of atmospheric scattering (the height to which such equipment could probe was limited only by its sensitivity). A Q-spoiled ruby laser was used as the transmitter. The output energy of the latter was about 6 J for each shot, which consisted of five giant pulses in a 5-μsec interval. The laser output beam passed through a rapid shutter which closed within 150 μsec after the shot and so cut off any fluorescence from the ruby. The beam was optically collimated to give a half beam-width of about 0·15 mrad and transmitted vertically. The receiving system for the light scattered back by the atmospheric constituents was essentially a 50-cm diameter astronomical telescope. The half beam-width of the receiving cone was adjusted to be about 0·2 mrad by altering the size of the aperture in the focal plane. The light passing through the aperture was filtered by interference filters of either 20 Å or 3 Å band-width, and then detected by a photomultiplier. The output of the photomultiplier was photographically recorded from an oscilloscope screen Each detected photon produced a pulse which was recorded as a y deflexion on a time base displayed in the form of a raster.

After a series of shots, the total number of photons counted at each height (h in km) was corrected for the background count. The product Nh^2 was then proportional to the scattering power of the atmosphere at that height. A typical night's result of scattering power versus height is shown in Fig. 1.

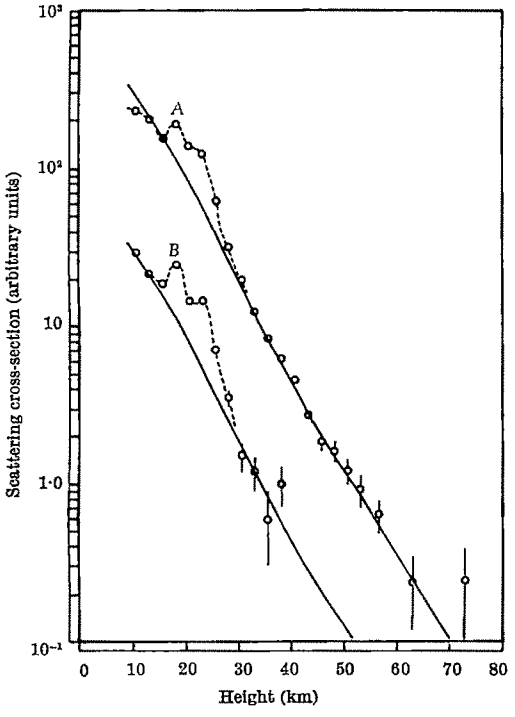


Fig. 1. Variations of scattering cross-section with height. A, Direct; B, with polarizing filter. ———, Standard atmosphere, ---O---, experimental observations

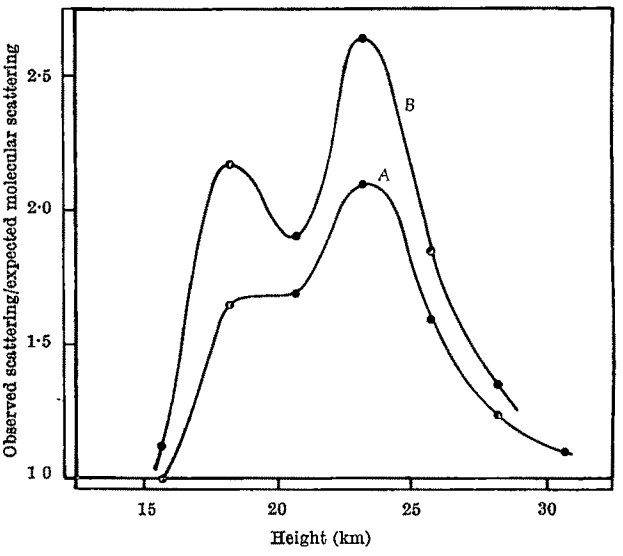


Fig. 2. Variations with height of the ratio of observed scattering to expected molecular scattering. A, Direct; B, with polarizing filter

The experimental points are shown in curve A, in conjunction with the theoretical curve of the amount of Rayleigh scattering to be expected from the molecules of the atmosphere, assuming the expected properties of the atmosphere above Jamaica. The ordinate scales have been chosen such that the experimental points and the curve fit at about 30 km. Excessive scattering occurs in the 15–30 km region. This is similar to the results obtained by Fiocco and Grams[4]. In our results, however, there is evidence of two peaks of extra scattering, one at 18 km and the other at 25 km; such a finding is in accordance with the results obtained by Elterman and Campbell[5] using the search-light technique.

In order to examine these peaks in more detail, efforts were made to accentuate their existence. The laser beam was highly polarized ; aerosol or dust particles could be expected to depolarize the returned signal more than air molecules. Consequently, a series of shots were fired with a polarizing filter over the detector. The filter was orientated in such a way as to reduce the signal received from the molecular scattering to a minimum. The results of this series of experiments are shown in curve B of Fig. 1. It will be seen that the molecular scatter was reduced by a factor of about twelve while the dust layer signal was only reduced by about eight. This point is emphasized in Fig. 2, where the ratio of total scatter to expected molecular scatter is shown.

It should be noted that in this series of experiments, our transmitting and receiving beams were very narrow and did not overlap completely below 10 km. Observations can easily be made at lower levels by increasing the receiving beam width.

The results from between 30 km and 60 km offer a means of determining the scale height and hence the temperature in this region. The scale height is found to change from 6·1 km at about 35 km to 7·6 km at about 45 km, which indicates a temperature change from 208° K to 260° K—as would be expected. The results from above 60 km are as yet only very approximate.

Fiocco and Smullins[3], who worked near Boston, Mass., where conditions may be different, have reported evidence for scattering from regions between 80 and 120 km during or after meteor showers. We have not as yet made observations at the time of meteor showers. The signal which we received from around 30 km was about 100 times as great as that received by Fiocco and Smullins, so that any signal from the 80–120 km region detected by them should have been easily recognized by our equipment. However, although there was a slight indication that there was

more scattering from these levels than would have been expected from simple Rayleigh scattering by air molecules, no scattering comparable with that observed by Fiocco and Smullins was, in fact, detected. We intend to make observations in Jamaica during the forthcoming meteor showers to see if similar echoes are detected. We shall also be able to improve the accuracy of our observations at greater heights by reducing the signal-to-noise ratio by cooling the photomultiplier.

The results described here are of a preliminary nature and the full capabilities of the equipment have not yet been attained. In spite of the brightness of the daytime sky, reliable measurements have been made in daylight up to about 30 km and will be extended to 40 km in the near future. This means that it should be easy to observe steadily the aerosol layers for 24 h a day—given cloudless days. It is of interest to note that we had to 'tune' the laser thermally in order to avoid the water–vapour absorption band at 6943·8 Å.

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Transmission of Indian Mica between 300 and 1,000 mμ and its Visual Classification

India produces ruby and green muscovite mica. Depending on the 'stains' and inclusions each is visually classified into a number of groups, namely, 'clear', 'good-stained', 'fair-stained', 'stained', 'heavily stained', 'densely stained', 'black-dotted', 'black-spotted', etc. It has been reported[1,2] that any visual classification has little bearing on the electrical properties of mica. Little information is available on the transmission of natural mica in the region of 300–1,000 mμ. Popper[3] has reported that the absorption in the ultra-violet region of the Congo green muscovite is more than that of the Rhodesian ruby and synthetic mica. Spectral absorption for Russian muscovite and phlogopite micas in the region of 220–1,200 mμ was studied by Grum-Grzhimails *et al.*[4] and Melankholin[5]. To our knowledge, no such investigation of the spectral transmission of Indian muscovite has yet been reported. This communication reports our investigations of the optical transmission of Indian muscovite and its correlation with the visual classification.

Samples of different grades of muscovite were obtained from the Bihar, Madras and Rajasthan regions—the three main mica-producing states of India. The samples were thoroughly cleaned with benzene (B.D.H.) and stored in a desiccator for 24 h before any optical measurements were undertaken. Spectroradiometric measurements were carried out in the region of 300–1,000 mμ using a Hilger 'Uvispeck' spectrophotometer provided with a quartz prism. Transmission curves for various mica samples are given in Figs. 1–4.

Table 1. Chemical Analysis of Ruby and Green Muscovite Mica

	Ruby muscovite mica (per cent)	Green muscovite mica (per cent)
SiO_2	43·87	43·86
TiO_2	0·24	0·22
Al_2O_3	36 00	33·27
Fe_2O_3	1 73	5·29
FeO	0 25	0·70
MgO	0 53	1·14
K_2O	9 78	9 31
Na_2O	0 88	0 40
L O 1	7·15	7·03

Transmission of ruby muscovite between 300 and 1,000 mμ is more than for green muscovite mica (Figs. 1 and 3). Our findings, that the 'cut-off' frequency for the green muscovite is greater than that of the ruby variety, are in full agreement with those of Popper[3]. Popper[3] and Grum-Grzhimails *et al.*[4,5] have reported that cut-off frequency in the ultra-violet region depends on the quantity of iron present in the sample. The higher cut-off frequency as well as the lower transparency for green muscovite can therefore be attributed to a higher iron content, as is evident from the chemical analysis (Table 1). The content of FeO and Fe_2O_3 in the ruby and green muscovite is not linearly related to the absorption coefficient. It is

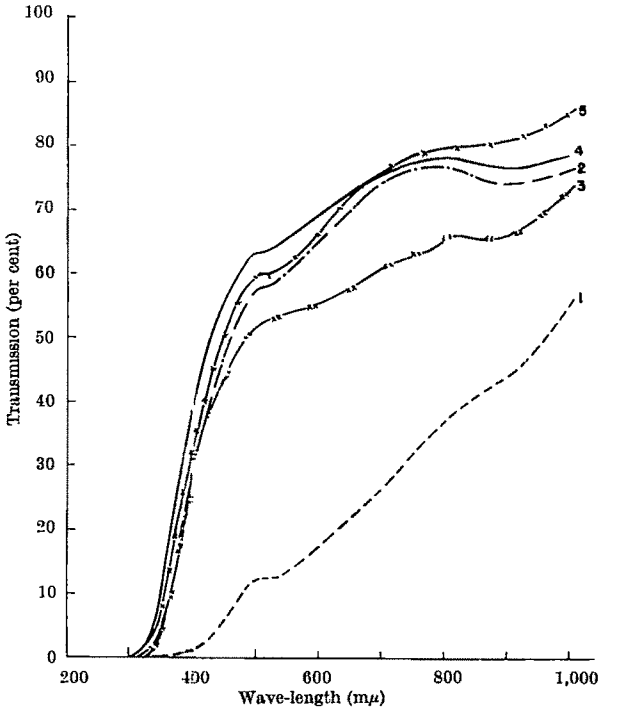


Fig. 1. Madras mica: (1) green (0·310 mm thickness); (2) ruby clear (0 310 mm); (3) green (0 219 mm); (4) ruby *A Q* (0 207 mm); (5) ruby *BQ* (0 207 mm)

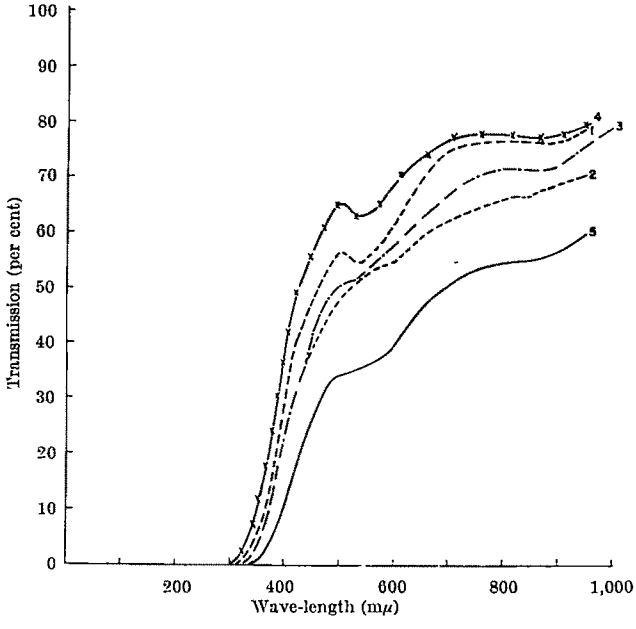


Fig. 2. Bihar ruby mica. (1) stained (0 263 mm thickness); (2) densely stained (0 263 mm); (3) black-dotted (0 501 mm), (4) stained *B* (0·219 mm); (5) densely stained (0 482 mm)

possible that the absorption spectra of muscovite might depend on the lattice parameters, especially on the presence of other elements in the octahedral layer.

Transparency in the ultra-violet region of the green muscovite of the same origin varies from sample to sample. In Fig. 3 it can be seen that two samples of green muscovite from the Rajasthan region which have similar thickness, differ in transparency, although the cut-off frequency is more or less the same. Green muscovite of the same visual quality but from Madras and Rajasthan regions differs in transparency and cut-off frequency in the ultra-violet region (Fig. 3). If the transparency is an indication of the iron content as advocated by Popper and others[3,5], it may be concluded that samples of green muscovite of the same quality from different regions, or even from the same region, differ in iron content. The cut-off frequency of ruby muscovite (clear) from the Bihar region is lower than that from the Rajasthan region, although the difference in transparency is small.

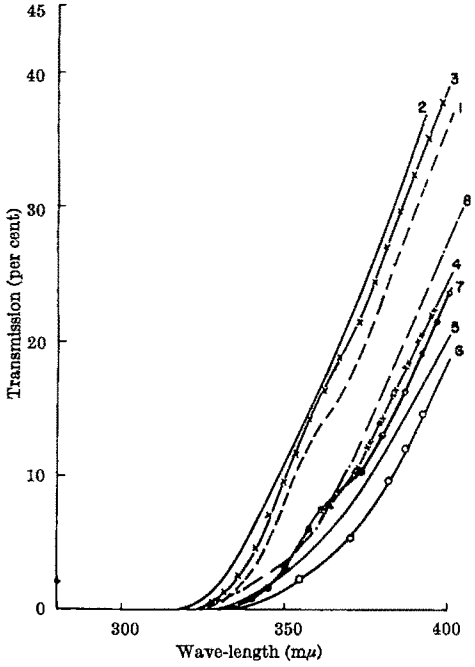


Fig 3. (1) Rajasthan green clear (0 196 mm); (2) Rajasthan ruby clear (0·196 mm); (3) Rajasthan green clear (0 190 mm); (4) Rajasthan ruby clear (0 310 mm); (5) Rajasthan heavily stained (0 311 mm); (6) Rajasthan green clear (0·335 mm); (7) Madras green clear (0 335 mm); (8) Bihar ruby clear (0·321 mm)

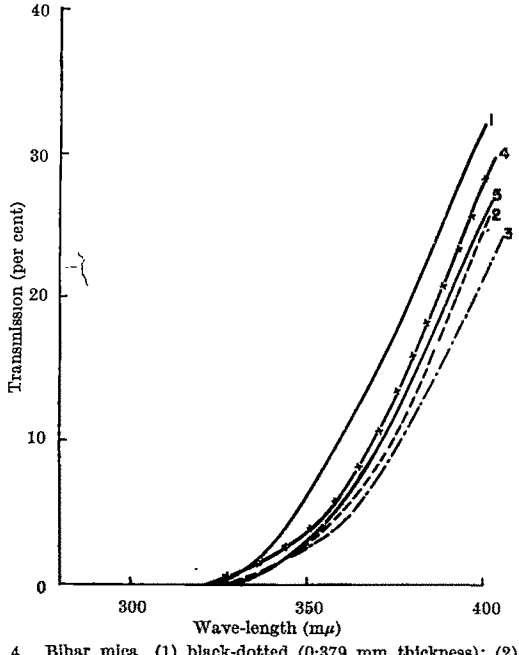


Fig. 4. Bihar mica (1) black-dotted (0·379 mm thickness); (2) fair stained (0 364 mm), (3) stained (0·363 mm), (4) ruby clear (0 312 mm), (5) Rajasthan ruby clear (0·312 mm)

Stains and other inclusions affect the transparency of muscovite and there appears to be some relationship between transparency and the visual classification of mica. Inclusions do not affect the cut-off frequency but, in general, the greater the 'stain'—that is, the lower the mica in the visual grade—the less transparent is the mica (Figs. 1 and 2). 'Black-dotted' mica appears to be anomalous in this respect; even though it is thicker it transmits more light than does 'densely-stained' mica—a superior quality mica according to the visual classification. As regards transparency in the ultra-violet region, 'black-dotted' mica is superior even to 'fair-stained' and 'stained' quality mica.

The transparency of various 'fair-stained' muscovite in the ultra-violet region, like 'stained' and 'fair-stained' (Fig. 4), ruby *AQ* and ruby *BQ* (Fig. 1), does not differ very much. These micas may, therefore, be grouped in the 'stained' class, as small variations in air or gas inclusions do not have appreciable effect on transparency.

The nature of the transmission curves for both ruby and green muscovite is similar irrespective of the presence of stains and inclusions. The curves show narrow low maxima (absorption) at about 525 and 875 mμ. Green muscovite shows some inflexion at about 360 mμ. These may be due to the presence of iron minerals in the muscovite lamina. As the nature of the iron minerals in the mica lamina was not identified, the foregoing hypothesis may be considered inconclusive.

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Visualization of Low-speed Flow using Suspended Plastic Particles

Photography of particles suspended in a fluid often gives a more complete and easily comprehensible record of the events associated with the passage of fluid around an object than can be achieved through measurements of force, velocity, or pressure. In practice, however, smoke trails and most other methods of flow marking[1–4] are both cumbersome and non-quantitative. In response to the problem of recording flow patterns around airfoils at Reynolds numbers of about 100, we have devised a new technique, using plastic particles suspended in a revolving bowl of brine and illuminated by pulses of light. This system produces photographs of steady flow patterns at Reynolds numbers between 30 and 300, with both speed and direction of flow indicated in the pictures (Fig. 1).

For use as flow markers, particles must (1) be of convenient size, (2) reflect light, and (3) be of a density suitable for suspending in the fluid. The following method produced particles slightly denser than water having an intense yellow colour in visible light and a bright yellow fluorescence under ultra-violet illumination. A slurry of the compounds listed in Table 1 was poured into gelatine capsules and polymerized at 60° C overnight. Since the 'STC' sank to the bottoms of the capsules, the upper, colourless portions of the plastic as well as the gelatine envelopes were cut away and discarded. The remaining bright yellow plastic was crushed in a cold mortar or

Table 1. COMPOSITION OF THE PLASTIC

(1) Hexyl methacrylate, 95 per cent by volume. Function: monomer. Source: Rohm and Haas Co., Washington Square, Philadelphia, Pa.
(2) Ethylene glycol dimethacrylate, 5 per cent by volume. Function: monomer; increases hardness by cross-linking. Source: Sartomer Resins, Inc., P.O. Box 56, Essington, Pa.
(3) α,α-Azodiisobutyronitrile, 0·5 per cent by weight dissolved in the monomer mixture. Function: catalyst. Source: Eastman (No. 6400), Rochester, N.Y.
(4) 'STC' about 20 per cent by weight of the monomer mixture. Function: colour; 'STC' is a 'fluorescent brightening agent' soluble in organic solvents. Source: Geigy Industrial Chemicals, Saw Mill River Road, Ardsley, N.Y.

powder mill and the particles sifted to sort them by size. Fig. 1, for example, was made with particles 0·2–0·5 mm in diameter.

Fig. 2 illustrates the flow chamber used to move fluid past the models; the bowl itself was a transparent 'Perspex' cylinder, 4 in. in diameter and 2·5 in. high. (The use of a revolving bowl was suggested by B. Howland in a personal communication, and the arrangement of bowl and motor drive was similar to that of Howland, Pitts, and Gesteland[5].) Particles were held in suspension by adjusting the density of the fluid; the final solution contained about 19 g of salt per 100 c.c. of aqueous solution. A slight vertical density gradient helped to keep the particles from sinking to the bottom or rising to the surface. An individual bath was serviceable for up to 2 days; the particles were recovered by filtration and re-used, generally without drying. Initially, the dry particles had to be shaken in degassed water or brine to remove adhering air bubbles.

Airfoils were made of transparent 'Perspex' in order to minimize shadows in the photographs. These models were held in the brine by a brass support at the axis of rotation of the bowl. Both the size of the model and its distance from the centre of the bowl as well as the rotation rate were chosen to give the desired Reynolds number. Light from either a mechanical or an electronic stroboscope passed through a curved slit perpendicular to the camera axis and parallel to the direction of flow; thus the light struck the particles as the latter traversed a plane which crossed the model. Use of a stroboscope gave a dotted line for the path of each particle, and consequently both speed and direction of flow at all points around a model were recorded. A General Radio Co. 'Strobotac', type 1531-*A*, provided adequate illumination at flashing rates up to 12/sec for photographs taken at *f*/5·6 on Eastman 'Tri-*X*' film. Supporting structures were coated with non-reflective black paint to reduce scattered light.

The present scheme for flow visualization may be modified to extend its usefulness. The composition of the particles (Table 1) represents only an empirical solution to a specific problem. Methacrylate monomers with side-chains shorter than hexyl produce denser and harder polymers; longer side chains yield lighter and softer plastics. The choice of colouring substances is limited only to those that can be either suspended or dissolved in the monomer mixture without inhibiting polymerization. The mixing of fluids from separate sources may be studied with particles of different colours.

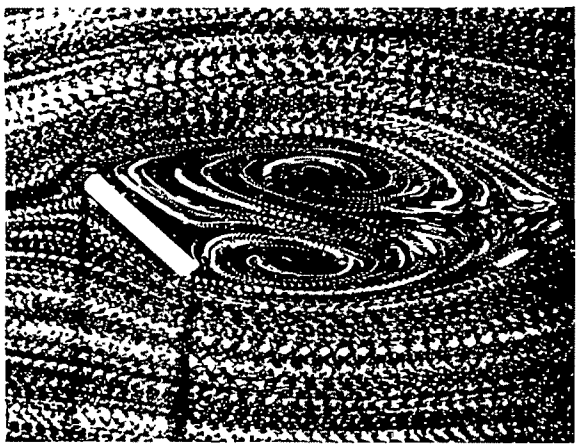


Fig. 1. Flow around a stalled airfoil at a Reynolds number of 140. The location of the model has been marked on the photograph, since the actual model was transparent. The shadows below the edges of the airfoil are caused by the bevelling of these edges. Chord: 8·5 mm. Flashing rate: 11 7/sec. Exposure: 4 sec

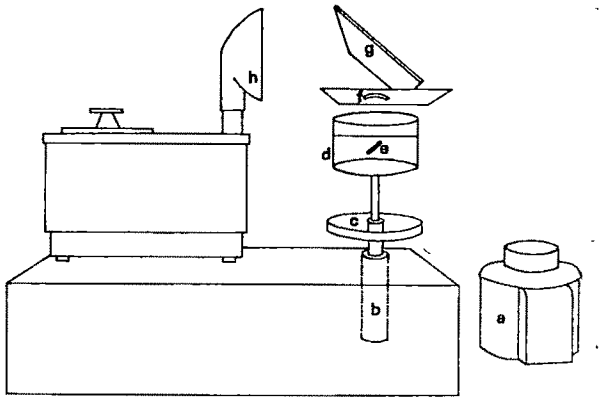


Fig. 2. The flow chamber and associated components. *a*, 'Variac' speed control, *b*, variable speed motor; *c*, flywheel; *d*, 'Perspex' bowl; *e*, model; *f*, curved slit in cardboard baffle; *g*, mirror; *h*, lamp housing of 'Strobotac'. The camera (not shown) was aimed at the model on a line normal to the plane of the diagram

Similarly, other parameters of the system may be varied. Higher Reynolds numbers may be achieved using either higher flow speeds or larger models, while lower velocities or the addition of glycerin to the fluid reduce the Reynolds number. Since the suspension of plastic particles is extremely stable, operation at very low speeds presents no special problems. Intensity of illumination is not limiting either: a mechanical stroboscope using a revolving sector disk can be used; while this variant gives dashes instead of dots on the photographs, it permits the use of a lantern slide projector as a high-intensity light source. Extraneous reflexions from incident illumination may be avoided, if necessary, by using an ultra-violet light source together with a suitable filter for the camera lens. The basic arrangement, with continuous illumination, should be well suited to cinematography for demonstration of the temporal structure of turbulence and related phenomena. Similarly, if force is measured simultaneously, changes in the appearance of flow around an object could be compared with the net reaction of the fluid on the object.

This method of flow visualization should also be convenient for laboratory investigations of the microhabitats encountered by limnologists and biological oceanographers.

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Dispersion Relation in Liquid Helium

THE normal mode representation[1] of the excitations in liquid helium describes the thermodynamic behaviour below the lambda point as the vibrational modes among a temperature dependent population of diatomic rotors. The excitation spectrum has now been obtained from the

theory on the assumption of an observed empirical relation.

The complete partition function, including phonons, is:

$$f = \Sigma \frac{(v + n - 1)!}{v!(n-1)!} \exp\left(-\left(v + \frac{n}{2}\right)\frac{h\nu_0}{kT}\right) \times \Sigma \exp\left(-\left(v + \frac{1}{2}\right)\frac{h\nu_0}{kT}\right)$$

v is the vibrational quantum number, n the number of dimensions and ν_0 the fundamental frequency of the harmonic oscillator. States above the ground state may be summed to give:

$$f = (1 - \exp(-h\nu_0/kT))^{-(n+1)}$$

From this expression, the thermodynamic functions may be obtained if n is defined as the number of states per atomic pair of thermally excited rotors:

$$n = \frac{\sum_{2,4\ldots} (2J + 1) \exp(-J(J+1)B/kT)}{\sum_{0,2,4\ldots} (2J + 1) \exp(-J(J+1)B/kT)} W$$

where B is the constant for a rigid rotor composed of two helium atoms with nearest neighbour spacing. W then is the number of ways pairs can be formed with nearest neighbour spacings ($W = 60$). The partition function can be re-expressed as:

$$f = \sum_v \omega \exp(-vh\nu_0/kT)$$

$$\omega = \frac{(v + n)!}{v!\,n!}$$

The excitation may be defined by the following relation:

$$\sum_v \exp(-\varepsilon/kT) \equiv \sum_v \omega \exp(-vh\nu_0/kT)$$

Equating terms and rearranging:

$$\exp(\varepsilon) = \exp(vh\nu_0 - kT \log \omega)$$

$$\varepsilon = vh\nu_0 - kT \log \omega$$

An additional relation must be used to eliminate the temperature from the dispersion relation. A comparison of the empirical dispersion relation with the internal energy leads to the observation that $c_2 p = kT$ at the upper limit of the phonon region and again at p_0 and T_λ (where c_2 is the velocity of second sound near 1° K). Above the lambda temperature the heat capacity rapidly returns to the Debye value, and above p_0 the dispersion curve is straight and parallel to the low-temperature phonon line (Fig. 1). In the normal mode representation, the lambda point is achieved when the number of excitations equals the number of particles. In the dispersion relation, ω will achieve a maximum value and become constant. The excitation energy will thereafter be linear with momentum, having a slope determined by the velocity of first sound.

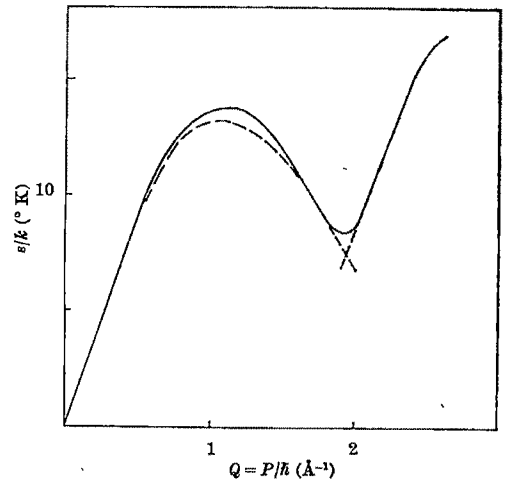


Fig. 1. Dispersion relation. Continuous curve, neutron scattering; broken curve, present theory

The equivalence of momentum and temperature is therefore assumed:

$$\frac{kT}{c_2 p} = \frac{c_1 kT}{c_2 vh\nu_0} = 1$$

The dispersion relation then takes the form:

$$\varepsilon = vh\nu_0\left(1 - \frac{c_2}{c_1}\log\omega\right)$$

The degeneracy is a function of n, which is temperature dependent. The same substitution renders n a function of v.

$$n = \frac{\sum_{2,4\ldots} (2J + 1) \exp(-J(J+1)Bc_1/c_2 vh\nu_0)}{\sum_{0,2,4\ldots} (2J + 1) \exp(-J(J+1)Bc_1/c_2 vh\nu_0)}$$

The resulting excitation spectrum is compared with the results of neutron spectroscopy[2] in Fig. 1.

The minimum of the dispersion curve at p_0 then represents the maximum value of ω, which will correspond to the case in which the number of particles equals the number of states. From this, the coherence length can be estimated. At p_0, $v = 4$ and $n = 27$ giving $\omega = 35{,}000$. The number of atoms participating is then 70,000. If these form a sphere, the radius would be 26 atoms or a distance of 100 Å. Such a size approaches the size of the vortices reported by Rayfield and Reif[3].

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Important Feature of Time Modulation

Time modulation, where information is conveyed by changing the time between similar parts of the modulated wave-train rather than by the frequency of occurrence, has a feature which is of great importance at the de-modulation stage. This can best be demonstrated by considering a common method of de-modulating a frequency-modulated wave-train and comparing it with a method which is particularly simple to use where the wave-train is time-modulated. Fig. 1 shows a frequency modulated wave-train with a step change in frequency; a common and representative method of de-modulation is shown in Fig. 2. The box represents a device which produces a pulse of current (di) of short and controlled duration for each pulse of the input wave-train. The mean current flowing through R is, therefore, $n \times di$ where n is the pulse-recurrence frequency. The mean potential across R is $n \times di \times R$ and is proportional to the mean value of the pulse frequency and hence represents the original modulation waveform. The value of c does not affect the mean value of current but has a marked effect on the transient performance of the circuit. If it is assumed that the current pulse has a negligible duration compared with the repetition frequency, then the waveform across the capacitor will be as shown in Fig. 1. The fall in potential between pulses is represented by the expression $v = V(\varepsilon - t/cR)$ where v is the capacitor potential at any time t after an input pulse at which time $v = V$. The potential of the capacitor, therefore, falls exponentially with time with a time constant given by the product of c and R. In most situations it is desirable to recover the original modulating waveform undisturbed by components of the carrier, and the difficulty is to choose $c.R$ so that the carrier frequency components (ripple) are at an acceptable level while maintaining a good transient performance of the de-modulated waveform. Often a simple resistance–capacitance filter will not give sufficiently good performance and elaborate low-pass filters must be used. The filters have, up to the present, always used

inductive elements which are not as convenient to use as resistive–capacitance components (they must in general be specially wound) and cannot be made in 'integrated circuit' form. (This method of construction involves making the components and their interconnexions at the same time, often on a common surface.)

Time modulation and de-modulation may be thought of in geometrical form. The modulator (Fig. 3) contains two essential features; a section which causes a potential to rise linearly with time (the reference waveform) and a voltage comparator. The input or modulating voltage must be arranged to be of one polarity only; if it is of either polarity a fixed bias must be added to it to make it conform to this restriction. The circuit is arranged so that when the reference waveform reaches the same potential as the input voltage (as sensed by the voltage comparator) it is returned to zero within a negligible time-interval and, at the same moment, an output pulse is emitted. Thus the time between output pulses is a linear function of the magnitude of the input waveform, and time modulation is achieved. The de-modulator contains a similar device to the modulator and creates a potential which rises at the same rate as the reference waveform in the modulator. The de-modulator waveform is called the generating waveform (Fig. 3). If an incoming pulse has just occurred, the generating waveform will, as will be explained later, be at zero and will have just started to rise. When the next pulse occurs, the generating waveform will have reached the same potential as the reference waveform in the modulator. This new input pulse first connects the generating waveform (via an electronic switch, Fig. 4) to a storage capacitor and then returns the generating waveform to zero. The constants of the circuit can be arranged so that the potential of the storage capacitor is brought into equilibrium in a time short compared with the pulse-recurrence time. It can also be arranged that the output circuit connected to the storage capacitor draws so little current that the potential of the latter is virtually unchanged during the pulse-recurrence time. The storage capacitor, therefore, takes on the value of the generating waveform at the time of an input pulse and holds this value until the next one occurs. If there has been no change in the modulating waveform between pulses there will be a negligible change in the potential of the storage capacitor. Thus the de-modulator has a 'ripple' voltage proportional to the difference in modulating potential between carrier pulses.

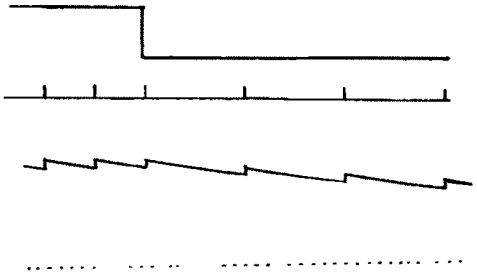


Fig. 1. Frequency modulation waveforms. Top: modulation waveform Middle: carrier wave-train. Bottom: de-modulated waveform

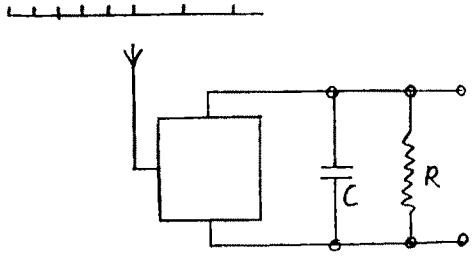


Fig 2. Frequency de-modulator. Block diagram showing input wave-train

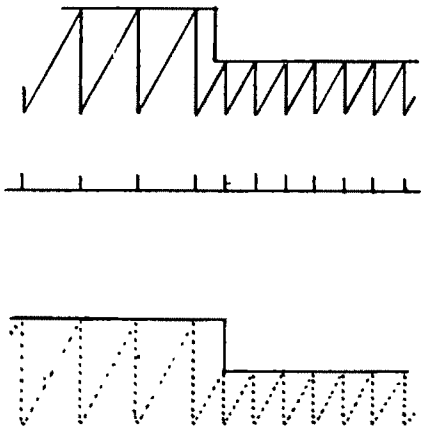


Fig 3 Time modulation waveforms. Top: modulation waveform in contact with reference waveform. Middle: carrier wave-train. Bottom: de-modulated waveform in contact with (dotted) generating waveform

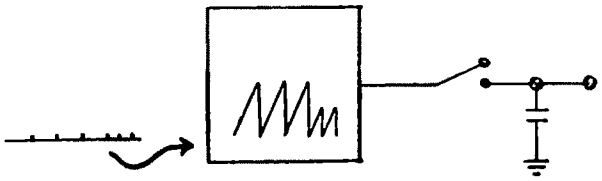


Fig. 4. Time de-modulator: Block diagram showing input wave-train, generating waveform electronic switch and storage capacitor

The system can be described in general terms as one in which the modulating waveform is sampled at various instants and the value transmitted to the de-modulator, which immediately assumes this value at its output and holds it until the next value is transmitted. It conveys the maximum information that can be transmitted with a simple pulse-train and the de-modulation makes the only assumption that can be generally true. The system may be realized in various forms and is very suitable for circuits using transistors as the active circuit elements. As it uses no inductive elements it is suitable for 'integrated circuit' construction. The performance of a simple form of the system (26 transistors in total for modulator and de-modulator combined) is shown in Fig. 5. The mean period of the modulated wave-train is 10 msec (~ 100 c/s) and the time deviation is ± 2 msec. The top row of waveforms (Fig. 5) shows the modulating waveforms which are, reading from left to right, 10 c/s sine wave, 10 c/s square wave, electrocardiograph-type waveform[1] with a period of 1 sec. The middle row shows the de-modulated waveforms with no additional smoothing, while the bottom row shows the effect on the de-modulated waveforms of a resistive capacitive time constant of 5 msec. It can be seen that for many purposes no additional filtration is needed after the storage capacitor and hence the system has a very good transient response. If some additional filtration is needed, however, a very simple filter is all that is required. If the modulating frequency is made much higher than the 10 : 1 ratio of Fig. 5, then additional filtration may usually be omitted. The electrocardiograph-type waveform demonstrates the lack of ripple at low amplitude changes and the system is most suitable for this important type of medical recording, where much of the useful information is contained in the low-level changes of the wave and little in the 'spike' ('*R*' wave) itself.

Though the de-modulating system has been described in terms of time modulated waveforms, it could be used with frequency modulated information and thus extend the advantages of the system to existing frequency modulated magnetic tapes. If the de-modulator were simply used on frequency modulated material, there would be an error

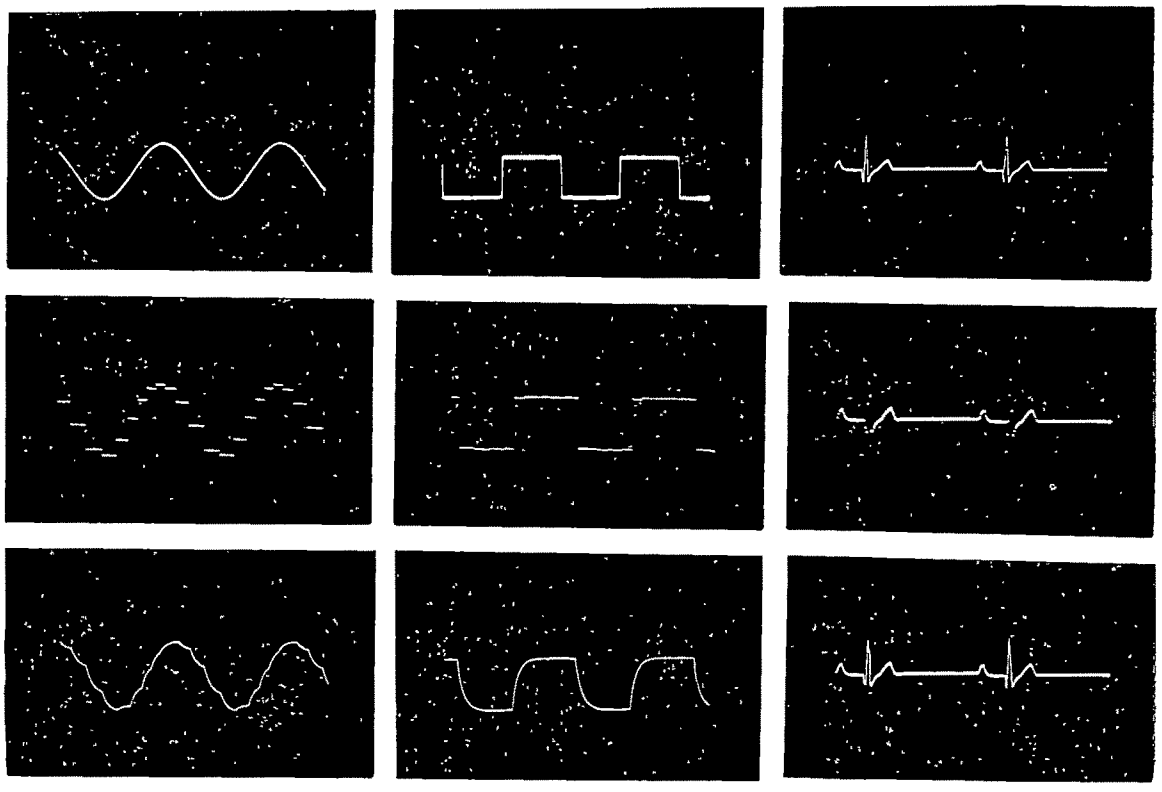


Fig. 5. Carrier mean period of 10 m/sec. Top row. modulating waveforms (10 c/s sine, square wave and 1 c/s electrocardiogram-type waveform). Middle row: de-modulated waveforms, no additional filtering Bottom row: de-modulated waveforms, 5 msec time constant filter

since frequency modulation is a function of $1/t$ (where t is the periodic time) rather than a direct function of t. This might not be too serious were the frequency deviation small, but if it were large the errors would probably be unacceptable. The errors can be eliminated by causing the generating voltage to have a value proportional to $1/t$. Work is in hand to design such a device.

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METEOROLOGY

Hydrogen Content of the Air

A series of experiments carried out by Glueckauf and Kitt in the 1950s (ref. 1) showed that hydrogen was present in the atmosphere to the extent of about 0·6 parts per million by volume, and that the concentration was not constant. It was not clear whether or not the variations were correlated with meteorological conditions.

The distribution of atmospheric hydrogen is of interest, since there is a considerable influx of protons into the upper atmosphere in the form of the solar wind. It has been suggested[2] that a proportion of terrestrial water originated as protons from the Sun. On the other hand, it is likely that some of the free hydrogen produced by photolysis of water and hydrocarbons in the high atmosphere is able to escape from the Earth. Possible sources of hydrogen at the Earth's surface include natural gas, first suggested by Paneth in 1937, coal mines and industrial activity.

Various methods have been investigated here for detecting very small concentrations of hydrogen in a volume of air sufficiently small to be collected from an aircraft or balloon. A method was also required which would give a continuous record of the hydrogen concentration at a fixed station, and an apparatus which appears to be capable of satisfying both these requirements has now been developed. It has been used to give an almost continuous record of the hydrogen concentration in Cambridge air from September 17, 1965, until the time of writing.

The apparatus is based on the phosphoric acid electrolytic hygrometer, described by Goldsmith[3]. After being dried with silica gel and P_2O_5, a slow stream of air is drawn over heated palladous oxide, and then past the hygrometer. At 300° C, PdO oxidizes hydrogen and carbon monoxide, but not, so far as is known, hydrocarbons. The whole apparatus, apart from preliminary drying agents, is enclosed in a stainless steel tube to avoid contamination from adsorbed water. A zero reference is provided by drawing the incoming air through a bed of heated alumina granules, coated with palladium, for 50 min in every 100. (This material is supplied as 'Deoxo' for removing oxygen from a stream of hydrogen,

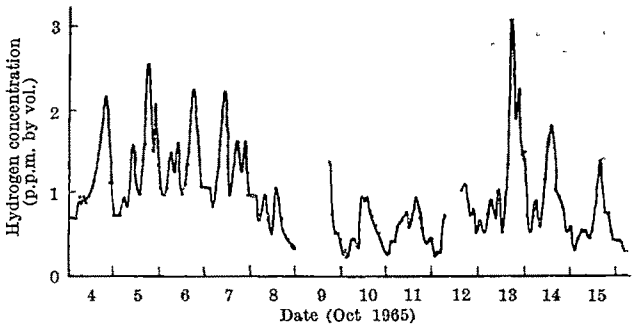


Fig. 1 Each point represents one 100-min cycle. The break on Oct. 9, 1965. was caused by a faulty time switch. On Oct. 12 the instrument was tested for sensitivity to methane

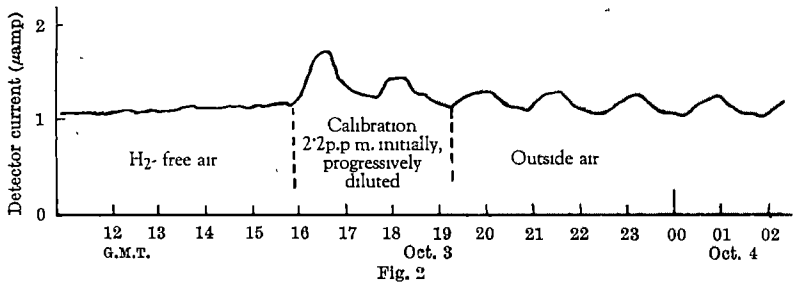


Fig. 2

but has been found equally effective the other way round.) The resulting trace on a pen recorder connected to the hygrometer is a square wave, distorted by the overall response time of the instrument, which is about 30 min.

It has been found that the hydrogen content of the atmosphere varies considerably. Extremely high values (> 2 p.p.m. by volume) are confined to the day and evening, and could well be attributed to exhaust fumes from the nearby road. During September 29–October 4 the apparatus was out of action while the P_2O_5 drying agent was changed, and the detector 'dried out' and calibrated with a sample of 0·027 c.c. of hydrogen mixed in 12 l. of hydrogen-free air (Fig. 2). It will be seen that the signal-to-noise ratio is adequate for atmospheric hydrogen measurement, while the cycling method eliminates errors due to long-term drift in the d.c. amplifier or detector. The results since then are shown in Fig. 1.

On October 12 the instrument was tested for sensitivity to methane, and found to be at least 10 times less sensitive than to hydrogen. A more precise test is intended later.

During October 4–7 the weather was fine, with clear nights, and mist in the mornings. A depression was moving past to the south, and south-easterly winds predominated. The night of October 7 was cloudy, and there was no mist on the mornings of October 8–13. During this time an anticyclone was centred over the Shetlands, with mainly north-easterly winds and cloudy nights. On October 13 and 14, fronts associated with a depression near Iceland passed over, and there was mist on the morning of October 14. Pressure began to rise again about midnight on October 14.

As far as can be judged from the short records available, it appears that anticyclonic conditions are associated with a low mean hydrogen concentration, and particularly with very low concentrations during the night. The possibility of contamination from vehicle exhausts cannot yet be ruled out, but the continuity of the readings from Sunday, October 10, to Monday, October 11, suggests that it is not serious. The wind was blowing from the direction of the road on both days. Large peaks of hydrogen concentration in the evening seem to be confined to nights on which mist forms. There is a marked diurnal variation, with a minimum about 0400 G.M.T. on most days. Whether this is of natural or artificial origin cannot yet be determined.

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GEOLOGY

Age of the Anorthosites in the Norwegian Caledonian Chain

The age of the anorthosites in the Caledonides of Norway has been much discussed, and both a pre-Caledonian and a Caledonian age has been considered[1]. This communication directs attention to a possible interpretation of the age problem, based on observations in the Möre region, central Norway, and the Bergen district, southern Norway.

According to my investigations in the Möre region, we have here an important Pre-Cambrian Caledonian stratigraphic sequence[2]. Dykes and lens-shaped bodies of metadolerites are found in the lower part of this sequence, and genetically related amphibolites occur as layers in the middle part. Titaniferous iron ore deposits occur both in the metadolerites and in the amphibolites[3].

In the eastern part of the More region, near the border of the Cambro–Silurian Caledonian Trondheim region, lenses and layers of anorthosite occur in the upper part of the Pre-Cambrian sequence[4]. Titaniferous iron ore concentrations are also found in the anorthosites.

The Bergen District. The gneiss area on the coast west of Bergen can be interpreted as being the core of a Caledonian anticline[5]. The axis of the anticline has a east-south-easterly direction, and the plunge of the fold is eastward.

The gneiss core dips to the east below a schist formation (the minor Bergen arc[6], in which the city of Bergen is situated), and the schists dip below a gneiss formation. There is no distinct boundary between the schist formation and the gneisses, and beds of mica schist and quartzite occur in the gneisses, and layers of gneiss are found in the schist formation. Bodies of metadolerite and amphibolite occur in this sequence. Magnetite and ilmenite can be present in considerable amounts within the bodies.

The gneiss formation dips to the east under an anorthosite formation. Titaniferous iron ore deposits occur in the anorthosites.

A Cambro–Silurian Caledonian schist sequence (the fossiliferous major Bergen arc) lies east of the anorthosite formation. Here we have a depression of the plunge of the anticline, and farther to the east anorthosites, gneisses and schists once more follow.

In my opinion, the gneisses, schists and anorthosites west and east of the fossiliferous Cambro–Silurian sequence form the Pre-Cambrian part of a Caledonian stratigraphic sequence. The schists are traditionally considered Cambro–Silurian, but there is much evidence in the field concerning the connexion between the schists and the gneisses. Correlations have been made between the Cambro–Silurian sequences in the Trondheim region east of the Möre region and in the Bergen district[7], and a correlation can also be made between the Pre-Cambrian sequence in the Möre region and in the Bergen district.

According to this interpretation of the Möre region and the Bergen district, metadolerites, amphibolites and anorthosites, with titaniferous iron ore concentrations, have a corresponding stratigraphic position in the two areas. Metadolerites and amphibolites are found in the lower and middle parts of the Pre-Cambrian Caledonian stratigraphic sequence, and the anorthosites occur in the upper part of the sequence. They represent basic products of the early Caledonian igneous activity in late Pre-Cambrian time[8].

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Phyllocarid Crustacean Fauna of European Aspect from the Devonian of Western Australia

A remarkable collection of about 400 specimens of phyllocarid crustaceans was made in 1963 by H. A. Toombs of the British Museum (Natural History), and K. Buller and E. Car of the Western Australian Museum. The material was collected at two localities from calcareous siltstone concretions in the Gogo Formation which yields a lower *Manticoceras* Zone (*Timanites angustus* Subzone) goniatite fauna of Frasnian 1α age, Upper Devonian[1-3]. The localities are: 1, Virgin Bore area, Gogo Station, 125° 54′ E., 18° 35′ S., and 2, Bugle Gap, 126° 02′ 15″ E, 18° 41′ S., both in the Fitzroy Basin, Kimberley Division of Western Australia. Preliminary identifications of the phyllocarids, together with the relative frequency of occurrence, are as follows:

Locality 1: *Montecaris lehmanni* Jux, abundant; *Montecaris sp. nov.* ?, a very rare, heavily tuberculate form; *Concavicaris sp. nov.* 1 *aff. C. elytroides* (Meek), rare; *Schugurocaris sp.*, rare.

Locality 2: *Concavicaris spp. nov.* 1 *and* 2, *Schugurocaris sp.*

The fauna of the Gogo Formation is stated to be "characterized by *Buchiola* and other pelecypods, numerous *Tentaculites*, small, straight nautiloids, ostracods and coccostean and crustacean remains, [the ammonoids] *Timanites* and *Koenenites*"[2]. The ammonoids and Foraminifera have recently been described, and Radiolaria and conodonts are also known to occur[3,4]. In addition to most of the foregoing, however, the locality 1 fauna in the present collection includes bryozoans, gastropods, a single specimen of a eurypterid best referred to *Rhenopterus* (determined by Dr. C. D. Waterston), and the following fish (determined by H. A. Toombs): antiarchs and arthrodires (under study by Dr. R. S. Miles), palaeoniscids (to be studied by Dr. B. Gardiner) and rhipidistians, as well as coprolites and problematica.

The phyllocarid fauna is important not only in being a unique association of genera, but also in being of European aspect. Thus *M. lehmanni* is otherwise known only from the Frasnian 1α of the Rhenish Schiefergebirge[5]. *Montecaris* is recorded from the Gedinnian and Famennian of Czechoslovakia, the Lower Frasnian of the Transvolga region of the U.S.S.R. (? = *Baituganocaris* Krestnovnikov) and possibly from the Upper Devonian of British Columbia[6]. *Eleutherocaris*, the other member of the Montecaridinae, is known only from the Frasnian Naples fauna of New York State, U.S.A. *Schugurocaris* (? = *Phasganocaris*) was described from the Lower Frasnian of the Transvolga/Urals[7], although it may occur in the Ludlovian of British Columbia and the Gedinnian and ?Emsian of Czechoslovakia[8]. *Concavicaris elytroides* occurs in the Famennian–Lower Mississippian of Oklahoma and Kentucky, but the genus ranges from Eifelian to Pennsylvanian of Czechoslovakia and the U.S.A.

The overwhelmingly European character of the Western Australian Devonian has been stressed by Teichert[9,10], and is apparent even in this small phyllocarid fauna. North American affinities are also indicated by the closely related genera and species mentioned here, but this is probably due to the derivation of both Australian and American faunas from common European stocks. Close affinity between the American and European ammonoid faunas is most clearly developed in the Frasnian[11]. Teichert's theory that the Australian faunas migrated from central Germany via south-east Asia has been criticized by Neaverson[12] but reaffirmed by Veevers[13].

The Australian and European phyllocarids mentioned here occur in platy, silty limestones or calcareous siltstones deposited in still waters in inter-reef basins[14,16]. Sedimentation was fine grained and continuous, and benthos was thereby inhibited. A platy sedimentary breccia, similar to those in the Schiefergebirge[15], has been collected from locality 2 and suggests that similar off-reef slumping took place.

This work, and the fuller description which it is hoped will follow, is being supported by National Science Foundation grant *G*24045 and the University of Glasgow.

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METALLURGY

Enhancement of Ductility in α-Uranium

Circumstances have been identified where metals and alloys undergo large plastic extensions, that is, 100–1,000 per cent elongation, before rupture[1,2]. Such materials are termed[2] 'superplastic' and can be extremely sensitive to changes in strain-rate[3], thus having a high value of the exponent, n, in the relationship between strain-rate $\dot{\varepsilon}$ and stress σ described by $\dot{\varepsilon}^n = k\sigma$, where k is a constant. Generally, n has values $< 0{\cdot}3$ in conventional tensile or creep tests even at temperatures approaching the melting-point; but certain non-ferrous alloys[3], notably those near eutectoid composition in the aluminium–zinc system which extend by several times their original length, have values of n up to $\sim 0{\cdot}7$.

Where n approaches unity, deformation tends to viscous (Newtonian) flow and, in this event, large elongations are possible since there is no enhancement of the rate of diminution of cross-sectional area. A common circumstance where metals deform by viscous flow is where vacancy creep[4] occurs, but a rapid rate of straining is generally impossible even at temperatures close to the melting-point since stresses must be sufficiently small to prevent dislocation movement.

One method of making polycrystalline uranium of random orientation flow viscously at temperatures away from the melting temperature, for example, $< 450°$ C, is to irradiate it with neutrons[5]. Under stresses about $Y/100$ and irradiation at 100° C, α-uranium exhibits a steady-state creep rate of $\sim 10^{-7}$ h^{-1} and approximately obeys a pseudo-creep law of the form, $\dot{\varepsilon} \simeq (\sigma/Y)\dot{\varepsilon}_g$, where Y is the yield strength of the material and $\dot{\varepsilon}_g$ the irradiation growth rate of individual crystals. This equation corresponds to a strain-rate exponent of unity and has been demonstrated experimentally by Roberts and Cottrell[5], who used small springs of uranium wire to facilitate measurement of the slow creep-rate. A demonstration of large elongations with tensile forces in these circumstances would not be practical despite the closeness of n to unity. (Calculations based on their results give $n \sim 0{\cdot}8$ using low-deflexion formulae to deduce the appropriate stresses and strain-rates.)

It is demonstrated here that viscous flow can be induced in lightly stressed materials with anisotropic coefficients of thermal expansion, by subjecting them to temperature changes sufficient to create 'spontaneous plasticity'[6]. It is expected, therefore, that under tensile forces and

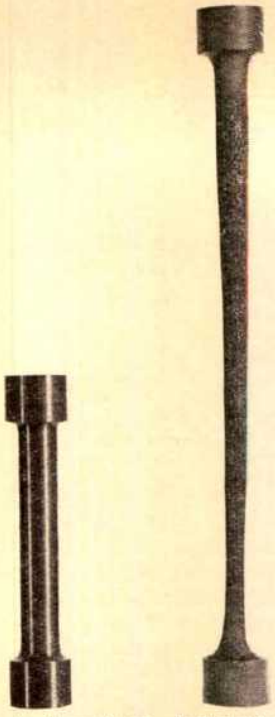


Fig. 1. Uranium tensile specimens. Left, before testing; right, after ~500 cycles between 400° C and 600° C at 11,500 g mm^{-2}

under these conditions any cross-section of a specimen will reduce in area at the same rate, independently of its initial magnitude, to produce extension without enhancing necking.

Superplastic flow in α-uranium is strikingly illustrated in Fig. 1 where the specimen has undergone ~170 per cent elongation. To achieve this the uranium specimen was held under constant stress (~1,150 g mm^{-2}) and the temperature changed from 400° C to 600° C and back to 400° C every 20 min for 7 days. The stress-level was chosen from work[7] in which a linear proportionality between strain-rate and applied stress was established for applied stresses < 2,600 g mm^{-2} and well below those generated internally.

An additional feature of this remarkable ductility in uranium is that mechanisms limiting ductility in normal tensile or creep testing are not relevant to the present case. For example, the continuous reversal of temperature creates conditions where some grain-boundary sliding is likely which reverses in direction at each half-cycle. No evidence of pronounced porosity was metallographically revealed.

Note added in proof. Extensions of 300 per cent without failure or signs of necking have since been achieved. This elongation should be compared with the failure at 55 per cent elongation of a similar specimen when tested under isothermal conditions at 600° C with the same stress.

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Crack Initiation during the Fatigue of β-Brass

Metallographic observations on the fatigue damage of β-brass in reversed bending have been reported[1]. A characteristic type of damage was observed in which short markings appeared, normal to, and sometimes on both sides of, some grain boundaries. These markings did not have the same traces as slip within the grain and formed adjacent to, but not in, the grain boundaries.

Further work has been performed on 48·25 at. per cent zinc β-brass fatigued in reversed bending both at room and liquid nitrogen temperatures. A sheet was hot-rolled to about 1·2 mm and specimens cut out in a jig. These were then recrystallized by a short anneal at 830° C and quenched into iced 10 per cent caustic soda. Surface layers were ground off, and specimens electropolished for 6 h in orthophosphoric acid. This ensured an unworked surface, as shown by the etch-pit distribution. A soft-iron pole-piece was attached to the free end of the specimen, which vibrated at resonant frequency under the influence of an alternating magnetic field. The specimen geometry resulted in a frequency from 100 to 300 c/s. The applied stress could not be measured using this arrangement, but after an initial period of work-hardening, a constant frequency was maintained, until fatigue-cracking started.

Cross-slip was frequently observed at room temperature, together with some curved and intersecting slip traces. At −196° C cross-slip was much less frequent, and slip traces tended to be straight.

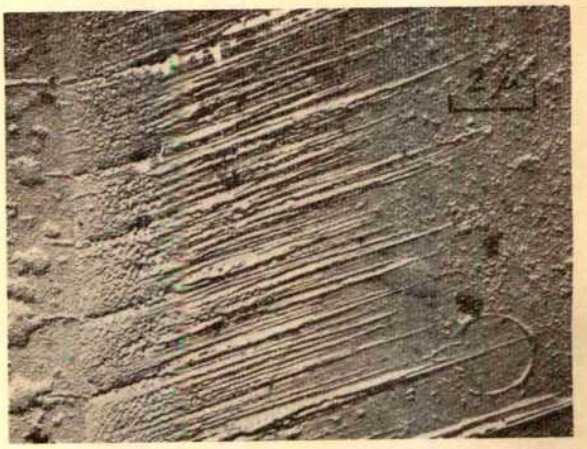


Fig. 1. Electron micrograph of region of short slip markings and cracks next to a grain boundary in β-brass (46·95 at. per cent zinc) fatigued at room temperature. C/Pt replica

At room temperature the more frequent mode of crack initiation was intergranular, either from triple points or along grain boundaries ending at the edge of the specimen. Short markings next to grain boundaries were seen as a second mode of crack initiation. These consisted of very fine markings of the type shown in Fig. 1, extending about 10μ from the grain boundary, but not necessarily on the active slip plane remote from the boundary, interspersed with 'persistent' markings or cracks often at regular intervals. Two surface analyses of these markings showed that they were incipient cracks lying on or close to {110} planes. Both slip and short markings were observed either on one side only or on both sides of the boundary. Cracks longer than others on one side of the boundary were always associated with longer cracks in the second grain. The occurrence of matching cracks across a boundary seemed to be determined by the correspondence of {110} planes in the two crystals. If {110} planes correspond, cracks may easily propagate across the boundary; if not, propagation will be difficult despite stress concentration at the crack

tip. These cracks appeared to form in succession, probably by a process producing slip intensification and a crack by the classical mode of fatigue-crack formation, in which cross-slip is known to be important[2].

The regularity of spacing of short cracks next to the boundary in β-brass seems to indicate that they are formed by the progressive relief of stresses in the grain boundary region. Occasionally one of these cracks propagated, in which case its direction changed a short distance from the boundary in the manner observed by Forsyth[3], such that the direction was influenced by the normal applied stress. Even if a crack had been initiated in the grain boundary, it was frequently found to propagate in a transcrystalline manner.

Specimens were fatigued at −196° C by standing the specimen clamping block in liquid nitrogen inside a Dewar flask. Failure always occurred by the propagation of a cleavage-type crack on {110} planes through the specimen. Again these planes did not always correspond with the active slip planes, which are variously reported as exclusively {110} (ref. 4), and almost exclusively {211} (ref. 5) at −196° C. Intergranular crack initiation was absent. A series of parallel cracks was observed from some grain boundaries (Fig. 2). These cracks had a spacing of about 10μ or more and the longer ones propagated a considerable distance into the grain. Shorter markings were also observed and, on sectioning on a second surface, no cracks were observed, but regions of transformation product were seen to correspond with the markings on the specimen surface (Fig. 3). It has been shown[6] that martensite may be induced in stable β-brass by deformation, so that the occurrence of transformation product indicates an increase in the stress in this region. No martensite was found associated with cleavage-type cracks.

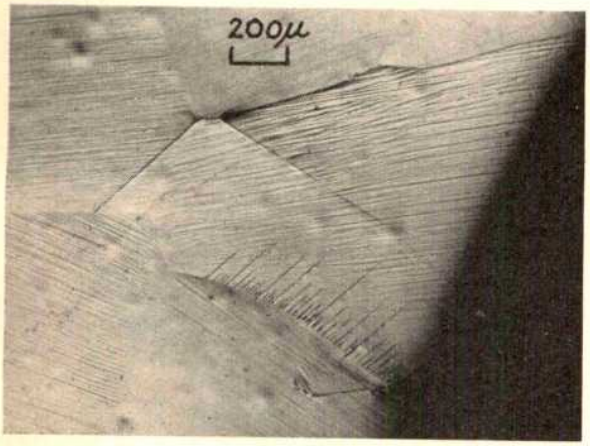


Fig. 2. β-brass (48·25 at. per cent zinc) fatigued $7{\cdot}5 \times 10^6$ cycles at −196° C showing cleavage-type cracks on {110} planes

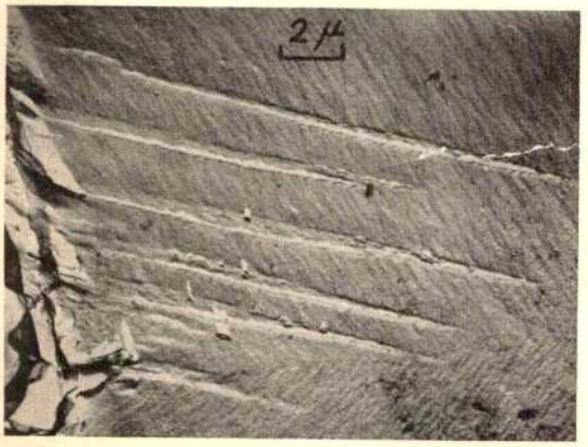


Fig. 3. Same specimen as in Fig. 2. Section through a boundary showing short markings. Transformation product, presumably martensite. Electron micrograph, C/Pt replica

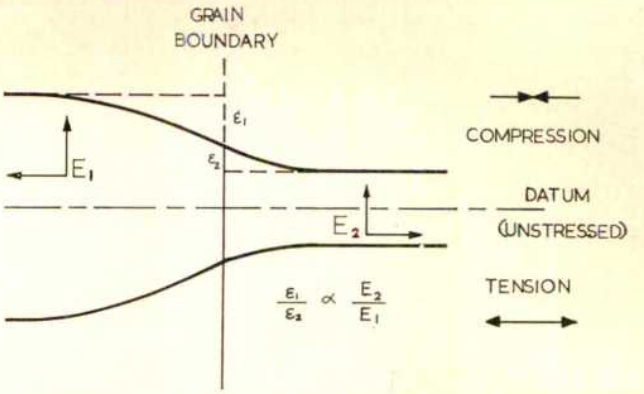


Fig. 4. Simple model to show the origin of elastic stress concentration at a grain boundary in an elastically anisotropic solid

It is proposed that stress concentrations of a sufficient magnitude to produce slip and cracks can arise at a grain boundary if the crystals composing it have a 'soft' and a 'hard' orientation to the applied stress. McManus[7] has investigated the change in the elastic moduli of β-brass with temperature and composition. For a 48·25 at. per cent zinc alloy his results give values of $0{\cdot}249 \times 10^{12}$ and $1{\cdot}94 \times 10^{12}$ dynes/cm² for Young's modulus measured in the <100> and <111> directions, respectively. The low value of $E_{[100]}$ implies weakness to (110) [$\bar{1}$10] shear, as pointed out by Zener[8]. The details of the stress distribution in the neighbourhood of the boundary between two anisotropic crystals will be complex, but a simple model may be postulated which can approximate to these conditions.

Fig. 4 shows the edge of a bicrystal composed of one 'soft' and one 'hard' crystal. On compression the two crystals deform elastically to differing extents, but to maintain coherency across the boundary the soft crystal will deform less in this region and the hard crystal more than in regions remote from the boundary. The magnitude of the lateral deformation in the grain boundary region will be inversely proportional to the ratio of the moduli in that direction. In tension the sign of the stresses will be reversed, but their magnitude will be the same as in compression. Such a stress concentration may be relieved in two ways. Either a crack can be initiated in the grain boundary between the two crystals, or a series of parallel cracks can form normal to the boundary. A grain boundary crack will be a more efficient means of stress relief, and its initiation will be particularly favoured if the boundary terminates in the edge of the specimen. Once the crack has formed, propagation criteria become important and the crack may spread on a transcrystalline path. Short cracks normal to the boundary can be formed when the boundary is limited by triple points. Once such a crack has formed, the stress distribution near it will be changed, and the stress concentration moved along the boundary, allowing more slip to occur and new cracks to form. Short slip markings are sometimes found associated with cracks along the grain boundary. It is possible that an intergranular crack may produce a stress concentration favouring the formation of short slip markings, or that fracture may be completed in a region of short cracks next to the grain boundary by the propagation of an intergranular crack. Short slip markings will be most pronounced a short distance from the boundary because coherency constraints between the two grains will limit the formation of large slip steps at the grain boundary itself. The occurrence of cleavage-type cracks at low temperatures in β-brass is consistent with the increase in brittleness of body-centred cubic metals with falling temperature.

In order to test the importance of elastic stress concentrations at grain boundaries in β-brass during fatigue, the orientation of a number of crystal pairs the mutual boundary of which shows fatigue damage is being determined and examined, with the view of differentiating between the conditions necessary to initiate a crack in an intergranular manner, or to initiate short cracks next to grain boundaries.

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CHEMISTRY

Matrix Isolation of Unstable Halogen Radical Ions

We have recently obtained spectroscopic evidence for the formation of reduced metal valency states in γ-irradiated 5·4 M H_2SO_4 glasses at 77° K (reaction (1)

$$M^{n+} + e^-_m \rightarrow M^{(n-1)+} \qquad (1)$$

where M^{n+} = Mn^{2+}, Co^{2+}, Ni^{2+}, Ga^{3-}, Ag^+, Cd^{2+}, In^{3+} and Tl^+)[1]. In the course of this work we have also investigated non-reducible solutes such as sodium chloride and sodium bromide.

Chloride. At relatively high concentrations of sodium chloride (0·1–1·0 M), γ-irradiation at 77° K produced the optical absorption shown in Fig. 1 *A*. The broad band with λ_{max} = 444 mμ and $G\varepsilon^{444m\mu}$ = 3,000 ± 400 is associated with the sulphuric acid, and has been assigned[1,2] to an oxidized radical entity, designated $SO_4^{-}\cdot$, which is formed by either direct action of the γ-rays or electron transfer to OH radicals or, possibly, H_2O^+ ions. The peak with λ_{max} = 335 mμ was observed only when chloride was present in the glass and increased with [NaCl]. In addition sodium chloride reduced the yield of $SO_4^{-}\cdot$. On warming, the 335 mμ absorption increased (Fig. 1 *B*) in the region 135°–150° K, and this is attributed to an increased yield of the entity, rather than a change of extinction coefficient with temperature, since the enhanced absorption persisted on re-cooling the glass to 77° K. Fig. 1 also shows that this change on warming was accompanied by a decrease in the $SO_4^{-}\cdot$ absorption. At 150°–154° K, the 335 mμ band decayed and a new absorption with λ_{max} = 230 mμ (Fig. 1 *C*) was formed, which was still present, although with smaller optical density, at room temperature (Fig. 1 *D*). Re-cooling the system to 154° K gave the larger absorption again (Fig. 1 *C*).

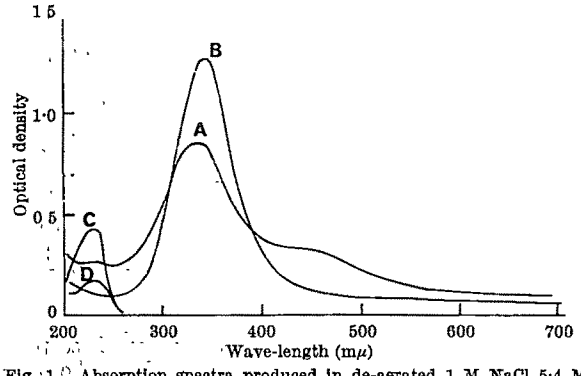


Fig. 1. Absorption spectra produced in de-aerated 1 M NaCl 5·4 M H_2SO_4 by γ-irradiation (1·14 × 10^{19} eV ml.$^{-1}$) at 77° K. *A*, *B*, *C* and *D* are the absorptions at 77°, 135°, 154° and 293° K respectively

The absorption with λ_{max} = 335 mμ is very similar to that reported by Anbar and Thomas[3], in the pulse radiolysis of dilute sodium chloride solutions at *p*H 3, and attributed to Cl_2^-. The assignment of the absorption in the glass phase to this entity seems reasonable, and is consistent with the reduction in the yield of $SO_4^{-}\cdot$, which suggests the formation of a new oxidized species. An electron transfer from a chloride ion to a $SO_4^{-}\cdot$ radical is thought to account for the additional Cl_2^- formed on warming. For [NaCl] = 0·5 M, $G\varepsilon^{335m\mu}_{Cl_2^-}$ (glass phase, 77° K) = 2,600 ± 300 and in view of the room temperature value for $\varepsilon^{\lambda max}_{Cl_2^-}$ of 12,500 ± 1,000[3], this corresponds to a value for $G_{Cl_2^-}$ of ~ 0·2. The high solute concentrations required to give Cl_2^- suggest that it is only formed in cases where two chloride ions are adjacent in the glass and no migration is necessary. Hornig and Osberg[4] have shown by infra-red studies that crystalline HCl exists as antiparallel zigzag chains of hydrogen-bonded molecules, at temperatures below 98° K. If the chloride in H_2SO_4, which exists partly as HCl at room temperature, forms similar chains on freezing to 77° K, a situation will occur in which the chlorine atoms are suitably positioned for the formation of Cl_2^-. The way in which Cl_2^- decays at 150°–154° K is uncertain, but if Cl_2 is a product, equilibrium (2) will exist, and the absorption represented in Fig. 1 *C* may be due to a mixture of

$$Cl_2 + Cl^- \rightleftarrows Cl_3^- \qquad (2)$$

Cl_2 and Cl_3^-[5]. Furthermore, the reversible change in the magnitude of the absorption on warming may be a result of the temperature dependence of the equilibrium constant.

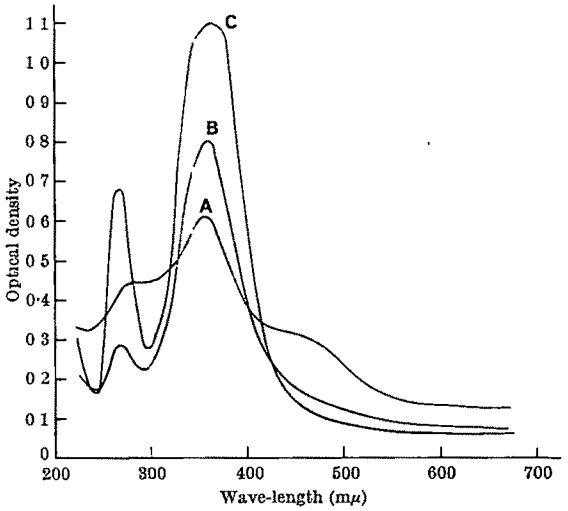


Fig. 2. Absorption spectra produced in de-aerated 0·4 M NaBr 5·4 M H_2SO_4 by γ-irradiation (1·14 × 10^{19} eV ml.$^{-1}$) at 77° K. *A*, *B*, and *C* are the absorptions at 77°, 132° and 143° K respectively

Bromide. The behaviour of γ-irradiated NaBr – H_2SO_4 glasses was found to be similar to that described for NaCl – H_2SO_4 glasses. The absorption produced by irradiation at 77° K is shown in Fig. 2 *A*; and the new absorption with λ_{max} = 355 mμ can be correlated with the liquid phase spectrum of Br_2^- obtained by the pulse radiolysis of bromide solutions[6]. Warming to 130°–145° K enhanced the 355 mμ peak and the absorption present as a shoulder (λ_{max} 270 mμ) at 77° K was also observed to increase (Fig. 2 *B* and *C*). This latter absorption is attributed to Br_3^-, on the basis of its similarity to that reported in the liquid phase[6]. At 150°–155° K the 355 mμ absorption decayed while that due to Br_3^- increased further and remained on warming to room temperature.

Iodine. Sodium iodide is oxidized by concentrated sulphuric acid at room temperature and the strong optical absorption of the product I_3^-, made spectroscopic work impossible at high solute concentrations. However, glasses made from 5·4 M sulphuric acid saturated with iodine ($[I_2] \sim 10^{-3}$ M) gave on γ-irradiation at 77° K a weak new absorption in the region of 380 mμ, which is where an absorption due to I_2^- has been observed in liquid water[7]. The formation of mobile electrons in γ-irradiated acid glasses has already been established[1,8] and in this case it appears that a simple reduction is occurring (reaction (3)).

$$I_2 + e^-_m \rightarrow I_2^- \qquad (3)$$

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Stability of a Radioactive Drug—Tetrasodium 2-Methyl-1,4-naphthaquinol Diphosphate labelled with Tritium

This communication describes the decomposition by self-irradiation of the radioactive drug tetrasodium 2-methyl-1,4-naphthaquinol diphosphate labelled in the 5,6 and/or 7 positions with tritium. The possible radiotherapeutic use of this labelled compound in the treatment of certain types of cancer is at present under investigation by Prof. J. S. Mitchell and colleagues at the University of Cambridge[1,2].

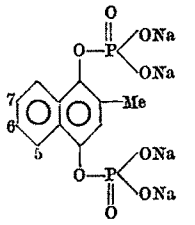
The peculiar property of radioactive organic compounds to undergo decomposition by self-irradiation on storage presents a difficult, but not an insoluble, problem in the use of such compounds. This property is particularly evident with tritium compounds, where almost all the β-radiation energy is self-absorbed by the compound.

Some of the governing principles and modes by which decomposition by self-irradiation can occur have already been published[3–5]. Decomposition by natural decay of the tritium atoms to helium-3 cannot be controlled, but fortunately this effect contributes little (less than 6 per cent per annum) to the total decomposition in relatively small molecules such as the naphthaquinol diphosphate. It is the secondary effects which are the more important and difficult to control; that is, the interaction of the labelled molecules with activated species such as free radicals or the excited products of primary decay. For radiotherapeutic use tritiated tetrasodium 2-methyl-1,4-naphthaquinol diphosphate is required at the highest possible specific activity, a requirement at which such secondary decomposition effects are most pronounced.

No method has yet been found to eliminate completely the decomposition, but certain measures can be taken to minimize its effect[6]. These are: (*a*) reduction of the effective radiation dose to the compound; for tetrasodium 2-methyl-1,4-naphthaquinol-T diphosphate this is done by storing the compound in aqueous solution; (*b*) lowering the temperature, thereby minimizing secondary (temperature dependent) effects by reducing the thermal activation energy of the excited species; (*c*) the addition of free radical scavengers; this method was not investigated for the tritiated drug which was required at high chemical purity.

A few results concerning the stability of tetrasodium 2-methyl-1,4-naphthaquinol diphosphate, labelled in the 6-position with tritium and stored in aqueous solution at 0° C, were published in 1962 (ref. 7). The results of a more extensive investigation are summarized in Table 1.

Table 1. Decomposition of Tritiated Tetrasodium 2-Methyl-1,4-naphthaquinol Diphosphate in Isotonic Saline Solution



Position of tritium atoms	Specific activity (c./mmole)	Radioactive concentration (mc /ml.)	Storage temp. (°C)	Storage time (months)	Energy absorbed (rads)	G(-M)	Decomposition (per cent)
6	27	390	2	10	$3\,5 \times 10^7$	0·3	75
6	29	390	2	3	$1·1 \times 10^7$	0·3	25
6	29	390	−80	3	$1·1 \times 10^7$	0·5	40
6	27	2	−196	6	$1·1 \times 10^6$	0·06	10
6	27	240	−196	6	$1·3 \times 10^7$	0·06	10
6	27	2	−196	6	$1·1 \times 10^5$	0·03	5
6	27	266	−196	6	$1·4 \times 10^7$	0 08	12
6, 7	10	1	20	0·33	3×10^3	8	23
6, 7	10	1	20	0·17	$1·5 \times 10^3$	7	10
6, 7	54	300	−40	1	$2·7 \times 10^6$	1	50
6, 7	20	247	−196	3·5	$7·8 \times 10^6$	0 14	9
6, 7	55	360	−196	3	$9\,7 \times 10^6$	0 06	9
6, 7	59	655	−196	2·75	$1·6 \times 10^7$	0·07	10
5, 6, 7	87	560	2	6	$3·0 \times 10^7$	0·2	95
5, 6, 7	76	360	2	3	$9\,7 \times 10^6$	0·1	20
5, 6, 7	76	360	−80	3	$9·7 \times 10^6$	0·14	30
5, 6, 7	65	344	−80	0·5	$1·6 \times 10^6$	1·5	45

The tritiated drug was stored under nitrogen in sterilized isotonic saline solution, and the radiochemical purity determined after various time intervals, by paper chromatography (Whatman No. 1) using *n*-butanol : water : acetic acid (12 : 5 : 3) and *n*-propanol : ethanol : water : conc. ammonia (30 : 30 : 39 : 1) as the eluting solvents.

The rate of decomposition in solution at room temperature (20° C) at concentrations above 100 mc./ml. is approximately 10 per cent in 24 h, at these high specific activities. The rate is reduced to 1–2 per cent per day if the radioactive concentration is lowered to 1 mc./ml. It is therefore essential to keep solutions of the drug cool. However, an interesting observation is that the decomposition rate in frozen solutions (at temperatures down to − 80° C) is greater than in solution at + 2° C, a phenomenon also observed with solutions of tritiated thymidine[6,9]. The reason for this increased (and erratic) decomposition in the frozen state may be due to 'clusters' of radioactive molecules in the ice giving rise to local pockets of highly concentrated radiation. On several occasions when examining frozen solutions of the tritiated quinol, brown areas were observed in the ice as if decomposition was occurring at definite centres. A similar explanation for the increased decomposition rate of tritiated thymidine in frozen solutions has been proposed by Apelgot *et al.*[10–12].

Storage of the tritiated drug in solution at liquid nitrogen temperature (−196° C) reduces the rate of decomposition by a factor of at least five and at this low temperature the decomposition rate is almost independent of the radioactive concentration in the range studied.

Less than 10 per cent tritium is actually lost from the molecule by exchange into the aqueous solution during 1 year storage, but the decomposition of the tritiated tetrasodium 2-methyl-1,4-naphthaquinol diphosphate gives rise to three major decomposition products which have not yet been identified.

In the use of this tritiated drug, necessarily required at very high specific activity for radiotherapy, these problems of decomposition by self-irradiation should be borne very much in mind by the cautious experimenter.

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Chromatographic Separation of Argon and Oxygen using Molecular Sieve

THE chromatographic separation of argon and oxygen is difficult, and existing methods depend on one of the following techniques:

(1) Separation on a molecular sieve 5 Å column at 18° C (ref. 1) and at −80° C (refs. 2–4).

(2) Chromatographing argon and oxygen as a composite peak on molecular sieve 5 Å at 100° C; removal of the oxygen with a reducing agent allows the argon peak only to be developed[5–7].

(3) Separation of argon and oxygen on a column of firebrick loaded with 8 per cent by weight of blood at 20° C (refs. 8 and 9).

It was noticed[10] that after a molecular sieve column was heated above the normal reactivation temperature of 300° C a partial separation of argon and oxygen resulted at a column temperature of 25° C. The operating parameters for optimum separation of argon and oxygen and the constancy of resolution with time have been investigated at this Laboratory using a molecular sieve 5 Å column. A helium ionization chromatograph[11] was used for this work.

The chromatographic column (5 ft. × 3/16 in. internal diameter stainless steel tube) was packed with Linde molecular sieve 5 Å (calcium alumino-silicate, 30–60 mesh) which had been previously heated to 400° C in air. The column was coupled to a helium ionization chromatograph and the molecular sieve heated to a minimum of 400° C for 24 h with a pure helium purge. Higher activating temperatures (for example, 500° C) do not appear to increase the efficiency of separation and the use of normal cylinder helium (no purification) does not give the required degree of reactivation.

A column treated in the foregoing manner gave the following relative retention times:

Hydrogen	Argon	Oxygen	Nitrogen	Methane
1·00	1·57	1·89	2·89	4·07

for a column temperature of 75° C and a helium flow rate of 67 ml./min. It was found that slight deterioration in resolution occurred over a 5-day period. This deterioration produced an increase in the argon retention time and a decrease in the oxygen retention time. Reactivation of the column at 400° C for 16 h gave the original retention times.

The effect of 'swamping' a small peak by a large amount of one component was found to be negligible for the range of volumes tested. A return to baseline between the argon and oxygen peaks was found for amounts of 20 μml. argon and 200 μml. oxygen and for 200 μml. argon and 19 μml. oxygen.

The sensitivity for both argon and oxygen was of the order of 1 μml. where the sensitivity is defined as five times the noise-level. A detector voltage of 500 V was used.

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Microwave Stimulation of Chemical Reactions

DURING an investigation of the effects of microwave energy on organic reactions, a novel application of such radiation has been disclosed. One well-known difficulty in the examination of solid-state pyrolytic reactions is that, perforce, for each particle, the products move from the site of their production against a positive temperature gradient. For any sizable particle, therefore, the pyrolysate is subject to further decomposition and, in many cases, it is impossible to isolate the primary products.

It has now been found that certain organic solids, notably coal and some similar substances, are effectively decomposed by microwaves and that the nature of the cracked product is different from that produced by purely thermal means. The reason for this difference is, I believe, because the microwaves decompose the material from the 'inside' outwards.

Small pieces of gas coal, about 1 c.c., are more or less instantaneously decomposed into a heavy tar precursor and a soft carbonaceous residue.

A Philips 7292 magnetron has been used and our equipment is a development of that of McTaggart[1]. The solids (1–3 g) are placed across the field in a wave-guide, within silica tubes, through which flows a slow current of nitrogen; the pressure is atmospheric. In the case of a typical gas coal, on switching on the anode current, within 2 sec heavy brown vapours were evolved which 'flowed' down the exit tube. The fog could not be effectively condensed by a liquid air trap but was collected by a packed plug of cotton wool. When allowed to deposit on surfaces, the fog coalesced to an orange varnish-like film, whereas, 'upstream' from the sample, the interior of the containing tube was covered for a short distance with a fine brown powder which, under the microscope, appeared as resinous particles. Immediately adjacent to the sample, the wall of the tube was covered with a carbon mirror, the inner surface of which was coated with fine carbon black. The residual coke was soft and friable. We have been unsuccessful, so far, in isolating the more conventional liquid products, such as are contained in 'light oil', etc.

The duration of the complete decomposition is so short (about 15 sec) that a conventional thermocouple placed outside the walls of the reaction tube does not have time to respond, although, if the power (700 W) be left on for 20–30 sec, the coke is raised to incandescence. Presumably, at lower power, uniform slow heating can be achieved.

With all coals, the decomposition was immediate and complete. With oil shales, there was often an induction period before reaction, whereas essentially aliphatic substances, such as torbanites, appeared transparent.

At this stage, only preliminary work has been done, but it is obvious that some organic substances are highly absorbent of microwave energy. As the energy can be directed and made to penetrate, it is thought that microwaves may provide a new tool with which to explore certain pyrolysis reactions without some of the inherent disadvantages of more conventional means.

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Free Energy Functions and Deviations in Thermochemical Data Storage for Inorganic Compounds

THE compilation of the results of measurements of thermochemical properties is a task which is growing rapidly in magnitude as our experimental skills are advanced. Whereas a few years ago the reliable data were few, the rate of publication has now achieved a scale requiring the preparation of specialized abstracts.

In considering means for preserving the results, the twin aspects of ease of access and accuracy of presentation often work against one another, and the earlier methods of using polynomial equations with temperature as a variable are becoming superseded by tables of the so-called free-energy function. The proponents of this latter method[1] argue that the function:

$$\text{free-energy function} = -\left[\frac{G^\circ_T - H^\circ_{298}}{T}\right]$$

varies smoothly with temperature and thus interpolation is simple when only a small number of values of the free energy function at a few chosen temperatures, that is, at 500° intervals, are given.

This is true, but the free-energy function does introduce a severe measure of inflexibility into the system of data storage. This is because the entropy at 298° K and the heat capacities which were available at the time of the compilation are indissolubly locked into the values of the free-energy function which are tabulated. A change in any one of these renders the values inaccurate and therefore the tabulations useless.

The polynomial method of presentation has the merit of clearly demonstrating which data were used, while the free-energy function does not. On the other hand, it leads to very tedious calculations the value of which is sometimes doubted before one begins, because the experimental data are not very accurate.

Ellingham[2] proposed a very elegant solution to the problem when he showed that a simple two-term equation gave a close approximation to the actual values of free energy changes for a number of simple reactions. This procedure has the merit of being not very time-consuming in application, and being a sufficient approximation in most instances in the light of the data which are available.

It is proposed here to elaborate a simple method of using data in which a high precision can be obtained for the values of free-energy changes when the data will allow, in which the relative importance of the degrees of elaboration can be compared, and in which new and more accurate data can be immediately incorporated.

It is obviously useful to separate the contributions of low-temperature calorimetry (up to 298° K) to our knowledge from the high-temperature data. These measurements are usually made quite independently of high-temperature measurements both in location and in time, and hence they are likely to change at a different time. We thus choose as our starting point a simple two-term free-energy equation based on ΔS°_{298} and ΔH°_{298}:

$$\Delta G^\circ_T = \Delta H^\circ_{298} - T\Delta S^\circ_{298} \qquad (1)$$

Provided there are no heat-capacity contributions above room temperature, this equation provides a simple means of calculating high-temperature free-energies. However, there always will be such contributions and we shall define the 'free-energy deviation' which is the difference between the correct value and that predicted by equation (1) in terms of heat-capacity equations. Kelley[3] has recommended from a survey of the available data the use of a heat-capacity equation of the general form:

$$Cp = \alpha + \beta T + \gamma T^{-2} \qquad (2)$$

We shall use this form in evaluating the 'free-energy deviation function':

$$\text{free-energy deviation} = \frac{1}{T}(H_T - H_{298}) - (S_T - S_{298})$$

$$= \frac{1}{T}\int_{298}^{T} Cp.\mathrm{d}T - \int_{298}^{T}\frac{Cp}{T}.\,\mathrm{d}T$$

Obviously for a chemical reaction:

Δ free-energy deviation = free-energy deviation (products) − free-energy deviation (reactants).

Calling now the ratio $298/T$ by the symbol θ and using Kelley's equation for the heat capacity:

$$\text{free-energy deviation} = \alpha(1 - \theta + \ln\theta) + \beta T\left(\theta - \tfrac{1}{2} - \frac{\theta^2}{2}\right) + \frac{\gamma}{T^2}\left(\frac{1}{\theta} - \frac{1}{2\theta^2} - \tfrac{1}{2}\right) \qquad (3)$$

or for the polynomial:

$$Cp = \alpha + \beta T + \gamma T^2 + \delta T^3 \ldots \nu T^{n-1}$$

the general term in free-energy deviation is:

$$\nu T^{n-1}\left\{\frac{\theta^{n-1}}{n-1} - \frac{\theta^n}{n} - \frac{1}{n(n-1)}\right\} \qquad (4)$$

If the free-energy deviation function is multiplied by the temperature, then the value of the free energy which must be added to equation (1) to get the accurate value is obtained. The functions in θ are universal functions to all systems whereas the individual values of α, β, γ, etc., belong to each substance characteristically. Table 1 shows the values of the θ functions multiplied by temperature at 200° K intervals and these should be used in a graphical form to aid interpolation.

An example of the use of these figures is as follows:

For the free energy of formation of La_2O_3 (*c*) from lanthanum solid and oxygen, Coughlin[4] presents the following data:

For $2La(c) + \frac{3}{2}O_2 \rightarrow La_2O_3(c)$

$\Delta H^\circ_{298} = -428,570$ cal ; $\Delta S^\circ_{298} = -72{\cdot}62$ cal/deg.

Heat capacities:

$La_2O_3\,(c)$; $Cp = 28{\cdot}86 + 3{\cdot}10 \times 10^{-3}T - 3{\cdot}28 \times 10^5 T^{-2}$ cal

$2La\,(c)$; $Cp = 12{\cdot}34 + 3{\cdot}2 \times 10^{-3}T$ cal

$\frac{3}{2}O_2\,(g)$; $Cp = 10{\cdot}74 + 1{\cdot}50 \times 10^{-3}T - 0{\cdot}60 \times 10^5 T^{-2}$ cal

and derives the equation:

$$\Delta G^\circ_T = -431,120 - 13{\cdot}31\,T\log T + 0{\cdot}80 \times 10^{-3}T^2 + 1{\cdot}34 \times 10^5 T^{-1} + 112{\cdot}36T \text{ cal.} \qquad (5)$$

This yields at the melting-point of lanthanum (1,153° K):

$\Delta G^\circ_{1153} = -347,290$ cal $\Delta H^\circ_{1153} = -425,290$ cal

$\Delta S^\circ_{1153} = -67{\cdot}57$ cal/deg.

Using equation (1) we would find:

$$\Delta G^\circ_{1153} = -344,770 \text{ cal}$$

A difference of 2,520 cal has appeared between the value calculated from the simple equation using 298° K. data only and the more complicated equation (5). For many purposes this is a small difference which can be neglected, especially since the uncertainties in the original data are often as big as this.

Table 1 FREE-ENERGY CONTRIBUTIONS USING KELLEY'S HEAT CAPACITY FUNCTION: $Cp = \alpha + \beta T + \gamma T^{-2}$ cal

$T°$ K	$T(1-\theta+\ln\theta)$cal	$T^2\left(\theta-\frac{\theta^2}{2}-\frac{1}{2}\right)$	$\frac{1}{T}\left(\frac{1}{\theta}-\frac{1}{2\theta^2}-\frac{1}{2}\right)$
400	−2	$-0{\cdot}48\times10^4$	$-1{\cdot}74\times10^{-4}$
600	$-11{\cdot}8\times10$	$-4{\cdot}60\times10^4$	$-8{\cdot}16\times10^{-4}$
800	$-29{\cdot}8\times10$	$-12{\cdot}63\times10^4$	$17{\cdot}5\times10^{-4}$
1,000	$-51{\cdot}8\times10$	$-24{\cdot}50\times10^4$	$-26{\cdot}9\times10^{-4}$
1,200	$-76{\cdot}6\times10$	$-40{\cdot}30\times10^4$	$-40{\cdot}3\times10^{-4}$
1,400	$-106{\cdot}9\times10$	$-60{\cdot}50\times10^4$	$-49{\cdot}5\times10^{-4}$
1,600	$-138{\cdot}6\times10$	$-85{\cdot}0\times10^4$	$-59{\cdot}0\times10^{-4}$
1,800	$-173{\cdot}6\times10$	$-112{\cdot}9\times10^4$	$-74{\cdot}4\times10^{-4}$
2,000	$-212{\cdot}0\times10$	$-144{\cdot}4\times10^4$	$-93{\cdot}0\times10^{-4}$

The fact that the correction involved by the use of heat-capacity data is so small can readily be seen by working out $\Delta\alpha$, $\Delta\beta$ and $\Delta\gamma$ from the heat-capacity equations, looking for the values of the θ functions at the appropriate temperature and working out the products. These are −4,040 cal for the first term, +593 cal for the second, and +858 cal for the third term. The balance −2,589 cal, obtained by this graphical procedure, is close to that calculated by the use of the polynomial equation (5). The procedure proposed here, however, shows the relative importance of each part of the heat capacity functions in contributing to the correction to the simple equation (1).

The present procedure has not only the advantage of making each contribution to the final value clear, but if at any time new data become available these can readily be used without destroying a lot of effort.

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Effect of Temperature on the γ-Radiolysis of Water Vapour

IT has been suggested recently[1] that observed yields[1,2] ($g(-H_2O) \sim 7 \pm 1$) from the radiolysis of water vapour in the presence of chemical scavengers could be explained by considering the energetics of charge neutralization of the hydrated species $H_3O^+(H_2O)_n$ (reaction 1) instead of the simple hydronium ion H_3O^+ (reaction 2):

	$\Delta H°$ (kcal/mole)	
$H_3O^+(H_2O)_n + e^- \rightarrow H + (n+1)H_2O$	> -120	(1)
$H_3O^+ + e^- \rightarrow 2H + OH$	$-(22–26)$	(2)

Support for this hypothesis is provided by the mass spectrometric investigations of Kebarle and Hogg[3] and of Knewstubb and Tickner[4], who have identified hydrates of the form $H_3O^+(H_2O)_n$, with $n = 1 - 7$, using α-radiation and electric discharges in water vapour. This communication presents preliminary evidence, principally from the radiolysis of water vapour containing 5 mole per cent *cyclo*hexane, with some supporting evidence from work with the system water/ammonia, which demonstrates the chemical consequences of reactions (1) and (2), and enables us to estimate the enthalpy change in the general hydration reaction:

$$H_3O^+ + nH_2O \underset{3b}{\overset{3a}{\rightleftarrows}} H_3O^+(H_2O)_n \qquad (3)$$

Values of $G(H_2)$ from the cobalt-60 γ-radiolysis of water vapour ($P \sim 1{\cdot}5$ atm.) were measured at a concentration of 5 mole per cent C_6H_{12}, which is sufficient to scavenge all the H atoms produced[2], and these are corrected for the contribution from energy absorption in C_6H_{12} itself, which was determined by irradiating pure C_6H_{12} over the same range of temperature (Fig. 1). The yield of H_2 from C_6H_{12} is constant ($G(H_2) = 7{\cdot}93 \pm 0{\cdot}19$) at temperatures from 120° C to 340° C and agrees with Freeman's data[5] ($G(H_2) = 8{\cdot}0$) for α-irradiation at 120° C, but it increases rapidly at higher temperatures. This observation, which was incidental to the main object of the work, is similar to the effect of temperature on the radiolysis of benzene[6].

With C_6H_{12} as scavenger the mean yields for H_2 from water vapour are $G(H_2) = 7{\cdot}0 \pm 0{\cdot}4$ over the temperature range 120° –240° C and $G(H_2) = 10{\cdot}6 \pm 0{\cdot}6$ at temperatures >300° C. We attribute the increased yield to a decrease in the stability of the ion-molecule clusters, with a change in the charge neutralization process from reaction (1) to reaction (2). If this is so, then below 240° C $G(H_2) = g(H) + g(H_2) + g(e^-)$, and above 300° C $G(H_2) = g(H) + g(H_2) + 2g(e^-)$ ($g(X)$ represents primary yields and $G(X)$ represents experimental yields). Thus the difference in the observed yields gives $g(e^-) = 3{\cdot}6$, which agrees with the theoretical value of $g(e^-) = 3{\cdot}57$–$3{\cdot}28$ based on $W(H_2O) = 28$–30 eV (refs. 7–9). The interpretation of data from work with H_2O/NH_3 is complicated by the possibility of proton transfer to NH_3, with a consequential change in the nature and energetics of the charge neutralization reactions, but the difference in H_2 and N_2 yields between 240° C and 340° C leads to a value of $g(e^-) \simeq 3{\cdot}5$. Moreover, yields for the disappearance of NH_3 in the system $H_2O/NH_3/O_2$ at 120° C and 300° C are respectively $G(-NH_3) = 6{\cdot}52 \pm 0{\cdot}16$ and $G(-NH_3) = 9{\cdot}83 \pm 0{\cdot}33$ with the difference giving $g(e^-) = 3{\cdot}3$. The results with added NH_3 thus support the conclusions from the work using C_6H_{12}. Increased pressure in the region of 300° C causes a reduction in the enhanced yield, an effect which is predictable from the equilibrium represented by equation (3), giving added confidence to the foregoing interpretation. Quantitative data on the effect of pressure will be published at a later date.

While the shape of the curve for $G(H_2)$ production from H_2O/C_6H_{12} as a function of temperature is not yet closely established, we can estimate the change in enthalpy involved in the general hydration reaction (3). On the basis of the foregoing ideas we write the general equation:

$$G(H_2) = g(H) + g(H_2) + 2xg(e^-) + (1-x)g(e^-) \qquad (4)$$

where x represents the fraction of ions present as H_3O^+ (or a low molecular weight hydrate) which gives reaction (2) on charge neutralization, and $(1-x)$ is the fraction of ions which are neutralized in reaction (1). By taking values for $G(H_2)$ in the temperature-sensitive region from the broken line in the figure, we have calculated relative equilibrium constants as a function of temperature:

$$K_{\text{Dissoc.}} = [H_3O^+]/[H_3O^+(H_2O)_n] \qquad (5)$$

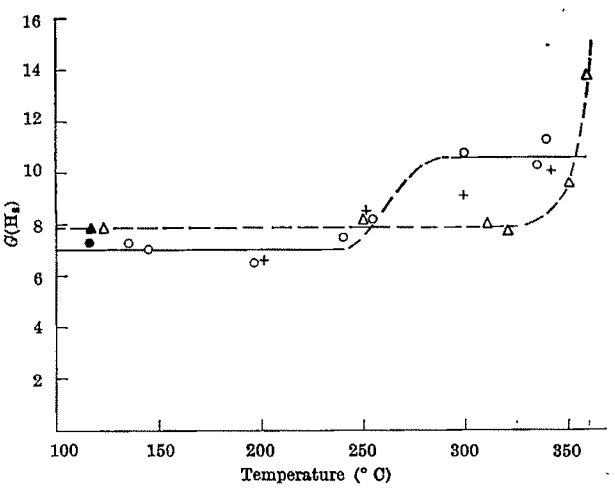


Fig. 1. Cobalt-60 γ-irradiation of water vapour as a function of temperature. Pressure ~1·5 atm; $[C_6H_{12}] \sim 5$ mole per cent. ○, Water vapour corrected for contribution from C_6H_{12}; △, pure C_6H_{12}; ●, ref. 2; ▲, ref. 5; +, H_2O/NH_3, $[NH_3] \sim 0{\cdot}7$ mole per cent

which leads to a value of $\Delta H^0 = 78$ kcal mole^{-1} on solution of the van't Hoff equation. This enthalpy change represents a minimum value for the heat absorbed in the endothermic process 3*b*, and we can write the general equation:

$$H_3O^+(H_2O)_n + 78 \text{ kcal} \rightleftarrows H_3O^+(H_2O)_{n-y} + yH_2O \xrightarrow{e^-} 2H + OH + nH_2O \quad (6)$$

where $y \leqslant n$.

Standard enthalpies of formation (kcal/mole) of the ground state in the gas phase are[10]: $\Delta H^0_f(H_2O) = -58$, (H) = 52, (OH) = 10, and the proton affinity of H_2O is 167–171 kcal/mole[11]. From these data and equation 6 we obtain $\Delta H^0_f(H_3O^+) = 138$ kcal/mole and $\Delta H^0_f(H_3O^+(H_2O)_n) = 38–58n$ kcal/mole. Thus from equation (3) the full hydration energy of H_3O^+ in the vapour phase is calculated to be ~ 100 kcal/mole which agrees with the best estimated value[12] for solvation of H_3O^+ in liquid H_2O, that is, ~ 100 kcal/mole. In view of the uncertainties involved the excellent agreement is probably fortuitous, since (*a*) we are considering only a general equilibrium constant represented by equation (5) instead of the individual stages:

$$H_3O^+(H_2O)_n \rightleftarrows H_3O^+(H_2O)_{n-1} + H_2O \rightleftarrows H_3O^+(H_2O)_{n-2} + 2H_2O \quad (7)$$

(*b*) we neglect any solvation energy of e^-, which will be small in the case of C_6H_{12}/H_2O vapour, and (*c*) the shape of the temperature-sensitive part of the curve is not closely established, although any change based on the existing data will not have a major effect on the estimated values for ΔH. The data of Kebarle and Hogg[3] indicate that each hydration step in the vapour phase is exothermic by ~ 17 kcal/mole, so that in the foregoing representation $n = 6–7$. This is probably a reasonable estimate on the basis of enthalpy changes, as additional H_2O molecules will not contribute significantly to the solvation energy. Moreover, additional molecules could be removed from the hydration shell at temperatures $< 240°$ C without resulting in a distribution of hydrates containing enough lower members to give an observable contribution from reaction (2) on charge neutralization. These data, together with those of Kebarle and Hogg, suggest that the H_3O^+ ion with one or possibly two molecules of hydration can give 2 H atoms and 1 OH radical in the vapour phase, on charge neutralization.

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Separation of Oxygen Isotopes by Gas Chromatography

Separation of some volatile isotopic molecules can be achieved either by gas solid chromatography on packed columns[1] or by gas liquid chromatography on capillary columns[2]. The small differences in vapour pressure of isotopes require columns with a high number of theoretical plates, which is more easily realized with open tubular columns.

Working at low temperature is recommended as the vapour pressure differences between isotopic compounds are usually enhanced; however, the use of partition chromatography is limited as it requires the stationary phase to be liquid. The use of adsorption capillary columns, recently reported[3], offers the advantage of working in any temperature range, and of obtaining the high resolving power such as is required for isotopic separations.

Glass capillaries for gas solid chromatography, prepared according to a method previously described[4], have been used for the separation of the isotopic molecules, oxygen-16/oxygen-18.

To prepare adsorbing surfaces, the inner walls of the glass capillaries were etched with an alkaline solution (20 per cent sodium hydroxide) for 6 h at 100° C, and washed to neutrality. The column was dried and activated under a flow of nitrogen at 200° C for 48 h. An internal layer of active silica (about 10μ thick) was uniformly distributed inside the column (170 m long and 0·28 mm internal diameter). The efficiency of this column, measured from the oxygen peak at the working conditions of the chromatogram shown in Fig. 1, is approximately 350,000 theoretical plates.

A low-temperature thermostat was used, which consisted of a metallic case containing the capillary column, a small electric heater, and a fan. The case was immersed in a Dewar flask containing liquid nitrogen. By changing the current supplied to the heater with a variable transformer it was possible to regulate the temperature inside the thermostat within the range $-190°$ to $-120° \pm 1°$ C. The temperatures were measured with an iron-constantan thermocouple. Pure nitrogen dried in a molecular sieve trap was used as carrier gas. An electron capture detector, operating in the d.c. system, was used to detect the presence of oxygen. An additional flow of 50 ml./min of nitrogen was added at the exit of the column as scavenging gas. By means of a special valve and a splitter at the column inlet, 0·1–0·3 ml. samples of each component were introduced in the ratio of approximately 1 : 100.

Table 1 shows the operating conditions and the results obtained at different temperatures. Retention volumes have been corrected for the 'column dead volume' measured from the oxygen peak obtained at room temperature. The values of the retention volumes ratios of the two isotopic molecules are compared with the ratios of the vapour pressures at the same temperatures[5].

Table 1

Temperature (° K)	Pressure (mm Hg)	Flow rate (ml./min)	$V_{R_{16}O_2}$ (min)	$V_{R_{18}O_2}/V_{R_{16}O_2}$	$P_{^{16,16}O}/P_{^{16,18}O}$
91·2	12·5	0·5	55·8	1·0036	1·0053
84·2	90	0·45	99·3	1·0065	1·0065
80·2	130	0·72	176	1·0107	1·0073

From a plot of a log of these ratios against $1/T$, it is possible to obtain the differences in the enthalpy and entropy changes between the isotopic species; for the chromatographic process these are, respectively:

$$\Delta H_{18_{O_2}} - \Delta H_{16_{O_2}} = 7·79 \text{ cal}$$

$$\Delta S_{18_{O_2}} - \Delta S_{16_{O_2}} = 0·1 \text{ e.u.}$$

From the data shown in Table 1 it is apparent that at the higher temperature the retention volume ratio for oxygen-18/oxygen-16 is smaller than the vapour pressure ratio for the mixed molecule, and consequently the separation obtained by gas chromatography is less than that expected from the relative volatilities; however, at lower temperature the differences are larger, and the separation factors achieved by gas chromatography increase more rapidly than the vapour pressure ratios. This behaviour might be explained by the fact that at low temperatures the differences in the heats of adsorption have a larger effect than the differences in the heats of condensation.

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BIOCHEMISTRY

Evidence of Complex Formation between Chlorpromazine and Different Xanthene Dyes

The formation of charge-transfer complex between flavin mononucleotide (FMN) and serotonin has been examined in detail[1], as a good example for a complex of biochemical interest and of characteristic colour change. Chlorpromazine can also form complexes with FMN and rhodamine *B*[11,12].

It is also known that at low temperature the xanthene dyes and ascorbinic acid form in pyridine a rather stable semiquinone[2]. This product results in the appearance of a new band in the absorption spectra of the stains at 410–430 mμ. This new band represents a blue shift.

The xanthene dyes are adequate substances to evoke different forms of photosensitization[3] for one photo-induced muscular contraction[4–7] which can be well inhibited by chlorpromazine[7].

We observed characteristic colour changes when chlorpromazine and eosin *Y*, erythrosin *B* or rose bengal were mixed. The colour change has been observed in water and in 96 per cent ethanol.

The change of the spectral absorbancy is demonstrated by the difference of extinction between the stain and stain–chlorpromazine mixture solutions in Figs. 1 and 2. The shape of the curve in ethanol suggests a solvent effect.

The peak of the new substance is shifted in a redward direction with about 30–40 mμ. In the case of rose bengal and erythrosin it is at 570 and 555 mμ respectively, so these substances cannot be identical with the semiquinone demonstrated by Zepalov *et al.*[2]. The low ability of eosin to react with chlorpromazine causes difficulties in measuring the spectral changes in the chlorpromazine–eosin mixtures.

As these stains can emit fluorescence at 580, 560 and 542 mμ respectively, we measured the fluorescence emission of the stains, exciting them with a mercury lamp (Beckman fluorescence attachment). We were able to measure the quenching of the fluorescence at the aforementioned wave-lengths. The quenching is demonstrated in Fig. 3 plotted against concentration of chlorpromazine. The rate of the quenching is in direct relationship with the photo-sensitor ability: rose bengal > erythrosin > eosin.

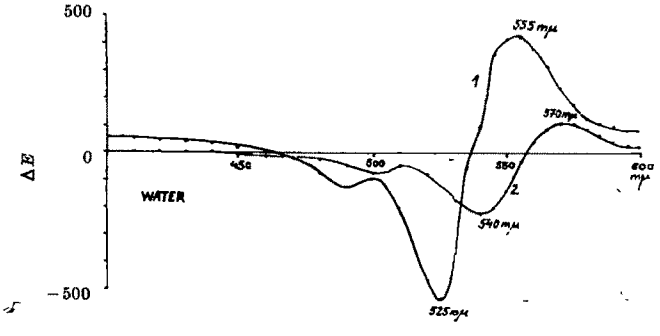


Fig. 1. Difference between extinction of the stain–chlorpromazine and stain solutions in water plotted against wave-length. Curve 1, *E*[25 μg/ml. erythrosin + 25 μg/ml. chlorpromazine], *E*[25 μg/ml. erythrosin]; curve 2, *E*[10 μg/ml. rose bengal + 10 μg/ml. chlorpromazine]; *E* [10 μg/ml. rose bengal]

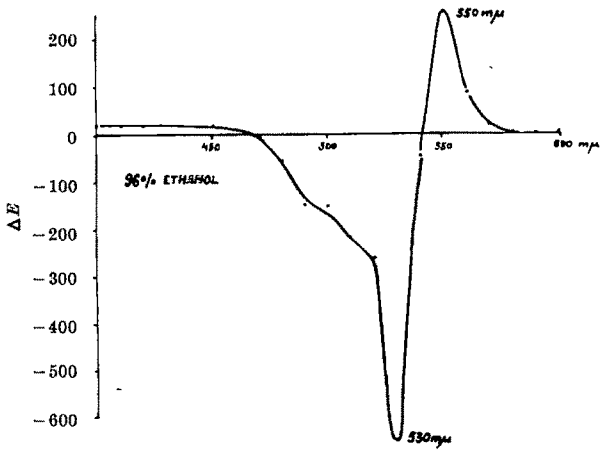


Fig. 2. Difference between extinction of erythrosin–chlorpromazine and erythrosin solutions in ethanol of 96 per cent plotted against wave-length. 25 μg/ml. rose bengal and 500 μg/ml. chlorpromazine

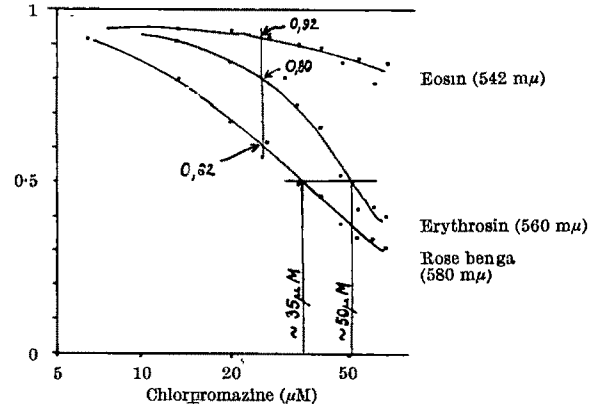


Fig. 3. Quenching of fluorescence of the stains at their peak by chlorpromazine plotted against chlorpromazine concentration Concentration of the stains was 25 μM Quenching of 50 per cent is at about 35, 50 μM chlorpromazine concentration in the case of rose bengal and erythrosin respectively. Quenching at 1 mol. ratio is 0 92, 0 80, 0 62 in the case of eosin, erythrosin and rose bengal respectively

The first investigations were carried out in filtered lake water, as the inhibitor effect of chlorpromazine against xanthene dyes-sensitized contraction had also occurred in water. Our chlorpromazine preparation in water above 35 μg/ml. showed opalescence, indicating its bad dispersity, so we measured the optical properties below the critical concentration and against chlorpromazine-blank. In ethanol the colour changes are smaller, and bleaching is higher than in water. These factors favour another reaction-kinetics.

Considering the observed phenomena two alternatives emerge. The bad solubility of chlorpromazine might indicate an adsorption connected with colour change. The good stability of the new complex after having mixed the participating substances contradicts this mechanism. There is another phenomenon which proves the formation of a new substance. One can observe two bands in the fluorescence emission spectrum of the rose bengal, at 580 and at 460 mμ. In the presence of chlorpromazine the fluorescence at 460 mμ increases and at 580 mμ decreases. The increase and decrease of fluorescence plotted against wave-length are demonstrated in Fig. 3. The decrease of fluorescence at 580 mμ is probably connected with the loss of the original stain concentration and with the increase of absorbancy of the new substance just at 580 mμ. The increase in fluorescence at 460 mμ probably originates from the complex substance alone or from chlorpromazine.

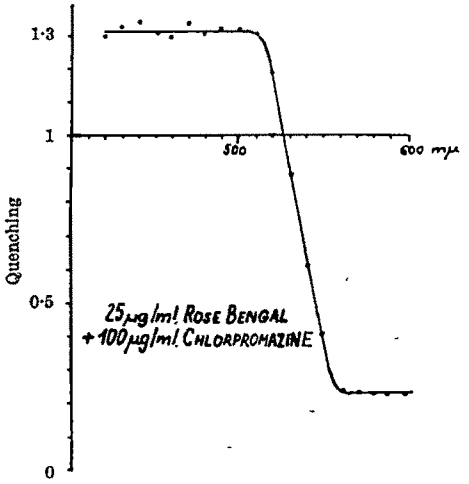


Fig. 4. Quenching and amplification of fluorescence of rose bengal by chlorpromazine plotted against wave-length

It is known that if flavin and adenine are attached similar shifts and quenching occur in the absorption and emission spectra of the flavin[8]. These changes are attributed to the formation of a complex[9]. The complex formation between FMN and dihydrothioctyl was found to be connected to similar spectral changes[10].

These changes were demonstrated as charge-transfer complex because no electron spin resonance signal was obtained[10].

Therefore, we suppose that the lilac coloration of the xanthene dyes–chlorpromazine mixture is due to the formation of charge-transfer complex and that there is sufficiently strong interaction between the participating substances to abolish the photosensitization by stains[7].

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Is the Antihaemophilic Globulin a Protein ?

The antihaemophilic globulin (AHG), in electric fields migrating in the β_2-fraction, and being precipitated from blood plasma by alcohol in fraction I (ref. 1) is generally considered to be a protein. Our laboratory has for a number of years been occupied with the problem of its purification[2,3] with the final aim of contributing to the elucidation of its structure, and, if possible, to the mechanism of its interaction with other blood-coagulation factors.

For a long time ECTEOLA-celluloses of various capacities were used in the purification experiments; later on we turned to DEAE-celluloses because of their greater protein-binding capacities. During these experiments it became clear that the higher the ion-exchange capacity, the higher the salt concentration which could be used during the absorption and washing stages of the chromatographic procedure, without the AHG leaving the column unadsorbed. In other words, AHG could still be adsorbed at relatively high salt concentrations, which permitted most of the proteins and other impurities to flow through the column unadsorbed. A rather high ion-exchange capacity, namely, 3·2 m.equiv./g, was then found in DEAE–'Sephadex *A*–25' (coarse). This enabled us to work at a sodium chloride concentration of 1·6 per cent. As long as the columns were operated at about their maximum AHG-binding capacity (fraction I from 100 ml. plasma per 15 g of DEAE–'Sephadex') the preparations obtained showed absorption spectra similar to those of proteins, but lacking the peak at 280 mμ (cf. ref. 4). When the amount of plasma per column was decreased, the protein character of the absorption spectrum disappeared altogether (Fig. 1).

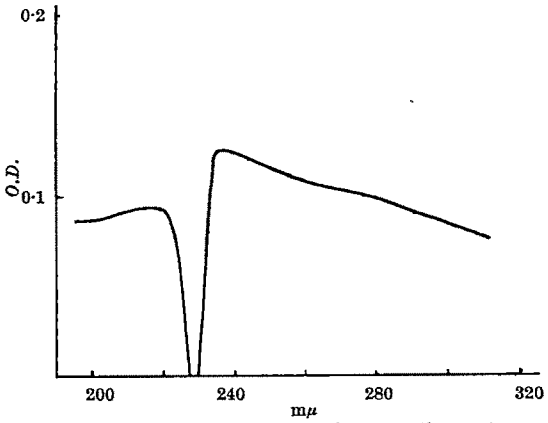


Fig. 1. Absorption spectrum of an AHG-preparation; 1·5× normal activity

Nevertheless, such preparations had biological activities of about 150 per cent (normal plasma = 100 per cent). Some of them were hydrolysed with 5·7 N hydrochloric acid at 105° C for 18 h. Two-dimensional paper-chromatograms[5], developed with ninhydrin, showed the presence of only 4 faint spots, which could not very easily be identified with any of the well-known amino-acids.

Since the homogeneity of the AHG-preparations is by no means certain, speculations about the amino-acids and the absorption spectra are not helpful; further investigations are necessary to clear up the present situation.

I thank Prof. Dr. S. van Creveld and Prof. Dr. W. H. H. Tegelaers for their advice.

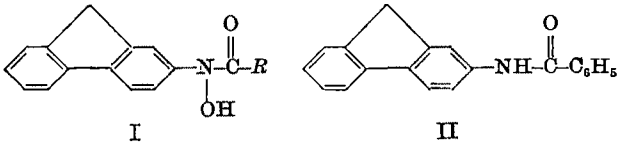
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N-Hydroxy-2-fluorenylbenzamide, an Arylhydroxamic Acid with High Carcinogenic Activity

For investigations of the mechanism of action of carcinogenic arylhydroxamic acids we have prepared the new hydroxamic acid, *N*-hydroxy-2-fluorenylbenzamide (*N*-OH-2-BAF) (I*a*), an analogue of the carcinogen, *N*-hydroxy-2-fluorenylacetamide (*N*-OH-2-AAF) (I*b*). We have tested this compound for its carcinogenicity and



I (*a*) $R = C_6H_5$ (*b*) $R = CH_3$ II

found it to be an extremely active agent. We wish to report here the results of tests in which the carcinogenic activities of *N*-OH-2-BAF, of its parent amide, *N*-2-fluorenylbenzamide (BAF) (II), and of *N*-OH-2-AAF (ref. 1) for the young female Holtzman rat have been compared.

N-OH-2-BAF, m.p. 187°–190° C (corr.), was prepared either by the partial catalytic (5 per cent Pd-C) hydrogenation of 2-nitrofluorene in ethyl acetate in the presence of benzoyl chloride or by the benzoylation of 2-fluorenylhydroxylamine[2] in pyridine. *N*-OH-2-BAF was purified through its copper chelate which formed when the compound was treated with cupric acetate in methanol. The chelate was decomposed with hydrogen sulphide in the usual manner. The compound was characterized by the elemental analysis (calculated for $C_{13}H_{15}NO_2$: C, 79·73; H, 4·98; N, 4·65; found: C, 79·44; H, 4·88; N, 4·77) and by its ultra-violet (λ_{max}^{MeOH} 303 mμ (ε, 21 100) with shoulders at 295 and 283 mμ; λ_{min}^{MeOH} 242 mμ (ε, 8 300)) and infra-red spectra (potassium bromide; ν_{O-H} = 3,160 cm^{-1}, $\nu_{C=O}$ = 1,610 cm^{-1}). BAF, m.p. 223°–224° C (corr.), was prepared by the benzoylation of *N*-2-fluorenamine in pyridine[3]. *N*-OH-2-AAF, m.p. 147°–148° C (corr.), was prepared by the standard procedure[1]. The carcinogenicities of the compounds were tested on young female albino rats (purchased from the Holtzman Rat Co., Madison, Wisconsin) which initially weighed 50–70 g. The animals were caged individually at 26° C and maintained on a 20 per cent casein diet[4], except those injected with *N*-OH-2-AAF, which were fed a grain diet[1]. The weight of each rat was recorded before each injection and once weekly after the injections had been completed. The compounds (4·5 mg/100 g body-weight) were administered intraperitoneally three times weekly for 4 weeks as a suspension in saline containing 1·75 per cent gum acacia[1]. Because of the short survival time of the rats treated with *N*-OH-2-BAF on this schedule 6 animals were given the compound (4·5 mg/100 g body-weight) twice weekly for 6 weeks by intraperitoneal injections in a separate series. Autopsies were performed on all animals immediately after death or killing. The tumours and certain other tissues (liver, kidney and lungs) were fixed in buffered formalin and sectioned. The sections (5–6μ thickness) were stained routinely with haematoxylin-eosin.

Table 1. CARCINOGENICITY OF *N*-2-FLUORENYLBENZAMIDE (BAF), *N*-HYDROXY-2-FLUORENYLBENZAMIDE (*N*-OH-2-BAF), AND *N*-HYDROXY-2-FLUORENYLACETAMIDE (*N*-OH-2-AAF) FOR THE YOUNG FEMALE HOLTZMAN RAT

Compound administered*	No. of rats used	Average amount of compound administered (mmoles/rat)	No of rats surviving At 8 weeks	No of rats surviving At 20 weeks	No. of tumour-bearing rats†	% Tumour incidence of rats surviving 8 weeks
None	12	—	12	12	0	0
BAF	12	0·20	12	12	1 (1)	8
N-OH-2-BAF	17‡	0·15	8§	3	8 (12)	100
N-OH-2-AAF	12	0 23	12	11	11 (26)	92

* All compounds (4·5 mg/100 g body-weight) were administered intraperitoneally in 0·9 per cent sodium chloride containing 1 75 per cent gum acacia (ref. 1).
† The numbers in parentheses are the total number of tumours
‡ Eleven rats received *N*-OH-2-BAF three times a week for 4 weeks and 6 twice a week for 6 weeks.
§ Nine animals died before 8 weeks with no incidence of tumours (average survival time equals 4·5 weeks).

The data of Table 1 show that tumours were present in all animals which received *N*-OH-2-BAF and which survived 8 weeks. 8/8 rats had intraperitoneal sarcomata which occupied the entire intraperitoneal cavity in several instances. Ascites were present in all these animals and hydronephrosis from ureteral obstruction was observed in two cases. The tumours invaded the intestinal wall, diaphragm, myometrium, lymph nodes and vascular spaces. Metastasis to the lungs was noted in one instance. The histological appearance of the tumours was similar to that of the intraperitoneal sarcomata obtained in adult male and female rats with greater amounts of *N*-OH-2-AAF than the quantities of *N*-OH-2-BAF used in the present experiments[1]. 3/8 Rats developed mammary tumours similar in appearance to the adenofibroma of the rat breast[5]. In contrast, the rats receiving BAF were free of tumours except for one animal which had an adenofibroma of the mammary gland. The low carcinogenicity of BAF observed here agrees with the assessment of its carcinogenicity by other workers[6]. 11/12 rats receiving *N*-OH-2-AAF had mammary tumours as previously described by Miller *et al.*[1]. No intraperitoneal sarcomata were noted in the young female rat with this dosage of *N*-OH-2-AAF confirming the results of Miller *et al.*[1]. Judging from the tumour incidence and the amounts of compound which induced neoplasia, *N*-OH-2-BAF appeared to be as potent a carcinogen as *N*-OH-2-AAF. In addition, *N*-OH-2-BAF depressed the rate of growth of the rats after the first injection and the depression persisted throughout the lifetime of the animals (Fig. 1).

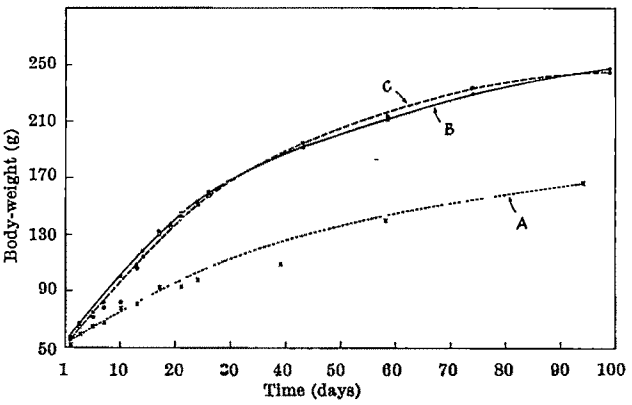


Fig. 1. The average weight gain of young female rats receiving *N*-hydroxy-2-fluorenylbenzamide (curve *A*) and *N*-2-fluorenylbenzamide (curve *B*) intraperitoneally in saline containing 1·75 per cent gum acacia at a dose-level of 4 5 mg of compound/100 g body-weight. Curve *A* represents the average weight of 17 rats at 7, 14 and 28 days, respectively, and of 12, 8 and 5 rats at 42, 56 and 90 days, respectively. Curve *C* is the average gain in weight of rats injected with the vehicle

These experiments indicate that an aromatic amide of low carcinogenicity (BAF) can be converted into a highly potent carcinogen (*N*-OH-2-BAF) by synthetic *N*-hydroxylation. We have reported previously that a non-carcinogenic metabolite of *N*-2-fluorenylacetamide, *N*-(7-hydroxy-2-fluorenyl)-acetamide[6,7], was transformed into the carcinogen, *N*-hydroxy-(7-hydroxy-2-fluorenyl)acetamide, by synthetic *N*-hydroxylation[8]. These results show clearly that arylhydroxamic acids are more active carcinogens than the respective parent amides, and they support the view that *N*-hydroxylation is essential in the metabolic generation of a molecular species which is more carcinogenic than the administered aromatic amide[1,8].

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Components of the Spore Wall of *Pithomyces chartarum*

The cell walls of fungi are known to contain large quantities of carbohydrate material, mainly composed of polymers of glucose, galactose, mannose and amino-sugars[1,2], but only in a few cases have the components been isolated from the walls and characterized. It is generally accepted that glycoproteins account for a considerable portion of the wall material in yeast[3], as they do in the hyphal walls of the filamentous fungus, *Pithomyces chartarum*[4]. Very little is known about the composition of spore walls; those of *Aspergillus oryzae* contain carbohydrate, protein and nucleic acid[5], and those of *Mucor rouxii* have carbohydrate, protein, lipid and pigment[6], the carbohydrate having been isolated as glucan, mannan and chitosan.

Spores, a gift from Dr. A. Smith, Ruakura Animal Research Station, New Zealand, were broken by disruption with glass beads in a Braun disintegrator (Shandon, Ltd., London), freed from cytoplasmic contamination by repeated sedimentation in 0·1 per cent sodium chloride and isolated after exhaustive dialysis followed by freeze-drying. The black pigmented product was shown to contain 45 per cent carbohydrate, 3·6 per cent bound hexosamine and 18·8 per cent protein (Table 1). Treatment with 10 per cent trichloroacetic acid failed to extract any material from the isolated walls (in contrast 15 per cent of the hyphal wall of the same organism was solubilized as phosphoglycoprotein under the same conditions[4]). Isolation of the cell wall components was achieved by extraction with anhydrous ethylenediamine at 37° C. On removal of the solvent, two chemically distinct fractions were obtained (Table 1), one soluble in ethylenediamine and soluble in water, *SWA* (4 per cent), the other soluble in ethylenediamine but insoluble in water, *SWB* (16 per cent). A residue, insoluble in ethylenediamine, was also recovered, *SWR*. Each fraction was shown to contain carbohydrate and protein. In addition the final residue contained all the wall pigment. The nature of the carbohydrate portion of each fraction was shown by examination of the acid hydrolysates using thin-layer chromatography. Each fraction contained glucose and glucosamine; in addition, *SWA* contained galactose and mannose. Ion-exchange chromatography of the acid hydrolysates of the three fractions showed the presence of fifteen amino-acids, in variable amounts, namely, asp, glu, ser, gly, ala, leu, ile, pro, val, lys, arg, thr, phe, his, met. Electrophoresis of the two extracted fractions on glass-fibre paper using formic–acetic acid buffer, 0·05 M, *p*H 2·0, indicated co-migration of the carbohydrate and protein in each case. In addition, *SWA* was chromatographed on a DEAE-cellulose column. Two fractions were obtained, one of which was not absorbed in *tris*-hydrochloric acid buffer, 0·001 M, *p*H 8·1, the other being eluted with the same buffer, 0·05 M, *p*H 8·1. The two glycoprotein fractions which were isolated from the spore walls were found to contain the same sugar and amino-acid components as the corresponding glycoproteins similarly extracted from the hyphal walls of the same organisms—the main difference being that the spore wall components contained less carbohydrate and more protein than the hyphal wall fractions. No evidence was found for the presence of the highly phosphorylated type of glycoprotein existing in the hyphal wall[4].

Table 1. Composition of Spore Wall Fractions

	Spore wall	*SWA*	*SWB*	*SWR*
Carbohydrate (per cent)	45 (40)†	50 (70)	61 (64)	43 (70)
Nitrogen (per cent)	3·3 (4·5)	4·3 (3·8)	4·0 (4·2)	3·1 (2·5)
Hexosamine[8] (per cent)	3·6 (10)	0·66 (1·9)	0·35 (4·6)	3·1 (10)
Protein (difference)	17·5 (25)	26·6 (22·8)	24·9 (23·8)	17·7 (10)
Glucose*	+	+	+	+
Galactose	+	+		
Mannose	+	+		
Amino-acids	+	+	+	+

* +, Present; −, absent or trace in acid hydrolysates.
† Figures in brackets are values for corresponding hyphal wall fractions, namely, whole wall, 2*A*, 2*B* and residue.

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PHYSIOLOGY

Reflex Activation of the Soleus Muscle of the Decerebrate Cat by Vibration

The primary afferent ending of the mammalian muscle spindle is highly sensitive to vibration[1–4]. In the presence of fusimotor activity it may discharge an impulse on each cycle of vibration applied to the muscle tendon, for vibrations of frequencies up to 300–400/sec and of amplitudes of a fraction of a mm (refs. 2 and 4). In the decerebrate cat, maintained stretch of an extensor muscle usually evokes from it a prolonged reflex contraction—the classical stretch reflex[5]. This reflex is generally believed to depend on the excitation of the primary endings of the spindle by the stretching of the muscle. Thus, it might be anticipated that vibration would also evoke a 'stretch reflex' in the decerebrate cat. The present experiments show that it does, indeed, do so.

The experiments were carried out on cats decerebrated under deep ether anaesthesia by transecting the mid-brain between the colliculi. The soleus muscle was investigated and the technique was generally similar to that described earlier[6]. The vibration was applied to the muscle tendon by connecting it to a vibrator (Pye-Ling, *V* 50), the steady current through which was modulated sinusoidally. The amplitude of vibration was determined by microscopic observation using a micrometer eyepiece. The contraction of soleus was recorded by connecting its tendon to an isometric myograph which was mounted on the end of the vibrator and through which the vibration was transmitted. The myograph utilized a semi-conductor strain gauge. Its maximum frequency of response was deliberately restricted to about 25/sec by electrical low-pass filters. This served to remove from its output the tension changes resulting directly from the vibration. The electrical activity of soleus was recorded from a pair of wires placed on its belly about 3 cm apart; virtually all the nerves in the leg, thigh and hip regions were severed except for that to soleus, so there was little doubt that the recorded activity did arise from soleus[6]. The leg was held by drills through the distal ends of the tibia and of the femur. The summed compliance of this fixation and that of the moving element of the vibrator was about 1 mm/kg; this comparative lack of rigidity is immaterial for the demonstration of the present effects.

Fig. 1 shows the typical reflex response of soleus to vibration. The initial length of the muscle was a few mm short of the maximum extension possible physiologically, but in this case there was no appreciable maintained tonic stretch reflex; the initial tension was 80 g wt. Vibration of 10μ amplitude at 200/sec elicited a contraction of 100 g wt. accompanied by appreciable electrical activity of the muscle. 100μ amplitude vibration caused a contraction of 500 g wt., accompanied by a massive

electrical discharge. There was no doubt that these effects were due to reflex excitation of the muscle, for they were abolished by cutting its nerve or by stimulating the central end of the cut peroneal nerve which reflexly inhibits the soleus motoneurones[6]. Fig. 2 shows this reflex inhibition of the response to vibration, demonstrating that the contraction cannot have been due to direct excitation of the muscle fibres by the vibration; following the inhibition there was a 'rebound' increase in the contraction of the muscle. It can be assumed that the receptors responsible for at least the major part of the vibration response lie in the muscle itself, for the rest of the hind-limb was widely denervated. More significantly, similar vibration applied to the denervated tibialis anterior muscle did not produce contraction of soleus, showing that the response was not due to spread of the vibration to receptors in the pelvic region which had not been completely denervated.

High-speed recording of the electromyogram showed that the minimum latency of the response was usually just under 10 msec from the beginning of the vibration when its amplitude was large (100μ). On cessation of the vibration there was always an abrupt decrease in the reflex within 10–20 msec. Sometimes, however, the contraction did not immediately return to its previous level, but remained larger for 1–2 sec. The reflex response to vibration was found for frequencies of vibration from 50 to 500/sec. Increasing the amplitude of vibration at any frequency increased the response up to an approximately constant maximum tension. Surprisingly, altering the frequency of vibration had relatively little effect on the value of this maximum. When the vibration was applied for periods of up to a minute, the reflex usually persisted throughout. The reflex still occurred after ablation of most of the cerebellum, including the whole of its anterior lobe, but it was abolished by section of the spinal cord at *C*1. It was also abolished by an anaesthetic dose of 'Nembutal' (40 mg/kg). In all this, its behaviour parallels that of the tonic stretch reflex elicited by maintained extension of the muscle. Thus, vibration appears to be another way of activating the tonic stretch reflex. In other experiments maintained reflex responses to vibration were seen also for the gastrocnemius and tibialis anterior muscles.

There is little doubt that the primary endings of the muscle spindles were being excited by the vibrations used[2,4], and this provides an adequate explanation for the observed effects. However, only direct recording of their discharges can show whether or not other muscle receptors are also excited, and thus whether they too may play some part in the observed reflex responses. If vibration should activate the primary endings virtually alone[2], then it would prove a useful tool for extending the investigation of their central effects. Recently, in man, vibration has been shown to produce interesting motor effects when applied to a muscle contracting voluntarily[7].

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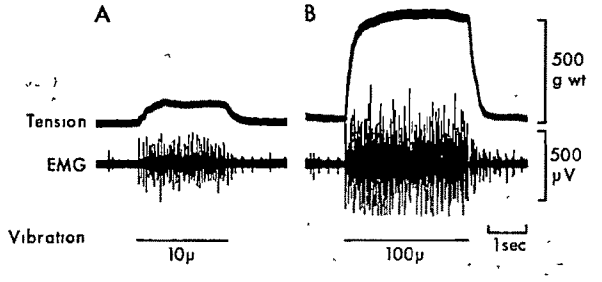


Fig. 1. Reflex response of soleus to vibration of its tendon at 200/sec. *a*, The peak to peak amplitude of vibration was approximately 10μ; *b*, amplitude, 100μ The vibration marker is diagrammatic

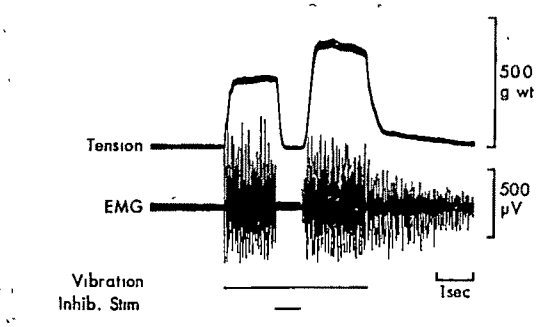


Fig. 2. Reflex inhibition of the response to vibration. During the bottom signal marker (diagrammatic) the reflex contraction of soleus elicited by 50μ vibration at 200/sec sec was inhibited by stimulating the ipsilateral peroneal nerve at 150/sec

Reversibility of an Electrical Correlate to the Process of Learning

The cockroach normally holds a chemically stimulated antenna with the opposite fore-leg during the process of cleaning. Immediately after the amputation of both fore-legs the insect is unable to hold its antennae with one of the middle legs. This, however, the cockroach can do about one week after operation.

In previous communications it was concluded that a cockroach with amputated fore-legs must learn to stand on three of the remaining legs when holding the antenna with one middle leg. It was also demonstrated that a nervous pathway impermeable to a single pre-synaptic stimulus (in 90 per cent of normal adult cockroaches) becomes permeable to this stimulus in all the insects at a certain moment during the process of learning[1,2]. The preparation investigated consists of a descending pathway from the oesophageal ganglion to one of the motor nerves of the hind leg. This pathway has synaptic connexions in the third thoracic ganglion.

The electrical response observed in 10 per cent of the cockroaches considered normal in a preceding communication[2] presented the characteristics of labile response, as described by Luco[3]. It shows a long and variable ganglionic delay (4·10 msec ± 0·232 *S.E.*, ref. 2). During learning, about the seventh day after amputation of the fore-legs, the response in all the insects was characteristic of a servile response[3]. It presented a short and constant delay (1·93 msec ± 0·078 *S.E.*, ref. 2). The pathway remained open so long as the learned reaction was being used, that is, as long as reinforcement was acting. In previous experiments this process was followed up to the fiftieth day after amputation.

The present communication deals with the reversibility of the modifications of responsiveness of this nervous pathway to single stimuli while the learned action is no longer performed, because newly formed fore-legs were used to grab the antennae. This situation was achieved by amputating both fore-legs in a series of 26 cockroaches during the period of moulting. Young cockroaches were operated on and after three to four moults the regrown forelegs were strong enough to hold the antenna for cleaning. From 15 to 20 days after this stage was reached, the two new fore-legs were amputated and both the behaviour and the electrical response of this nervous pathway were investigated using the previously described

technique[2]. This situation implies that for a period of about 20 days the insects were not using the middle legs for holding the antenna, which may be interpreted as a lack of reinforcement for about three weeks.

Twenty-one of the 26 cockroaches were able to use one of the middle legs for holding the antenna immediately after the amputation of the two regrown fore-legs. The remaining insects made unsuccessful efforts to grab the antenna during the first hours after operation, but within the first day they could use one of the middle legs. An improvement of the behavioural performance was evident through the first two post-operative days.

The electrical activity was studied *in vitro* immediately after operation and for a period of up to six days. Fig. 1 shows the change of the synaptic delay values and their standard errors through the whole period of the experiment. It will be noticed that, at the beginning, a labile response was present, that is, the synaptic values were long and variable. Four days after, these values corresponded to those described as characteristics of a servile response. As can be seen in Fig. 1, the decrease of the mean synaptic values is faster than the decrease of the dispersion of these values.

In the previous work[2] the process of learning was considered to be related to a change in the permeability of synaptic transmission. When the synapse was closed (90 per cent of normal adult insects), it opened during learning; this opening meant a servile response. If the labile response was present before learning (10 per cent of the cockroaches considered normal), it also became a servile response after learning.

As described in this communication, the labile response is always present in the group of cockroaches that had already learned the new motor behaviour and which for about 20 days had not performed the acquired motor reaction. The labile response can be interpreted as a trace of a learned behaviour that lasts for at least 20 days, during which there is no reinforcement.

From the results described here it is pertinent to suggest that whenever a labile response could be elicited by a single presynaptic volley, the nervous system had already reached a physiological condition that enabled the cockroach to use one of the middle legs for holding the antenna.

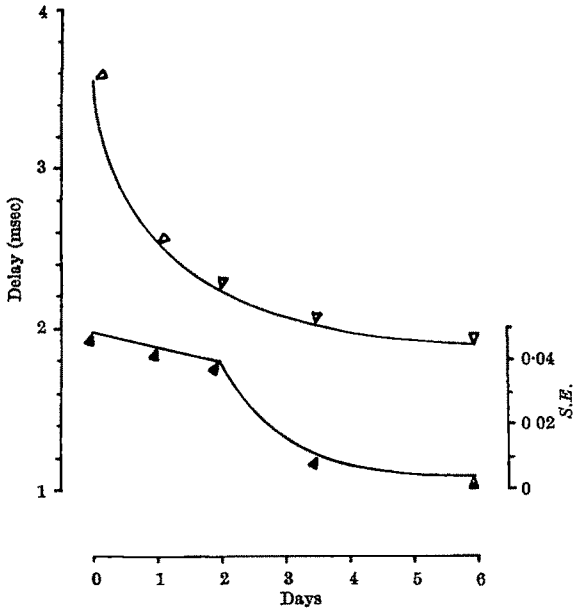


Fig. 1. Ganglionic delay[1,2] of an all or none response (*in vitro*) in a motor axon of the third thoracic ganglion and its standard error. A single electrical stimulus was applied between the œsophageal ganglion and the first thoracic ganglion. Abscissa, days after amputation of both regrown fore-legs. Ordinate on the left, delay in msec, △; ordinate on the right, standard error in msec, ▲. Each point represents the mean value of 300 measurements. The value *P* between two adjacent delay points (△) is less than 0 001

It can also be suggested that the transformation of a labile into a servile response represented an improvement of the system resulting in a better adjustment of the motor reaction.

On the other hand, it could be concluded that a 20-day period of disuse of the learned reaction produced a partial loss of the behavioural ability. This effect can be called the 'forgetting-process' and can be correlated to a change of a servile response into a labile one. Conversely, 3–4 days of reinforcement enable these cockroaches to achieve the highest behavioural efficiency. This 'relearning process' seems to be related to the transformation of a labile response into a servile one.

It is worth while emphasizing that the change of synaptic permeability appears as a smoothly graded process from a closed to an open synaptic transmission. Three situations have been considered to be operationally useful: (1) the lack of response; (2) the typically labile response; (3) the typically servile response. The lack of response may be interpreted as a response with a very prolonged delay; should this delay be shortened, a labile response may appear and, in case it reaches the shortest value, a servile response appears. Besides, the fact that the dispersion of the synaptic delay value is decreasing during the process of learning is another reason for suggesting that a graded transmitter efficiency is being built at the synapses during learning.

It has also been demonstrated[2,4] that a modification of permeability of this motor pathway can be achieved acutely by a short tetanic stimulation; but it lasts only for a short period. Should no response be present before a tetanic stimulation, a labile response may appear after the tetanus. When a labile response is recorded before a tetanus, the latter may change this labile response into a servile one. The main difference between the process achieved acutely and the process achieved during learning is that in the former case both building-up and decay of the process occur more rapidly.

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Fraction of the Motoneurone Pool activated in the Monosynaptic *H*-Reflexes in Man

The monosynaptic reflex response evoked in the cat by a maximal volley in the group I*a* afferent fibres from a synergic group of muscles has been measured as a fraction of total motoneurone pool of that muscle by Jefferson and Benson[1] and found to range between 4·3 and 26·5 per cent. Apparently there has been no measurement of the activated fraction of the motoneurone pool with the analogous *H*-reflexes that are evoked in the gastrocnemius-soleus muscle of man by stimulation of the group I*a* fibres in the popliteal nerve[2–4].

In the present series of experiments the popliteal nerve was stimulated by an electrode carefully placed on the skin overlying it in the popliteal fossa and the electrical responses were recorded from the soleus muscle by two surface electrodes. Both electrodes mounted in one rubber strip were located and cemented to the skin on the upper third of soleus muscle. In this way it was possible to avoid any shift of recording electrodes by muscle movement. Suitable earthed leads minimized artefacts, and the electrical responses were amplified and photographed in the conventional manner.

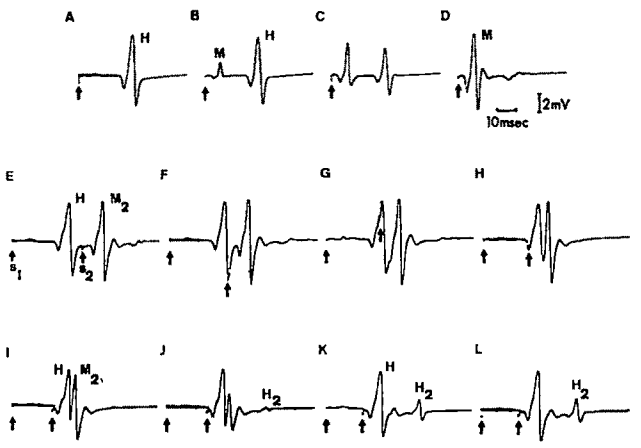


Fig. 1. Electrical response of m. triceps surae after electrical stimulation of N. tibialis post. *A*—*D*, Stimulation with increasing stimulation intensity. The progressive decrease of the amplitude of the *H*-reflex is shown. In *D* it can be seen that the strongest stimulation caused complete blockade of the *H*-reflex. *E*–*L*, Double stimulation. *I* shows the second *M*-response superposed on the latter part of the *H*-response. In *J*–*L* progressive increase of the H_2-response was evoked by the second stimulus. Time, 10 msec; amplitude, 2 mV

Presumptive evidence for activation of a large fraction of the motoneurone pool has been obtained in several subjects (5), in which the responses to graded intensities of stimulation gave a series such as that illustrated in Fig. 1*A*–*D*. The weakest stimulation evoked a large triphasic muscle action potential (the *H*-reflex) with a latency of 32 msec. With progressive stimulus increase (*B*, *C*) this spike declined as a much earlier triphasic spike developed. It is well known that this is due to direct stimulation of the motor nerve fibres to the gastrocnemius-soleus muscle, which have a higher threshold to electrical stimulation than the group I*a* afferent fibres[2–6]. Besides causing the early muscle action potential (the *M* response) with a latency of about 8 msec, the impulses generated in these motor fibres pass antidromically to the spinal cord and cause a blockade of the monosynaptic reflex discharge (the *H*-reflex) either by collision with this impulse discharge or by the refractoriness that the antidromic invasion induces in the motoneurones.

As shown in Fig. 1*D*, the strongest stimulation caused this blockade of the *H*-reflex to be virtually complete. The *M*-spike response produced in these circumstances can therefore be assumed to be due to activation of almost 100 per cent of the motor axons of the gastrocnemius-soleus muscle, and hence of the motor units of this muscle. The largest action potential of the *H*-reflex in Fig. 1*A* is a little smaller in size than the maximum *M*-spike of *D*. However, close inspection reveals that the triphasic *H*-reflex potential is composed of a more asynchronous response of the motor units, the interval between the initial and terminal positive waves being 6 msec as compared with 5 msec for the *M*-spike. This asynchronism can, of course, be attributed to the much longer neural conduction pathway for the *H*-reflex with the interpolation of a synaptic relay. It seems likely that the increased asynchronism of the constituent motor unit responses is sufficient to account for the smaller size of the *H*-reflex spike relative to the *M*-spike in Fig. 1*E*. Similar evidence for a virtually total activation of the motoneuronal pool has been obtained in several other subjects.

A crucial test of this presumed total activation is illustrated in Fig. 1*E*–*L*, the control *H* and *M* responses being in Fig. 1*A*, *D*. A maximal *H*-reflex was set up by a stimulus that was just at threshold for the most excitable motor axons, as may be seen by the extremely small initial *M*-spike. The testing stimulus evoked an almost maximal *M*-spike, as is demonstrated by the very small *H*-reflex that followed the *M*-spike. With progressive shortening of the interval by which the testing *M*-spike followed the *H*-response it is seen that there was no appreciable change in the *M*-spike until it was superposed on the latter part of the *H*-responses (*I*). Further advance of the testing *M*-spike stimulus then resulted in a rapid decrease of the *M*-spike and, in the last record of the series (*L*), the spike induced by the combined stimulation could be exactly superimposed on the control *H*-reflex response (the first response of *E*). Thus it is established that, within the limits of accuracy of this experiment, the *H*-reflex in Fig. 1*E* was occurring from 100 per cent of the motoneuronal pool. It will be noted in *J*–*L* that there was a progressive increase in the *H*-response (H_2) evoked by the second stimulus. This is, of course, to be expected, as refractoriness prevents the second stimulus from exciting the motor axons and so blockading the *H*-reflex as already described.

A similar result was obtained in another subject in which the *H*-reflex was almost as large as the *M*-spike. In that subject comparison of the *H*-reflex and *M*-spike sizes also indicated activation of virtually 100 per cent of the motoneurone pool by an *H*-reflex. However, there are many subjects in which a much lower activation —even less than 50 per cent—is indicated by the comparison of the *H*-reflexes and maximum *M*-spikes. The measurement of the motoneuronal fraction involved in *H*-reflexes should be a standard procedure in all investigations in *H*-reflexes. For example, in testing the effect of muscle stretch on *H*-reflex it was sometimes found that there was almost no change in sizes of the reflexes. This stability of the *H*-reflex, under conditions of presumed inhibitory or facilitatory influences, would be expected if motoneurones were so powerfully excited that all of them were supramaximally excited in the *H*-reflex.

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PHARMACOLOGY

Blocking of Cortical Inhibitory Synapses by Intravenous Lidocaine

It has been shown by various pharmacodynamic examinations that systemically administered local anaesthetics act only depressively on the central nervous functions: for example, the cortical after-discharge[1], the spinal reflex[2], the reticular activating system[3], the thalamic activating system[4], the direct cortical response[5], and the transcallosal evoked potential[6]. The reason why local anaesthetics in a toxic dose manifest a central nervous excitation or convulsion has so far remained obscure.

We recorded extracellularly unit firings of a cortical neurone at the sensory-motor area in unanaesthetized rabbits immobilized with gallamine. In some neurones the transcallosal stimulation (0·5 msec, 100 c.s and 10–20 V for 1–3 sec) induced complete blocking of spontaneous firings (Fig. 1*A*). Intravenous injection of a small dose of lidocaine (7 mg/kg) abolished or attenuated the effect of stimulation (*B*–*E*), and perfect recovery followed it (*F*). In other neurones the transcallosal stimulation caused a marked increase in firings, and lidocaine injected in the same dose did not block this effect, whereas a large dose (15 mg/kg) partly inhibited the excitatory effect.

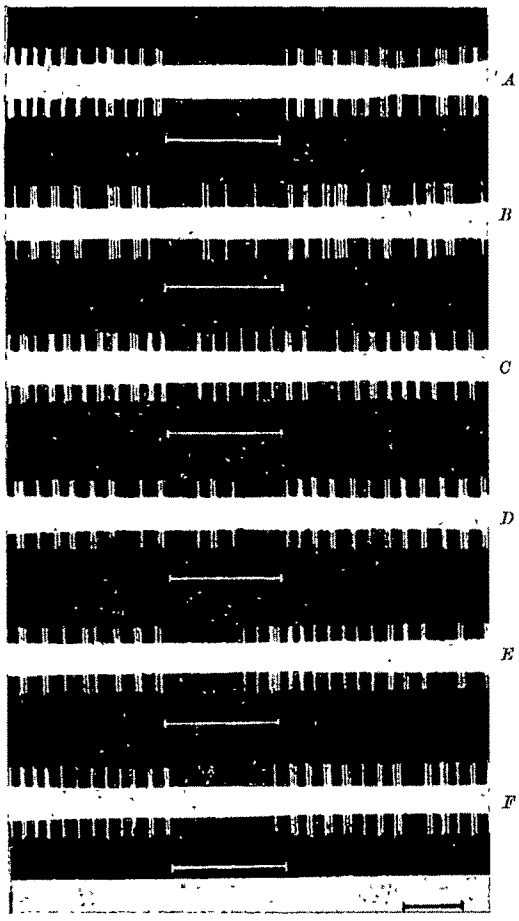


Fig 1. Effects of lidocaine on the inhibitory stimulation *A*, Control; *B*, 2 min, *C*, 4 min, *D*, 6 min; *E*, 8 min, *F*, 14 min after intravenous injection of lidocaine (7 mg/kg)

These results indicate that inhibitory synapses of cortical neurones are selectively blocked by lidocaine administered intravenously and excitatory synapses are more resistant to the drug. The mechanism of cortical excitatory action of local anaesthetics is thus interpreted, and the gap between behavioural observations and analytical experiments seems to be filled.

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Enhanced Adenosine Inhibition of Platelet Aggregation in the Presence of Cardiac Glycosides

Blood platelets suspended in plasma readily exhibit aggregation when small amounts of adenosine diphosphate are added[1]. Aggregation can also be produced by the addition of 5-hydroxytryptamine[2,3] since this has been shown to release ADP from platelets[4]. Born *et al.* have shown that adenosine, its analogues or related substances, when added prior to an aggregating dose of ADP or 5-HT will inhibit the aggregation and may prevent it altogether[1,5,6].

It has been suggested[7,10] that adenosine is, at least partially, destroyed by adenosine deaminase in the plasma and hence the inhibition of ADP-induced platelet aggregation which it produces is of short duration and rarely lasts longer than 20 min. The 2-substituted chloro analogue, 2-chloroadenosine, is a far more potent and prolonged inhibitor of ADP-induced platelet aggregation[7] and is resistant to adenosine deaminase.

Both adenosine and 2-chloroadenosine produce heart block when injected into the left atrium of the guinea-pig heart and, whereas this effect of adenosine is brief and transient, that of 2-chloroadenosine is more powerful and very prolonged. The heart block produced by adenosine can be prolonged by the simultaneous administration of cardiac glycosides, and Angkapindu, Stafford and Thorp[8] have suggested that these drugs act as adenosine deaminase inhibitors.

We have examined the effect of cardiac glycosides on platelet aggregation following substantially the same method as Born[1,5] using sheep platelet/plasma suspensions. The cuvette containing the plasma mixture was in a temperature-controlled chamber and stirring was very precisely controlled. The turbidity changes were recorded as percentage light transmission using a continuous potentiometric recorder.

Sufficient platelet-rich plasma was prepared at the commencement of each day to allow comparison to be made between adenosine inhibition alone at various time intervals after addition and similar experiments which included the cardiac glycosides added prior to adenosine.. All experiments on any one day were performed on the same batch of plasma and comparisons have only been made between results obtained in such an experimental series since the aggregation response varies from one platelet/plasma batch to another.

The concentration of platelets was checked by direct count and was usually within the range $1{\cdot}0 \times 10^6$ platelets per cu. mm. As the aggregating agent either ADP or 5-HT was used and similar results were obtained in both cases.

Fig. 1*a* shows a comparison between experiments in which 5-HT was used to cause aggregation in the presence and absence of ouabain.

It will be seen that this control response is the same whether ouabain is present or absent, and other experiments have shown that this glycoside alone has no influence on ADP- or 5-HT-induced aggregation of platelets. Fig. 1*b* shows the results obtained when adenosine is added 2

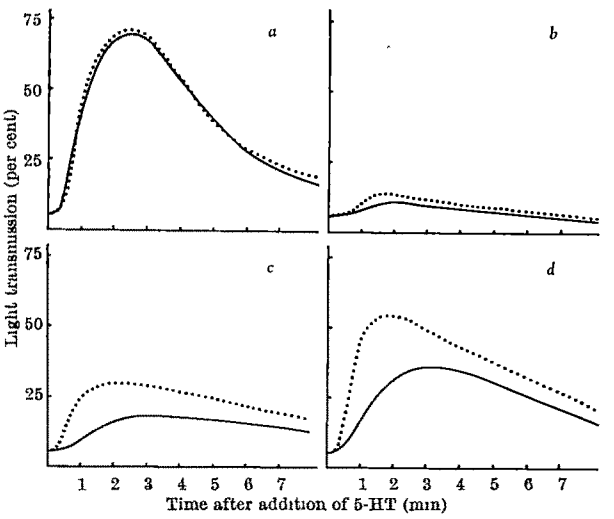


Fig 1. Aggregation curves for 5-HT (1·5 μM) added to platelet suspensions in plasma. *a*, Control responses; *b*, adenosine added (1·25 μM) 2 min prior to 5-HT; *c*, adenosine added (1·25 μM) 5 min prior to 5-HT; *d*, adenosine added (1·25 μM) 15 min prior to 5-HT. Responses (——) in presence of ouabain (100 μM); responses (.....) in absence of ouabain

Table 1. Percentage Inhibition of Platelet Aggregation when Cardiac Glycosides are added at Various Intervals of Time prior to 5-HT or ADP

Aggregating agent	Drug combination	Time interval (min) 2	5	10	15
5-HT (1·5 μM)	Adenosine alone (1·25 μM)	88	60	33	10
	Adenosine + ouabain (50 μM)	87	73	50	36
	+ ouabain (100 μM)	98	90	75	69
	Adenosine + *K*-strophanthin (40 μM)	87	68	36	11
	+ *K*-strophanthin (80 μM)	88	70	40	14
	Adenosine + digoxin (10 μM)	86	65	34	12
ADP (1·4 μM)	Adenosine alone (1·25 μM)	76	52	25	8
	Adenosine + ouabain (50 μM)	78	65	47	29
	+ ouabain (100 μM)	81	72	55	42
	Adenosine + *K*-strophanthin (40 μM)	75	52	30	13
	+ *K*-strophanthin (80 μM)	79	61	46	30
	Adenosine + digoxin (10 μM)	77	56	29	20

min before the 5-HT. It produces strong inhibition of aggregation which is influenced by ouabain only slightly. When 5 min elapses (Fig. 1*c*) between the addition of adenosine and 5-HT it will be seen that the presence of ouabain has resulted in a substantially smaller degree of aggregation than when adenosine is used alone.

Fig. 1*d* shows the effect after an interval of 15 min when the preservation of the adenosine is even more pronounced.

This effect has been shown with other cardiac glycosides, digoxin, strophanthin *K* and lanatoside *C*, but ouabain is more readily water soluble than other cardiac glycosides and hence can be used in higher concentrations. Comparative results for ouabain, *K*-strophanthin and digoxin are given in Table 1.

The percentage inhibition is measured by the steepness of the slope of the aggregation curve during the first 0·5 min after addition of ADP or 5-HT. It will be seen that the inhibition of adenosine destruction is dose dependent but in the case of digoxin it was not possible to obtain a sufficiently high concentration in the cuvette to exhibit more than the response to a low dose.

It is not possible to use organic solvents since these interfere with the aggregation process and hence all glycosides were used as solutions in saline buffered to *p*H 7·4.

Under the conditions we have used the aggregation curves are highly reproducible and the adenosine inhibition in all these experiments is greater in the presence of cardiac glycosides.

Recent investigations in this department by Dean and Maguire[9] have shown that ouabain is a competitive inhibitor of purified cardiac adenosine deaminase, and these results indicate that the deaminase in plasma is also inhibited by these drugs.

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HAEMATOLOGY

Role of ABO Erythrocyte Groups in Human Histocompatibility Reactions

The role of red blood cell group antigens in human histocompatibility has long been a matter of controversy[1–6]. It has recently been noted by Griffiths and Crikelair[7], and by Kuhns, Rapaport *et al.*[8], that a rise in anti-A and anti-B antibodies may occur following the exchange of skin homografts between blood group-incompatible subjects. This communication describes a series of experiments which provide suggestive evidence that erythrocyte group antigens may, on suitable immunization of recipients, play a significant part in conditioning the response of these subjects to first-set skin homografts.

In the course of a preliminary experiment, five volunteers (3 A Rh+, 1 B Rh+, and 1 O Rh+ subjects) received one intradermal injection of red cell membranes obtained from an A Rh− donor. Fifteen days later, the recipients were challenged with a skin homograft from the same donor and with a graft from another subject (who was O Rh+). The red cell membranes were prepared from whole blood collected in Fenwal bags through a cation-exchange resin chamber, which eliminated 90 per cent of the blood platelets. 100 ml. of a 6 per cent dextran (mol. wt. 200,000) were added to the blood, and the lowest portion of the red blood cell sediment was collected. This fraction was centrifuged three consecutive times, and each time the upper layer, containing a supernatant leucocyte layer, was removed. The remaining purified red blood cell preparation was haemolysed utilizing distilled water and the sodium monohydrogen phosphate method of Ponder. The final preparation did not contain any detectable leucocytes after several observations in counting chambers. Technique of graft application and criteria used for the determination of skin homograft rejection by gross and stereomicroscopic observations of the graft surfaces have already been described in detail[9–13].

None of the recipients of red cell membranes (sensitized in this experiment with a single intra-dermal injection) responded with accelerated rejection or white graft reactions. The two shortest skin homograft survival times in these recipients occurred, however, when skin homografts from a red blood cell group-incompatible A Rh− donor were applied to O Rh+ and B Rh+ recipients, respectively (Table 1).

Table 1. Response to Skin Homografts after Sensitization with One Intradermal Injection of Erythrocyte Membranes

Dosage of A-erythrocyte membranes injected	Blood group of recipients	First set skin homograft behaviour (days) grafts from (A−) donor	grafts from (O−) donor
$3·4 \times 10^9$	A+	16	14
$4·0 \times 10^9$	A+	10	10
$6·3 \times 10^9$	A+	17	17
$4·6 \times 10^9$	O+	9	17
$6·4 \times 10^9$	B+	9	10

Following this investigation, four recipients were sensitized with four consecutive weekly injections of $6·4–9 \times 10^9$ group A_2B Rh−. (An A_2B donor was selected to attenuate possible risks of intravenous injection of erythrocytes in the recipients.) The red cell preparations used to sensitize the recipients were obtained from defibrinated blood (thereby eliminating blood platelets in the final preparations). Leucocytes were removed by seven or eight successive sedimentations in dextran. No white cells were visible after several observations made in counting chambers. Two subjects were sensitized intradermally, and the other two received intravenous injections of erythrocytes. Eight days after the final injection, each recipient was challenged with six skin homografts obtained from donors other than the source of erythrocytes used to sensitize the recipients. Two of the skin homografts were obtained from donors of blood group A_1B; one graft was obtained from an A_2B donor, and three grafts were obtained from subjects of blood group O. The method of preparation of skin homografts, operative technique, and criteria for the determination of skin homograft rejection were as already noted[9–13]. Tables 2*A* and 2*B* outline the results of the experiment, and correlate them with the known blood group compatibilities existing between donors and recipients. A consistent difference in survival times was noted between the behaviour of ABO-compatible and ABO-incompatible skin homografts applied to these subjects. In the recipients of intradermal injections of AB erythrocytes (Table

2A), the skin homografts obtained from AB blood group donors which were applied to O Rh+ recipients were accorded a shorter survival time (mean survival time of 6·4 days) than grafts obtained from O donors applied to the same recipients (mean survival time of 11·1 days). The responses of subjects sensitized intravenously were even more pronounced (Table 2B). In these individuals, all homografts obtained from ABO blood group-incompatible donors were rejected in accelerated fashion. There were two white grafts, two mixed white graft reactions, and two accelerated rejection reactions (4–5-day rejection). All other grafts were accorded a longer survival time; however, four ABO blood group-compatible grafts have been rejected more rapidly on an average than after intradermal immunizations.

Results of this investigation indicate that the ABO blood group antigens may play a more important part in conditioning the response of human subjects to skin homografts than has been previously suspected. This observation is consistent with similar reports on the role of such antigens in histocompatibility reactions in other species[14–16]. It is also in accord with the description of alcohol-soluble ABO antigens in leucocytes, as well as in other tissues, and particularly in vascular endothelium and epidermis[17–20].

It is of interest that the homograft responses described in this communication occurred in the absence of significant rises of anti-A or anti-B antibody titres in the recipients. Only small rises in titres were noted after erythrocyte sensitization, and again after the application of skin homografts. Such antibodies were neutralized by soluble A and B substances, by heating to 70° C, or by treatment with mercaptoethanol. It would therefore appear that the accelerated and white graft rejection responses noted in the ABO-incompatible recipients sensitized with red blood cell antigens may have been associated with mechanisms other than humoral antibody, such as cellular immune factors, which have been previously shown to mediate homograft sensitivity in man[21]. The observed results also cannot be attributed to erythrocyte incompatibility systems other than ABO. Incompatibility for red cell group antigen D was only noted between one recipient and six donors of skin homografts in this investigation, and the homograft responses in this subject did not differ from those observed in the D-compatible subject immunized through the same route. With reference to the possible bearing of leucocyte or platelet incompatibility on the results obtained, the X^aX^b (ref. 22) or 4^a4^b (ref. 23) leucocyte groups, as well as the Ko^aKo^b platelet groups[24,25], were considered. The

Table 2A Response to Skin Homografts after Intradermal Sensitization with Four Successive Injections of Erythrocytes

	Erythrocyte groups			Blood group incompatibilities noted between the donors of erythrocytes and of skin homografts and their recipients												Survival time of skin homografts (days)
				Erythrocyte antigens										Leucocyte antigens		Platelet antigens	Serum antigens	
	ABO	Le	Se	ABO	Cc	D	Ee	Fy^a	MN	Ss	K	P		X^aX^b (4^a4^b)	$Y^aY^bY^c$† ($6^a6^b6^c$)	Ko^a Ko^b	$Gm^{a\,x\,b}$	
Recipient:	0	Le^{a-b+}	Se															
Donor of erythrocytes:	A_2B	Le^{a-b+}	Se	A_2B	—*	—	—	—	M	—	—	P		—	—	—	—	not done
Donors of skin homografts:	A_1B		Se	A_1B	—	—	E	—	M	—	—	P		—	—	—	—	6
	A_1B	Le^{a-b-}	Se	A_1B	—	—	—	—	M	—	K	P		—	—	—	$a\,x$	7
	A_2B	Le^{a-b+}	Se	A_2B	—	—	E	—	M	—	—	P		—	—	—	a	6
	0	Le^{a-b+}	Se	—	—	—	—	—	M	—	—	—		—	—	—	—	11
	0	Le^{a-b+}	Se	—	—	—	—	—	M	—	—	P		—	—	—	—	11
	0	Le^{a-b+}	Se	—	—	—	—	—	M	—	—	P		—	—	—	$a\,x$	11
Recipient:	0	Le^{a-b+}	Se															
Donor of erythrocytes.	A_2B	Le^{a-b+}	Se	A_2B	—	—	—	—	—	—	—	—		—	—	—	—	not done
Donors of skin homografts·	A_1B		Se	A_1B	C	—	—	—	—	—	—	—		—	—	—	—	6
	A_1B	Le^{a-b-}	Se	A_1B	C	—	—	—	—	—	—	—		—	—	—	—	8
	A_2B	Le^{a-b+}	Se	A_2B	C	—	—	—	—	—	—	—		—	—	—	—	5
	0	Le^{a-b+}	Se	—	—	—	—	—	—	—	—	—		—	—	—	—	11
	0	Le^{a-b+}	Se	—	C	—	—	—	—	—	—	—		—	—	—	—	10
	0	Le^{a-b+}	Se	—	—	—	—	—	—	—	—	—		—	Y^c	—	—	13

* No evidence of incompatibility. † The identity between the "Y" and the "6" systems is probable though not yet proved.

Table 2B. Response to Skin Homografts after Intravenous Sensitization with Four Successive Injections of Erythrocytes

	Erythrocyte groups			Blood group incompatibilities noted between the donors of erythrocytes and skin homografts and their recipients												Survival time of skin homografts
				Erythrocyte antigens										Leucocyte antigens		Platelet antigens	Serum antigens	
	ABO	Le	Se	ABO	Cc	D	Ee	Fy^a	MN	Ss	K	P		X^aX^b (4^a4^b)	$Y^aY^bY^c$ ($6^a6^b6^c$)	Ko^aKo^b	$Gm^{a\,x\,b}$	
Recipient:	0	Le^{a-b-}	Se															
Donor of erythrocytes:	A_2B	Le^{a-b+}	Se	A_2B	—*	—	—	—	M	—	—	—		—	—	—	—	not done
Donors of skin homografts:	A_1B		Se	A_1B	—	—	E	—	M	—	—	—		—	—	—	—	White graft reaction
	A_1B	Le^{a-b-}	Se	A_1B	—	—	—	—	M	—	K	—		—	—	—	$a\,x$	Mixed white graft reaction
	A_2B	Le^{a-b+}	Se	A_2B	—	—	E	—	M	—	—	—		—	—	—	a	4–5
	0	Le^{a-b+}	Se	—	—	—	—	—	M	—	—	—		—	—	—	—	10
	0	Le^{a-b+}	Se	—	—	—	—	—	M	—	—	—		—	—	—	—	6
	0	Le^{a-b+}	Se	—	—	—	E	—	M	—	—	—		—	Y^c	—	$a\,x$	7
Recipient:	0	Le^{a-b+}	Se															
Donor of erythrocytes:	A_2B	Le^{a-b+}	Se	A_2B	—	—	—	Fy^a	N	—	—	—		—	—	—	—	not done
Donors of skin homografts:	A_1B		Se	A_1B	C	D	E	Fy^a	N	—	—	—		—	—	—	—	White graft reaction
	A_1B	Le^{a-b-}	Se	A_1B	C	D	—	Fy^a	N	—	K	—		—	—	—	x	Mixed white graft reaction
	A_2B	Le^{a-b+}	Se	A_2B	C	D	E	Fy^a	—	—	—	—		—	—	—	—	4–5
	0	Le^{a-b+}	Se	—	—	D	—	Fy^a	N	—	—	—		—	—	—	—	10
	0	Le^{a-b+}	Se	—	C	D	—	Fy^a	N	—	—	—		—	—	—	—	6
	0	Le^{a-b+}	Se	—	—	D	E	Fy^a	N	—	—	—		—	Y^c	—	x	6

* No evidence of incompatibility.

recipients were selected in such a fashion as to avoid the possible bearing of incompatibilities in these systems on the responses of the subjects to skin homografts. Results of this study therefore appear to indicate that the question of the possible role of ABO antigens in conditioning the response of human recipients to tissue transplants deserves further investigation. Observations outlined in this communication may be of particular relevance to recent descriptions of rapid kidney homograft rejection in ABO-incompatible recipients. Of twelve renal transplants performed in such individuals, nine were accorded survival times of less than 30 days[26]; in some instances, graft cyanosis occurred almost immediately after completion of vascular anastomoses[27]. It may be of interest to re-evaluate these data in the light of experimental results described here—with particular reference to the possible bearing of repeated blood transfusions on the renal homograft reactions observed.

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Interactions of ε-Aminocaproic Acid with the Thrombin Clotting and Fibrinolytic Systems

Several reversible inhibitors of plasmin have been found to protect the latter from irreversible combination with antiplasmin within appropriate limits of time and concentration parameters. However, ε-aminocaproic acid (EAC) and several related compounds appeared to potentiate the destruction of plasmin by antiplasmin, even though they were able to stabilize the enzyme against autodigestion in the absence of antiplasmin[1,2]. Because of the complexities of the two-phase model system used for these examinations, it was difficult to decide whether the results could be explained entirely on a kinetic basis, or whether in addition some unique reaction involving EAC and related structures could be taking place. The present investigations of a single-phase clot lysis system provided unexpectedly dramatic visual evidence in favour of the latter possibility.

Fibrinogen, thrombin, plasminogens, plasmins, trypsin and inhibitors were isolated or obtained as previously described[1-3]. Clots were made in tubes in initial experiments with 20 units of thrombin/ml. and usually 2 mg fibrinogen/ml., and observed at 25° C. During the course of a study of effects of various inhibitors on lysis time of these clots by plasmin, it was soon noticed that some clots containing EAC became strikingly opaque over a period of time. Normal clots made in the buffer used[4], which had a *p*H of 7·4 and ionic strength of 0·3, remained clear indefinitely. A quantitative picture of this phenomenon was obtained by making the clots in spectrophotometer cuvettes and following the increase in absorbance at 350 mμ (Fig. 1). Analogous results were obtained with the same fibrinolytic levels of Kline-type human plasmin of similar purity, or with euglobulin-type human plasmins of higher specific activities which had been fractionated in DEAE-cellulose. Opacity did not develop in clots containing EAC by itself or in the presence of plasminogens or urokinase separately, nor was the absorbance of fibrinogen solutions affected by the plasmin–EAC combination. Clots containing 2 μg trypsin/ml. and 0·2 M EAC also showed no opacity up to the time of lysis of about 2 h.

Other inhibitors tested over a range of levels which did not induce opacity with plasmin include Trasylol, Kunitz pancreatic trypsin inhibitor, and soybean and lima bean trypsin inhibitors. Furthermore, addition of sufficient levels of these inhibitors to clots containing plasmin–EAC prevented the usual opacity development. In fact, higher concentrations of EAC itself greatly delayed the time of initiation of opacity development, and this effect was not due to any significant change of *p*H or ionic strength of the medium, as determined by glass electrode and conductivity measurements. Several other inhibitors related to EAC, for example, lysine and γ-aminobutyric acid, also induced opacity.

At a given EAC level, the initial rate of opacity development depended on the concentration of fibrin or plasmin, while the maximum opacity was a function of the fibrinogen concentration. A quantitative kinetic analysis of these relationships is obviously complicated because lysis with consequent disappearance of opacity took place at a faster rate as the EAC : plasmin ratio decreased, and the initial rate of opacity development decreased at higher EAC concentrations.

These results suggest that there is an enzymatic activity associated with plasmin which results in structural

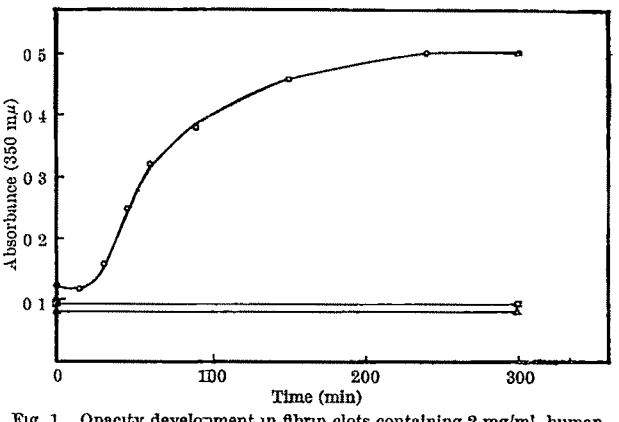


Fig. 1. Opacity development in fibrin clots containing 2 mg/ml. human fibrinogen and 20 units thrombin/ml. ○, Clot containing 70 μg/ml euglobulin-type human plasmin and 0·05 M EAC; □, clot containing 0·05 M EAC; ▲, clot with no additions

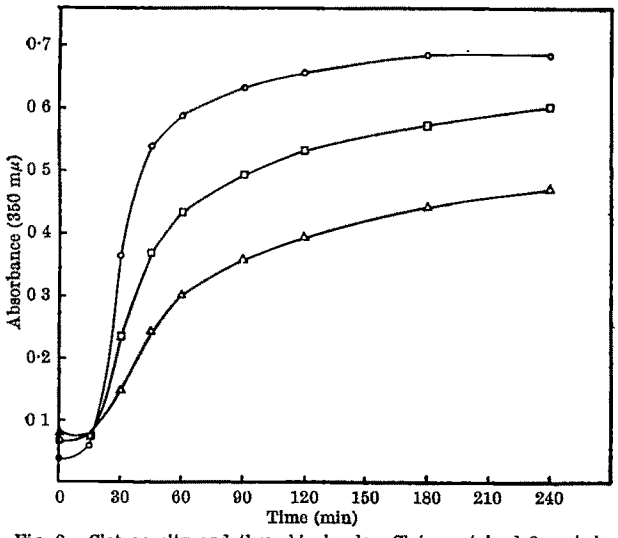


Fig. 2. Clot opacity and thrombin levels. Clots contained 2 mg/ml. human fibrinogen, 11 μg/ml. DEAE-purified human plasmin, 0·05 M EAC; and ○, 1 unit thrombin/ml.; □, 2 units thrombin/ml.; △, 5 units thrombin/ml.

modifications of the fibrin network in the presence of EAC and related compounds. This activity might involve a different site from the one concerned with fibrinolysis, since some other inhibitors of the latter do not induce opacity. However, since these inhibitors (as well as high levels of EAC itself) also prevent or retard opacity development with EAC, the possibility must be considered that the same site is involved and is catalysing some reaction which incorporates EAC into the clot structure, conceivably by amide bond formation; or there could be a limited hydrolysis taking place at bonds other than those split by thrombin, with large structural elements being aggregated by secondary forces. The structural implications of opacity in fibrin clots have been discussed by Ferry and Morrison[5].

When the thrombin concentration in the clots was reduced, additional complexities of the system were revealed and perhaps some insight as to mechanism was gained. As seen in Fig. 2, the development of opacity was inversely related to the thrombin concentration. In all experiments so far described, the order of addition of components was such that the fibrinogen was added last, which is our usual procedure in order to minimize fibrinogenolysis before the action of thrombin. It was now obvious that interaction of plasmin–EAC with fibrinogen sites had to be considered, by addition of thrombin after pre-incubation of fibrinogen, plasmin and EAC. The results of Fig. 3 strongly support the concept that the fibrinogen sites are indeed the susceptible ones. Further evidence that thrombin and plasmin–EAC are competing at the same sites is given in Table 1. Although plasmin–EAC significantly prolonged the clotting time, the resulting clots do not lyse for long periods of time; in fact, the seemingly paradoxical result was obtained that, in this range of thrombin concentration, the clots which formed more rapidly at the higher thrombin level were the first to lyse. This is understandable on the basis that the polymers resulting from thrombin action alone are more susceptible to plasmin hydrolysis.

Table 1. Effect of Plasmin and EAC on Thrombin Clotting Time*

Thrombin (units/ml.)	Plasmin (μg/ml.)	EAC (M)	Clot time (min)
1	—	—	13·5
2	—	—	5
5	—	—	2
1	—	0·05	15·5
2	—	0·05	8
5	—	0·05	2
1	11	0·05	30
2	11	0·05	11
5	11	0·05	3·5

* Plasmin and fibrinogen as in Fig. 2; fibrinogen was added last to these mixtures. With plasmin in the absence of EAC, clots did not form at any of these thrombin levels.

If pre-formed clots were made and plasmin and EAC added in a fluid supernatant, opacification could readily be seen developing at the interface and slowly spreading into the gel. Presumably, in any pre-formed clot structure, there are still sites available which have not been acted on by thrombin, and the latter is relatively immobilized in the meshwork of the gel.

Some information is available which allows speculation about the nature of the sites referred to here. At least with regard to action on synthetic substrates, the specificities of both thrombin and plasmin are directed toward arginyl and lysyl bonds; while the clotting action of thrombin is limited to arginyl bonds[6,7]. It might be postulated that plasmin action at arginyl sites is involved in the polymerization to a lysis-resistant structure in the presence of EAC, while concerted action at both arginyl and lysyl sites is required for hydrolysis. From structural considerations, it seems reasonable that EAC might interfere more at lysyl than at arginyl sites, and the experimental results are in harmony with this idea. Another implication of this hypothesis is that the plasmin autodigestive process would probably involve that part of the active centre related to lysyl bonds primarily. As the EAC concentration increases, interference at the arginyl sites does become evident, so that there is inhibition not only of lysis, but also of the reactions which lead to opacification. However, the arginyl sites acted on by thrombin alone must be differently situated, since EAC by itself has little effect on thrombin action.

It is obvious that there are additional complexities beyond this level of speculation. It appears that some reactions catalysed by thrombin are involved in the opacity phenomenon, and these reactions must be subsequent to the actions of plasmin (Fig. 3). Perhaps plasmin–EAC, while initially competing with thrombin (if present) for the latter's preferred sites, also by a limited hydrolysis in another area, exposes additional sites which can then be acted on by thrombin to produce the opaque, lysis-resistant structure.

Whatever the details may be, this interaction of EAC with the clot system only in the presence of plasmin makes it possible to understand better many previously puzzling

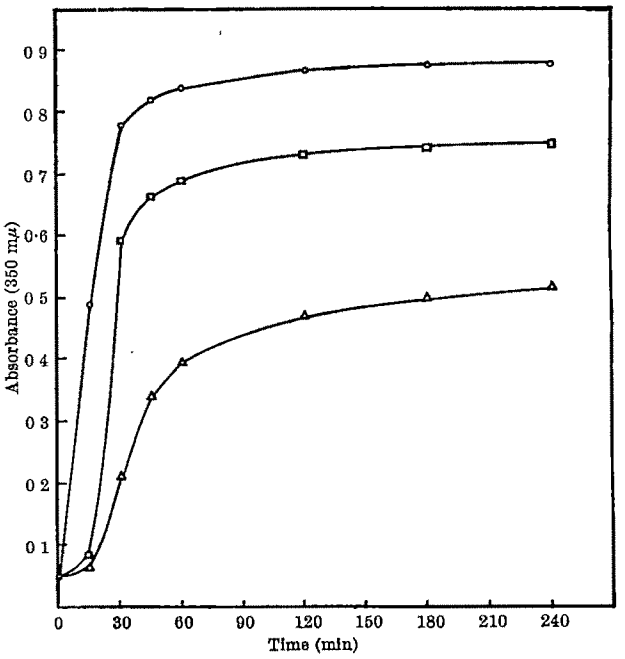


Fig. 3. Pre-incubation before thrombin addition. Clots contained fibrinogen, plasmin, and EAC as in Fig. 2; and 5 units thrombin/ml. added after pre-incubation of ○, 10 min; □, 5 min; △, 0 min

results in studies comparing EAC with other inhibitors, or comparing EAC inhibition of plasmin action on fibrin and on casein or synthetic substrates. For example, a more detailed interpretation is now possible for the increased destruction of plasmin by antiplasmin in the presence of EAC (refs. 1 and 2) if it is assumed that inhibition of fibrinolysis by EAC is largely due to interaction of EAC with the substrate rather than with plasmin. The EAC-modified clot is more resistant to lysis but the inhibition is reversible over a period of time. However, if an irreversible inhibitor such as antiplasmin is also present, it will remove free plasmin from the system before it can become effectively associated with the modified substrate. Whether or not a mechanism of this type should be called potentiated inhibition could be a matter of semantics or opinion, depending on whether one looks at the process or at the end result.

Additional interactions of EAC with the enzyme rather than with the substrate are, of course, not excluded. It can now be understood why EAC and other compounds which act by the same mechanism[2] may be stabilizers or enhancers of plasmin action at lower concentrations and inhibitors of fibrinolytic activity at higher concentrations, if in the former case interaction with the enzyme and inhibition of autodigestion are the more important effects, while in the latter case interaction with the substrate becomes sufficiently rapid or extensive to retard its digestion. Other reversible inhibitors which do not affect the fibrin substrate structure are able to insulate the plasmin from both autodigestion and antiplasmin for periods of time, during which fibrinolysis can take place[1,2]. With other substrates, casein, for example, much higher EAC levels in general are required for comparable inhibition, and this could be related in part to the fact that EAC does not interact with those substrates, or at least not in a way to inhibit their digestion by plasmin.

It is of interest that Donaldson[8] also suggested alteration of fibrin by EAC, on the basis of a decrease in urea solubility of clots containing EAC. While we have not been able to demonstrate this effect, such a result might depend on the type of fibrin used or other conditions of the experiments. The development of opacity described here might never be noticed if clots were made with preparations of fibrin which produce opaque clots originally, particularly at lower *p*H and lesser ionic strength; or if the thrombin : plasmin ratio were sufficiently high and the period of observation relatively short. It should be emphasized that the examinations reported here are concerned with interactions of human plasmin and human fibrinogen; our preliminary results with bovine plasmins and bovine fibrinogen indicate that the opacification phenomenon may not be readily observable with a bovine fibrinogen substrate. It is entirely possible that the largely physical changes leading to opacity may be mechanistically only indirectly related to the initial biochemical interactions which retard fibrinolysis.

Additional details and implications of these studies will be presented elsewhere.

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PATHOLOGY

Renal Handling of Glycine in Primary Hyperuricaemia

The recent demonstration that patients with primary hyperuricaemia are also hyperaminoacidaemic[1] has suggested the possibility that the presumptive renal tubular defect in the handling of uric acid by these patients[2] may be only one manifestation of a more generalized transport defect that involves other substances. The amino-acid glycine, a purine precursor, has been investigated with the view of determining whether the elevated serum level of glycine can be explained on a renal basis.

Thirteen adult male subjects were investigated. Five patients were normouricaemic (mean serum uric acid of 5·3 mg per cent (4·3–6·0)) with a mean age of 35 years (22–55), a mean creatinine clearance of 108 ml./min (81–125) and a mean serum glycine of 260 μmoles/l. (224–285). Eight had primary hyperuricaemia (mean serum uric acid of 8·3 mg per cent (7·2–9·7)), with a mean age of 43 years (22–52), a mean creatinine clearance of 108 ml./min (82–132), and a mean serum glycine of 283 μmoles/l. (231–313). None of the subjects in either group was acutely ill, none had demonstrable hepatic or gastrointestinal disease or abnormalities of renal function, or was taking any drugs. The methods used were as previously described[1].

Endogenous glycine clearances were determined in the morning after an overnight fast and were based on a 2-h renal clearance. At the end of this 2-h period, glycine was fed orally in 2- or 5-g amounts, or administered intravenously in a dose of 0·9 g. 2 h later, another urine was collected to measure the increment in glycine excretion. Blood was drawn at 15, 30, 45, 60, 90 and 120 min after the glycine dose to determine serum glycine-levels at these times.

In Table 1, endogenous glycine clearances and glycine to creatinine ratios are shown. Both functions are lower in the hyperuricaemic individuals as compared with the normal subjects. The comparison of glycine excretion after the administration of a glycine load between the two groups is shown in Table 2. This comparison demonstrates that, regardless of the dose or route of administration, the hyperuricaemic subjects excrete less of the administered glycine.

Data from the serum investigations can be summarized as follows: (1) in the hyperuricaemic subjects the serum

Table 1. Endogenous Glycine Clearances and Glycine to Creatinine Clearance Ratios in Five Normouricaemic and Eight Hyperuricaemic Subjects

Glycine clearance in ml./min		Clearance ratio of glycine to creatinine	
Normouricaemic	Hyperuricaemic	Normouricaemic	Hyperuricaemic
2·97	2·34	0·026	0 019
2 56	1·01	0·020	0 008
1·20	0 94	0·015	0·011
2·15	0·79	0·025	0·007
2·58	1·60	0·024	0 017
	1·92		0 017
	0 81		0·007
	1·58		0 019
2·29	1·37	0 022	0·013

Table 2. Glycine (moles) excreted during the 2-h Control Period and during the 2-h Post-glycine Load Period, and the Percentage of the Total Dose excreted during the Latter Period. Eight Experiments performed on Five Normouricaemic Individuals and Eleven Experiments on Eight Hyperuricaemic Subjects

	Normouricaemic			Hyperuricaemic		
Dose of glycine	2-h control glycine excretion	2-h glycine excretion after load	Percentage of dose excreted	2-h control glycine excretion	2-h glycine excretion after load	Percentage of dose excreted
5 g orally	65	695	1·0	17	93	0·1
	109	1,706	2·4	37	190	0·2
	92	1,089	1·5	34	277	0·4
	74	1,785	2·6	29	173	0·2
	45	350	0·5	105	554	0·7
2 g orally	70	382	1·2	47	89	0·2
	82	224	0 6	54	95	0 2
				80	164	0 3
				54	182	0 5
				58	183	0·5
0·9 g i.v.	75	193	1·0	69	91	0·2
	77		1·4	53		0·3

level of glycine tends to reach a peak earlier than in the normouricaemic subjects; (2) the peak concentration of glycine tends to be slightly but not significantly lower than the peak concentration attained by the normal subjects; (3) there is no correlation in either group between the peak serum level and the quantity of the glycine load excreted in the urine ($r = \pm 0{\cdot}23$, $P = 0{\cdot}5$); (4) the disappearance of the administered glycine from the serum proceeds at approximately the same rate in both groups. Table 3 illustrates most of these phenomena.

Table 3 Serum Data after 5 g of Oral Glycine in Five Normouricaemic and Five Hyperuricaemic Subjects

	Normouricaemic	Hyperuricaemic
Peak serum level		
Reached at 30 min	1	5
at 45 min	4	0
Mean peak serum Level (μmoles/ml.)	1·56 (1·09–1·91)	1·27 (0·97–1·66)
Mean serum 'half-life' of glycine	33 min	31 min

The results of these investigations thus show that the kidneys of the hyperuricaemic individuals under-excrete glycine—a defect parallel to, and more marked than, the presumed defect in the excretion of uric acid by these patients. Such a result might have been expected from the known similarity of renal mechanisms for the handling of amino-acids and uric acid in dogs[3] and in kidney slices from various animals[4], from the fact that a uricosuric drug (salicylate) is also aminoaciduric[5], and from the observation that aminoaciduric syndromes are accompanied by uricosuria (for example, Wilson's disease and the Fanconi syndrome).

Whether or not the increased rate of net uptake of glycine by the renal tubular cell (either decreased secretion or increased reabsorption) occurs in other body cells as well cannot be definitively ascertained from the data presented. The earlier occurrence of the peak level of serum glycine concentration is suggestive of this, and the similarity of cell membrane transport systems in several types of cells[6] would lead one to expect that an abnormality in amino-acid transport across the renal tubular cell membrane might be duplicated in the membrane transport systems of other cells.

If such a universal cell membrane defect were the case, a unitarian hypothesis for the aetiology of primary hyperuricaemia suggests itself, namely, that primary hyperuricaemia is a disease of membrane transport. There is an increased rate of net uptake by renal tubular cells of at least one purine precursor (glycine) leading to decreased excretion, and increased rate of net uptake of purine precursor by purine-producing cells leading to increased availability of purine precursor within these cells and increased purine production. Thus the 'renal retention' and 'overproduction' theories of the aetiology of hyperuricaemia could be reconciled.

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IMMUNOLOGY

Antigenicity of a Carboxymethylcellulose Bovine Serum Albumin Glycoprotein in Conventional and Germ-free Mice

During the course of studies on the biological properties of synthetic glycoproteins it seemed desirable to obtain information as to their antigenic properties. Micheel and co-workers showed that a number of compounds in which carbohydrate was covalently bound to protein were antigenic in their entirety when injected into rabbits[1,2]. Good antibody response to Pneumococcal Type III polysaccharide, the components[3] of which are structurally similar to those found in carboxymethylcellulose (CMC), has been shown in mice[4]. Since little is known with respect to the antibody response of germ-free animals in general and since mice are one of the easiest animal species to raise under germ-free conditions, it was hoped that this investigation would provide information with respect to the immunological responses of gnotobiotic mice in addition to the antigenic properties of CMC.

A synthetic glycoprotein containing CMC and bovine serum albumin (BSA) covalently bound to each other was administered to 14 Swiss mice which were housed in conventional quarters, and to 13 Swiss mice maintained in a germ-free environment in Fisher–Kewaunee–Horton units. The mice were approximately two months old when the injections were started.

The synthetic glycoprotein was prepared by adding crystalline BSA to a solution of CMC (mol. wt. 40,000) the carboxyl groups of which had been activated by 1-*cyclo*hexyl-3-(2-morpholinyl-(4)-ethyl)-carbodiimide metho-*p*-toluene sulphonate[5]. The linkages between CMC and BSA were of the amide type involving carboxyl groups of CMC and amino groups in BSA. The BSA not bound to CMC was removed by precipitation with $HClO_4$, with a final $HClO_4$ concentration of 20 per cent. The glycoprotein (BSA–CMC) was homogeneous when analysed electrophoretically in veronal buffer, *p*H 8·6, $\mu = 0{\cdot}1$, with a Tiselius-type model 38-*A* Perkin–Elmer electrophoresis apparatus equipped with a 6 ml. cell. The CMC/BSA ratio in the glycoprotein was 15. Full details of the preparation and properties of the glycoprotein will be presented elsewhere[6].

The glycoprotein (0·1 ml. of a 2 per cent solution using 0·85 per cent NaCl as the solvent) was administered to the mice via the tail vein twice weekly for a total of seven injections. Seven days after the final injection, the animals were bled retro-orbitally and killed. Serum was collected and its reactivity toward the following antigens was ascertained using the double-diffusion gel method[7]. The antigens were 2 per cent glycoprotein, 2 per cent BSA and 2 per cent CMC. Serum was also collected from four conventional and five germ-free mice to whom BSA had been administered and from four conventional and five germ-free mice to whom CMC had been given. BSA was administered in a manner similar to that described for the glycoprotein. An incomplete Freund adjuvant was used in conjunction with the CMC administration. The reactivity or BSA, CMC and glycoprotein to these sera was determined also by the double-diffusion gel method.

The reactivity of the synthetic glycoprotein antisera toward various antigens is summarized in Table 1. In both the conventional and germ-free animals, the glycoprotein was a potent antigen. In only one of the animals

Table 1. Immunological Response in Mice to Synthetic Glycoprotein and Reactivity of the Antisera to Various Antigens

Antigens	Glycoprotein antisera Conventional	Germ-free
2 per cent CMC	3/14	7/13
2 per cent BSA	12/14	12/13
2 per cent glycoprotein	13/14	12/13

in each group did the glycoprotein fail to elicit an immunological response detectable by the Ouchterlony procedure. The BSA and CMC moieties of the glycoprotein both served as antigens. Twelve of the 14 antisera obtained from conventional animals reacted with BSA and all but one of the germ-free animal antisera gave a positive reaction with BSA. Seven of the germ-free animal antisera precipitated in the presence of CMC whereas only three of the conventional animal antisera were positive in this respect.

Table 2. The Reactivity of Anti-BSA and Anti-CMC Sera to Various Antigens

Antigens	Anti-BSA sera	Anti-CMC sera
2 per cent CMC	0/9	2/9
2 per cent BSA	6/9	0/9
2 per cent glycoprotein	7/9	6/9

The reactivity of the anti-BSA and anti-CMC sera toward the three antigens employed in this examination is shown in Table 2. CMC gave a positive reaction with only two of the anti-CMC sera and BSA reacted positively with six of the nine anti-BSA sera. None of the anti-BSA sera reacted with CMC and no antibodies toward BSA could be detected in the anti-CMC sera. The synthetic glycoprotein formed precipitin bands with seven of the nine sera obtained from animals immunized with BSA and with six of the nine sera from mice injected with CMC.

The synthetic glycoprotein exhibited strong antigenic properties in both conventional and germ-free animals. Undoubtedly, the BSA moiety contributed greatly toward its antigenicity but the CMC portion also exerted some antigenic power, probably through a combination of factors, one of which may be the previously referred to structural similarity to Pneumococcal type III polysaccharide which has been shown to be a good antigen in mice[4]. Certainly the reactivity of the glycoprotein antisera with CMC is indicative of antigenicity of the polysaccharide moiety.

CMC did not appear to be a strong antigen, even when administered with the incomplete adjuvant. This may have been a result of the measuring device employed in this study since the anti-CMC sera, which gave only two positive reactions toward CMC, reacted to a greater extent with the glycoprotein, that is, six of the nine sera reacted positively with the glycoprotein. The apparent lack of antigenicity of CMC could be a function of its comparatively low molecular weight: 40,000. The minimum molecular weight of the glycoprotein as calculated from the ratio of the CMC and BSA content would be approximately 670,000. The compound of higher molecular weight should presumably be less subject to renal clearance.

The glycoprotein employed as an antigen in this examination was more resistant to attack by proteolytic enzymes, such as papain, than was the parent compound, BSA (ref. 5). This would indicate that the synthetic glycoprotein might retain its antigenic character longer than BSA. Wostmann has shown that BSA failed to elicit an immunological response in germ-free animals unless it was administered in conjunction with an adjuvant[8]. In our experiments, the synthetic glycoprotein was administered without any adjuvant being present and appeared to be a powerful antigen. This would indicate that the germ-free animal, when stimulated properly, is as capable of producing as good an immunological response as is the conventional animal. Bauer, Horowitz and Popper have shown that conventional and germ-free animals reacted in a similar manner when challenged by bacterial antigens[9].

Further examinations are in progress in which the antigenicity of CMC, BSA and glycoproteins of varying CMC/BSA ratios are being determined in germ-free animals.

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BIOLOGY

A Comparison of the Calcium Concentration of Submaxillary Salivary Glands

This communication reports the calcium concentration of submaxillary salivary glands of mammals including man. The submaxillary glands of the rat have been reported to contain more calcium than blood or other soft tissue[1]. The calcium concentration of the submaxillary glands of other mammals have not been compared using the same analytical procedures. The work recorded here was undertaken to determine whether the high concentration of calcium encountered in the rat submaxillary gland was characteristic of these glands in other mammals and man.

Submaxillary salivary glands were removed from rats, mice, rabbits, cats, dogs and sheep, following lethal doses of sodium pentalbarbital. Cattle glands were obtained from an abattoir immediately after slaughter. Human material was obtained at autopsy by the Pathology Department of Ben Taub Hospital, Houston, Texas.

Whole glands or representative portions of the glands were dried at 100° C and ashed at 500° C for 24 h. The ash was dissolved in a small volume of 1 N hydrochloric acid and a sufficient volume of distilled water to obtain a final concentration of 0·1 N hydrochloric acid. Standards of known amounts of calcium were treated in the same manner. Calcium analysis was done on appropriate aliquots of the dissolved ash by automatic EDTA titration[2]. Calcium concentration was expressed as milligrams of calcium per 100 g of wet weight tissue. Repeated analysis of aliquots of the same ashed samples gave results within the limits of the precision of the method used[2]. Analysis of the right and left glands of three male adults of each species was used for the results presented in Table 1.

Variations in the calcium concentration of the glands from the same species were to be expected since the calcium content of this tissue is affected by the extent of salivation prior to death[3]. There is a marked difference in the submaxillary gland calcium concentration of the species examined as indicated in Table 1. Cattle glands have the highest concentration while those of mice are the lowest in calcium.

Table 1. The Calcium Concentration of Submaxillary Salivary Glands of Various Mammals expressed as mg of Total Calcium per 100 g of Wet Weight of Tissue

	Calcium content (average ± *S.E.* of mean)
Mouse (Swiss albino)	10·3 ± 1·6
Rabbit	15·2 ± 2·1
Human	18·1 ± 2·0
Rat (Holtzman)	30·1 ± 1·9
Sheep	49·1 ± 3·1
Cat	63·8 ± 4·8
Dog	70·1 ± 4·2
Cattle	84·0 ± 6·1

Histochemical examination of submaxillary glands shows differences between rats, rabbits, human beings, dogs and cattle[4]. The variation in the metachromatic properties used for their differentiation is apparently related to the sialic acid content of the mucins extracted from the glands[5]. A calcium-binding salivary gland mucoid, containing sialic acid, hexosamine and protein, has been isolated from bovine submaxillary glands[6]. On the basis of the calcium concentration of 3·3 per cent, this mucoid, which represents approximately 1 per cent of the wet weight of the gland, would account for more than 30 per cent of the total calcium present in the gland. There also appears to be a direct correlation between the calcium concentration of the salivary glands as reported in this paper and the published values of sialic acid concentration of extractable mucins of human, cattle, dog and rat glands[7].

This work shows that the calcium concentration of submaxillary glands is markedly different in the species examined. In mice it is as low as that found in plasma, whereas in cattle it is 7–9 times this value.

In view of the fact that a bovine salivary gland mucoid has been reported to contain a high amount of calcium and that the sialic acid content of the mucoid of four species is in the same order as the calcium content of the submaxillary glands found in this communication, it is suggested that the calcium content of salivary glands is related to the sialic acid content of the mucoid present.

Some of this work was carried out at the Biological Laboratories, Rice University, Houston, Texas, and supported by a grant from the U.S. Public Health Service. I thank Prof. D. H. Copp of the Department of Physiology, University of British Columbia, for providing facilities for these investigations.

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Effect of *N*-Dimethyl Amino-succinamic Acid (*B*-995), a Growth Retardant, on Drought Tolerance

Recent information has shown that growth retardants such as (2-chloroethyl) trimethyl ammonium chloride (CCC) and 2,4-dichlorobenzyltributyl phosphonium chloride ('Phosfon'), applied as foliar sprays, can increase the drought tolerance of certain plants[1–3].

Another growth retardant, *N*-dimethyl amino-succinamic acid (*B*-995), has shown considerable promise as a retardant for fruit trees[4–6], but little information is available concerning its effects on drought tolerance. Greenhouse observations of growth reduction in several plant species indicated that *B*-995 may also increase tolerance to drought and this led to the work reported here.

In an initial experiment dealing with growth reduction in fruit trees, samples of fruit and leaves were taken from apple trees (variety 'Red Delicious') which had been treated with two sprays of 2,000 p.p.m. of *B*-995 six weeks earlier, and measurements of water deficit and transpiration rate were made. The water deficit of intact leaves was determined by obtaining their fresh weight, saturated weight after water absorption through the petiole for 24 h in a humid chamber at 100 per cent relative humidity, and oven-dry weight (70° C). Water deficit was calculated as the change in water content on saturation expressed as a percentage of water content at saturation. As shown in Table 1, leaves and fruit from treated trees had a lower water deficit than the controls. This occurred whether samples were taken from fruiting or non-fruiting spurs.

The leaves used to determine water deficit were also used for determination of transpiration rate. After the leaves had become fully turgid, they were removed from contact with water, exposed to uniform atmospheric drying conditions in the laboratory, and weighed after 30 min, and 1 and 6 h to determine transpiration rate. As shown in Table 1, the treated leaves lost water at a slightly lower rate than the controls. However, in subsequent tests using a leaf potometer in which water was supplied continuously to the leaf petiole and transpiration rate determined by weighing, it was found that in only 3 out of 10 trials (10 leaves/trial) had treated leaves transpired less and in the remaining trials there was no difference in rate of water loss from treated and control leaves.

A more detailed investigation was then initiated with sunflower (*Helianthus annuus*) as the test plant. Seedlings were grown in 5-in. plastic pots of silt loam soil until the primary leaves were completely expanded and then the seedlings were treated with three sprays of 2,000 p.p.m. at 5-day intervals. When a noticeable difference in size between treated and non-treated plants had developed, watering was discontinued and the plants were allowed to gradually dry out the soil in the pots. As the plants wilted, samples were taken periodically to determine plant water content, plant water deficit, and soil moisture content. Plant samples were obtained by cutting off the entire top (leaves plus stem) of each seedling at a point just below the primary leaves. Soil samples were obtained by unpotting the seedlings and taking approximately 200 g of soil.

The samples were taken at four stages of wilting, with an elapse of time between each stage. Both treated and check plants were selected at the same time as they appeared to dry out at the same rate. The plants were selected on a visual basis as follows: stage 1, slight wilting, all plants expected to recover after re-watering; stage 2, moderate wilting, most plants expected to recover; stage 3, severe wilting, approximately half the plants expected to recover; stage 4, very severe wilting, most plants not expected to recover. At each stage, half the plants were re-watered to compare the ability of treated and control plants to recover following wilting.

There was no dramatic effect of treatment on the moisture status of the plants or of the soil in the pots (Table 2). However, treated and control plants differed in their ability to recover turgidity and resume growth on re-watering. All plants recovered from wilting stages 1 and 2, and only 1 check died at stage 3. However, at stage 4 seven of ten checks died and only three of ten *B*-995 plants died. An attempt was made to determine the transpiration rate of individual leaves which had been placed with

Table 1. Effect of *B*-995 Foliar Spray on Water Deficit and Transpiration Rate of 'Red Delicious' Apple Tree Samples*

	Water deficit			Transpiration†		
	Leaves from non-fruiting spurs	Leaves from fruiting spurs	Fruit	30 min	1 h	6 h
Check	19·9	21·0	0·6	0·59	0·82	1·64
B-995	12 8	14 0	0 5	0·48	0·66	1·30

* All values are averages of 10.
† Rate determined for single leaves not in contact with water, results expressed as g water/g dry weight.

Table 2. Effect of *B*-995 on Moisture Status of Sunflower Plants and Soil at Four Stages of Wilting*

	Check			*B*-995		
	Plant water content†	% Water deficit	Soil water content	Plant water content	% Water deficit	Soil water content
Stage 1	317·3	38 5	3·5	317·5	41·7	3·5
Stage 2	229·6	51 5	3 0	251·2	52·3	2·4
Stage 3	203·9	60·0	2 9	203·7	59·3	2·9
Stage 4	187·7	59·2	2·1	154·2	58·9	2·8

* All values are averages for 10 plants.
† Percentage of dry weight

their petioles in water in a humid chanber (100 per cent relative humidity) and allowed to regain full turgor. Determination of transpiration rate of leaves at each stage was hampered, however, because the check leaves tended to re-wilt quickly when removed from the humid chamber. In fact, at stage 4, only 1 of ten check leaves regained turgor in the humid chamber, although 9 of ten *B*-995 leaves did.

In summary, it can be said that *B*-995 treatment does not appear to have a consistent effect on the magnitude of water deficit developed by plants subjected to drought or to delay the onset of wilting; however, the treatment does seem to enhance the ability of the plant to recover on watering after severe drought conditions.

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Short-term Temperature and Light Conditions associated with Auxospore Formation in the Marine Centric Diatom *Coscinodiscus concinnus* W. Smith

THE periodic appearance of auxospores in a uni-algal culture of the marine centric diatom *Coscinodiscus concinnus* W. Smith (Holmes and Reimann[1]) made possible an investigation of the temperature and light conditions under which auxospore formation occurs. By using the cross-gradient apparatus of Jitts *et al.*[2] I was able to expose potential auxospore forming cultures to 64 different light and temperature combinations simultaneously. The cells were examined daily by removing the culture vessels from the gradient block and scanning the contents with an inverted microscope.

Altogether three different experiments were run, each lasting 6–7 days. Cultures were used in which no auxospores or male gametes were visible initially. The population density of the culture used was adjusted with sterile media (see following) to yield approximately 100–200 cells per ml.—a density that was optimal for viewing under the inverted microscope. The incident light and temperature ranges were the same in each experiment, namely, 0·003–0·5 Langlies per min and 9·0°–26·7° C, respectively. However, different light and dark periods were used. In the first experiment the organisms were illuminated for 16 h daily, in the second for 12 h daily and in the third for 8 h daily. Guillard's[3] *f* medium, diluted with an equal volume of 80 per cent sea water collected near shore off La Jolla, and modified to yield a final concentration of 150, 250 and 25 μg at. of silicon, nitrogen and phosphorus per litre, was used in the first and third experiments. In the second experiment the silicon, nitrogen and phosphorus concentrations were reduced to 0·25 of that given here. The results of these experiments are summarized in Fig. 1, which includes observations on the presence of free-swimming cells (believed to be male gametes) in the experimental cultures as well as on auxospore occurrence.

The observations (Fig. 1) show that colourless uniflagellate male gametes developed and were released from the parent cells over a wide temperature (9°–26·7° C) and light (0·003–0·50 Langlies per min) range. This range probably somewhat exceeds the tolerance of the species for growth since the cells held at the extremes of light and temperature appeared very unhealthy in each of the experiments. The photo-period also influenced the range of conditions under which the gametes developed and were released. In the first and second experiments (16 and 12 h of light daily, respectively) the range of conditions suitable for male gamete formation was considerably more restricted than in the case of the shortest photoperiod (third experiment—8 h of illumination daily), where gametes appeared over almost the entire range of experimental conditions. A difference in nutrient concentration (second experiment) seemed to have little effect on the range of conditions under which male gamete formation occurred. The male gametes appeared in maximum abundance a day or two before auxospore formation, although co-occurrences were sometimes observed. In this latter case the peak abundance had passed and only a few living gametes were observed together with the auxospores.

Auxospore (zygote ?) formation occurred over a much more limited range of temperature and light intensity and is markedly influenced by photoperiod (Fig. 1). The results of the first and second experiments were rather similar in that auxospores formed only between 0·05 and 0·04 Langlies per min (see Fig. 1) and, with one exception, only between 21·6° and 24·1° C. Under the 8 h of daily illumination, however, a considerably wider temperature and light range was conducive to auxospore formation, namely, between 16·1° and 24·1° C and between 0·02 and 0·50 langlies per min. Furthermore, the shortest photoperiod (Fig. 1) accelerated the formation of auxospores. These were observed within 38 h after placing the cells on this light–dark cycle, whereas in the other two experiments, periods of 76 or more hours elapsed before auxo-

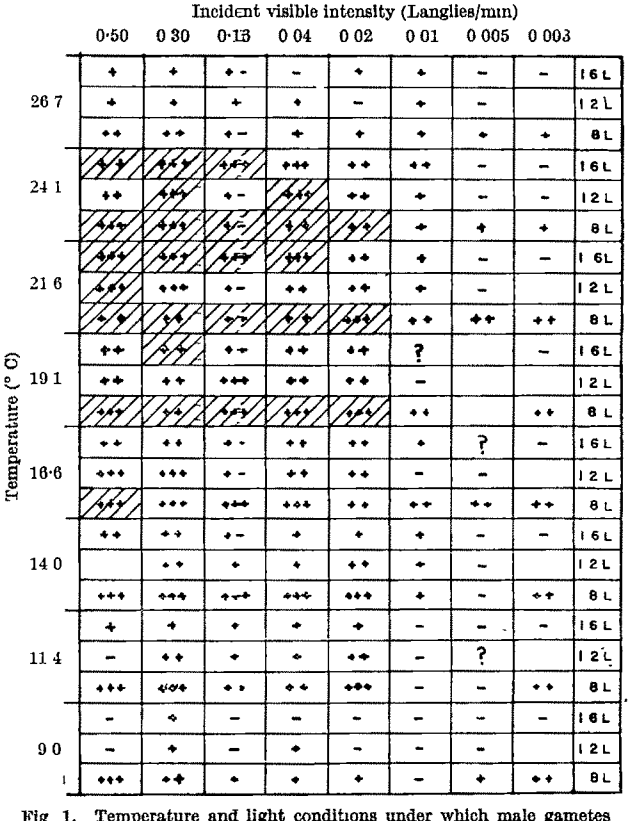


Fig 1. Temperature and light conditions under which male gametes and auxospores were observed in *Coscinodiscus concinnus* in three experiments with different photo-periods (16 light-8 dark, 12 light-12 dark, 8 light-16 dark, h daily). Male gametes, + + +, abundant; + +, common; +, rare; —, absent. The presence of auxospores is indicated by the hatched areas

spores formed. As was the case with male gamete formation, no appreciable effect of low nutrient concentrations (Exp. 2) was observed.

A number of factors have been reported to influence auxospore formation in centric diatoms[4–7] once the cells became physiologically capable of producing them[4,5,8]. Among these factors, light[4] and temperature[4,6] may be included. I know of no published investigation in which the effects of different photo-periods are discussed in this respect. Dr. Richard Norris of the University of Washington has informed me, however, that work carried out in his laboratory has revealed that short photo-periods will induce auxospore formation in *Ditylum brightwellii*.

An extrapolation of these experimental observations to the environment is perhaps premature, but the data suggest that in *C. concinnus* auxospore formation should occur near the sea surface where light intensity is high. Other things being equal, we would expect to observe this phenomenon under the broadest range of temperature and light during that portion of the year when the number of daylight hours is small.

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Oxygen and Phytoplankton Changes in the Newly Forming Volta Lake in Ghana

The Volta Dam was closed on May 18, 1964, initiating the transformation of a tropical river into one of the largest man-made lakes in the world. This lake will lie between latitudes 6° N. and 9° N. along the 0° meridian. Stretching more than 250 miles, it will eventually cover an area of 3,275 square miles and hold 120 million ft./acre of water. The maximum elevation of the water surface will be maintained at 276 ft. above sea-level and the seasonal variation in depth will be 8–10 ft.

The plankton and the various physico-chemical factors of the water are being investigated by members of the Volta Basin Research Project of the University of Ghana[1]. An intensive investigation has been undertaken of samples collected at Ajena, 2 miles north of the dam site at Akosombo. At first, samples were collected from the surface in mid-stream and, shortly after the closing of the dam, the middle and bottom depths were sampled in addition by using a Ruttner sampler.

A correlation between the amount of dissolved oxygen and phytoplankton has been observed since the closing of the dam. Dissolved oxygen was estimated by Winkler's method and expressed as a percentage of the saturation according to Truesdale, Downing and Lowden[2]. Phytoplankton was estimated by the sedimentation technique, using an inverted microscope, and expressed as the number of individual organisms per ml.

Before closing the dam, the river water was supersaturated with oxygen (110–170 per cent saturation) irrespective of the density of phytoplankton. The dam was closed at a time when a waterbloom due to a blue-green alga, *Anabaena aphanizomenoides* Forti, was rapidly declining. The immediate effect was an abrupt increase of this alga accompanied by a phenomenal excess of dissolved oxygen of 324 per cent saturation; such an excess is rarely observed and the cause is not well understood[3]. In this case it appeared to be due to the increasing phytoplankton which contributed more oxygen to a suddenly stagnated water which was already supersaturated. Later, the rapid decline of *Anabaena* continued and the dissolved oxygen rapidly decreased to as low as 16 per cent saturation on June 19. An increase of phytoplankton then followed and another blue-green alga, *Microcystis flos-aquae* (Wittr.) Kirchn., succeeded, and formed a peak on July 10; at the same time the dissolved oxygen increased to 36 per cent saturation. The subsequent decline of *Microcystis* was found to correspond with the decreasing

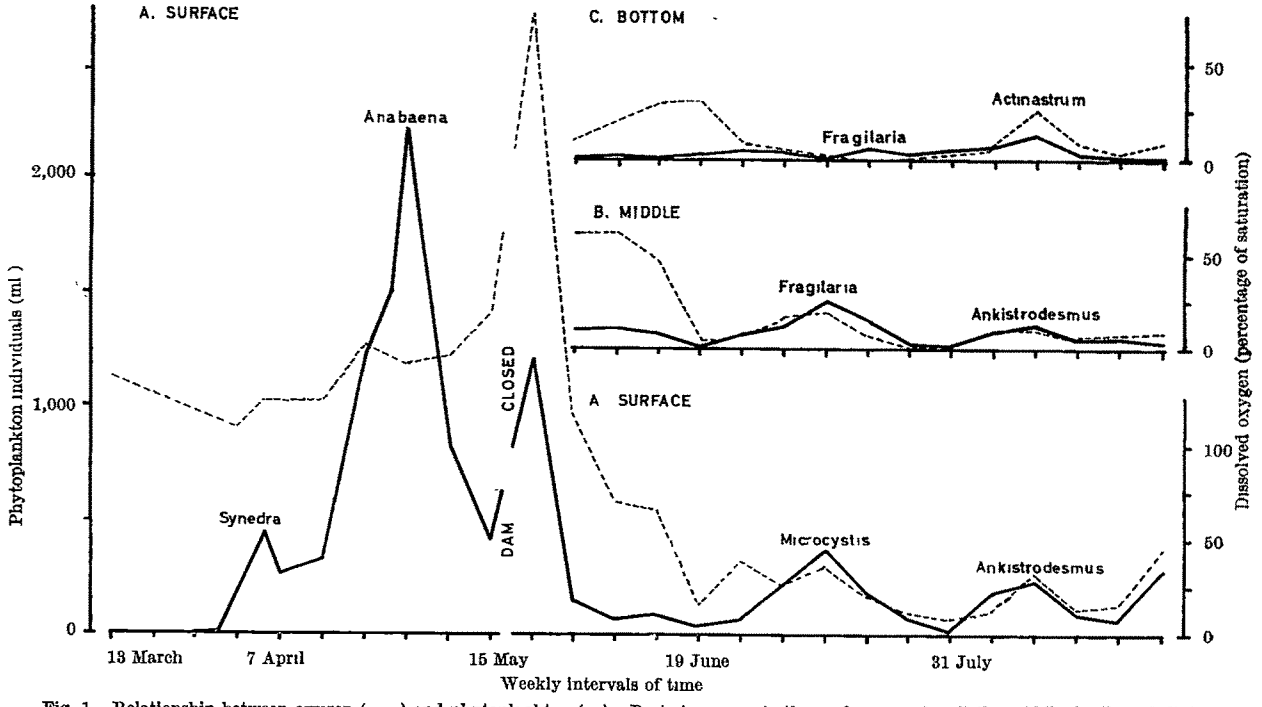


Fig. 1. Relationship between oxygen (- - -) and phytoplankton (—). Part *A* represents the surface samples, *B* the middle depth and *C* the bottom. The phytoplankton peaks have been labelled according to the dominant organisms of which they are composed

Table 1. Selected Data to show the Relationship between Oxygen and Phytoplankton Contents before and after Closing the Dam

	Before closing the dam				After closing the dam				
Dates	13/3/64	7/4/64	1/5/64	15/5/64	22/5/64	19/6/64	10/7/64	31/7/64	14/8/64
O_2 per cent sat.	134	124	141	170	324	6	36	9	33
Dominant phytoplankton per ml.	3	449	2,203	423	1,224	7	376	22	237
Names	*Ankistrodesmus*	*Synedra*	*Anabaena*	*Anabaena*	*Anabaena*	*Anabaena*	*Microcystis*	*Fragilaria*	*Ankistrodesmus*

oxygen content and by July 31 both were at their lowest. Later an increase of a green alga, *Ankistrodesmus falcatus* (Corda) Ralph., was associated with the increase of dissolved oxygen to 33 per cent saturation. It thus appears that the changes in the dissolved oxygen are intimately associated with the phytoplankton content. This is also true at the middle and bottom depths of the lake (Fig. 1, *B* and *C*).

The oxygen depletion was greatest on July 31 when the water was isothermal at 27·4° C through its depth of 24 m (≃ 79 ft.). So far there has been no establishment of permanent thermal stratification, as was also observed at Lake Kariba when its level was still rising[4].

I thank Prof. G. W. Lawson, Mr. R. S. A. Beauchamp, Prof. D. W. Ewer, and Dr. B. J. Harris, for their advice.

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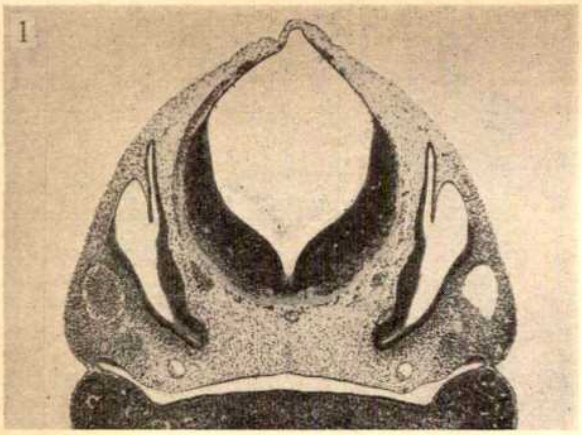
Influence of the Neural Tube on the Differentiation of the Inner Ear in the Mammalian Embryo

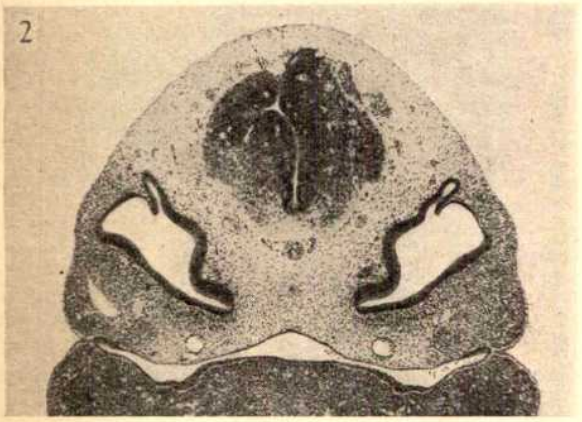
It has long been known from transplantation experiments that in amphibians and birds the differentiation of the otic vesicle into a normal labyrinth depends on the influence of the neural tube[1–4], but on account of the technical difficulties involved it has not been possible to extend these findings to mammals by means of similar experiments. The use of mutant mice is the most promising way of circumventing these difficulties. An analysis of the abnormalities of the inner ear in kreisler (*kr/kr*) mice[5] provided evidence that the neural tube plays the same part in the differentiation of the inner ear in mammals as in amphibians and birds. Confirmatory evidence was obtained from a study of dreher (*dr/dr*) mice[6]. It had been known for some time that, in this mutant, the differentiation of the inner ear was faulty. It was argued that, if the kreisler interpretation was correct, then in dreher embryos abnormalities of the neural tube in the region of the myelencephalon might precede those of the inner ear. This turned out to be the case[6]. Still further confirmatory evidence can be obtained by approaching the problems from the opposite direction, that is, by examining the inner ear in mutants where nothing is known about this organ but where the neural tube is known to be abnormal. Such an attempt is the subject of this report.

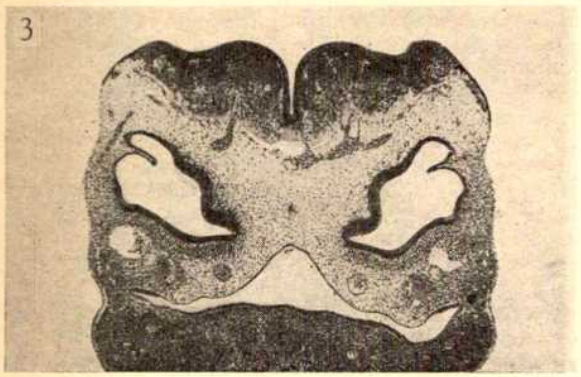
The mutants selected were Splotch (*Sp*)[7] and Loop-tail (*Lp*)[8]. The homozygotes in both these mutants have extensive abnormalities of the neural tube, and die during the middle or late stages of gestation. In *Lp/Lp* embryos, virtually the whole of the neural tube fails to close, and the neural tissue proliferates excessively. The abnormalities are similar in *Sp/Sp* embryos, but not so widespread, the neural tube often closing in the normal manner in the cervical and thoracic regions, and occasionally also in the cranial region. The material used was kindly supplied by Dr. Margaret C. Green and Dr. Margaret Dickie of the Jackson Laboratory, Bar Harbor, Maine, U.S.A., whose help is gratefully acknowledged. There were 27 embryos in all from the Splotch stock, 14 normal (+/+ or *Sp*/+) and 13 abnormal (*Sp/Sp*). Among the abnormals there were three in which the malformations of the neural tube were confined to the sacro-caudal region. Only 11 embryos were available from the Loop-tail stock, seven normal (+/+ or *Lp*/+) and four abnormal (*Lp/Lp*), but the results were so clear-cut that this number was considered quite sufficient. Since the differentiation of the otic vesicle does not begin until the 10-day stage and is complete in most essential respects by the 13-day stage, no younger or older embryos were examined.

The labyrinth was normal in the three *Sp/Sp* embryos in which the malformations of the neural tube did not extend to the cranial region. In all the other *Sp/Sp* embryos the differentiation of the otic vesicle was abnormal right from the beginning, although its position appeared to be normal. At the 10-day stage the point of origin of the endolymphatic duct is shifted backwards and upwards, and the duct itself is short and conical instead of being long and tubular. This difference becomes more pronounced at the 11-day stage, the duct growing backwards and outwards, and not vertically upwards as in the normal (Figs. 1 and 2). At the 12-day stage the cochlear tube can be seen growing in the medial direction. The vestibular part of the labyrinth now lies lateral to it, whereas its normal position is behind and above the cochlea. At the 13-day stage the vestibular part may even extend in front of the cochlea. Not a single structure in the labyrinth of *Sp/Sp* embryos is normal. The coiling of the cochlear tube is very poor, and the sacculus and the utriculus, in so far as they can be distinguished, bear little resemblance to the normal. The semicircular canals are present, but their planes, points of origin and relationship to the rest of the labyrinth are all abnormal. The anatomical situation is much the same in *Lp/Lp* embryos (Fig. 3), except that the abnormalities are even more severe: the endolymphatic sac in later stages grows to vast proportions, and the formation of the semicircular canals may be completely or partially suppressed.

In both the mutants the neural tube (or the neural plate) is believed to be the site of gene action. Its abnormalities appear before those of the inner ear. The latter cannot be explained away as the result of retardation or ascribed to mechanical causes. They may be regarded as secondary effects of the genes concerned, produced through the agency of the neural tube. This is evident from the normal structure of the labyrinth in the three *Sp/Sp* embryos in which the neural tube was normal in the cranial region. When considered in conjunction with previous work on kreisler[5] and dreher[6] mutants, the results of this investigation provide strong evidence that in mammals the differentiation of the inner ear is controlled by the neural tube in the same way as in amphibians and birds. Whether the neural tube exerts a direct influence or whether it works indirectly through its control of the movements and divisions of the facial-







Figs. 1–3. Transverse sections through the inner ear of an 11-day normal embryo (Fig. 1) (× 40), its *Sp*/*Sp* littermate (Fig. 2) (× *c.* 42) and an *Lp*/*Lp* embryo of the same age (Fig. 3) (× 40). Note that although the neural tube has failed to close in both *Sp*/*Sp* and *Lp*/*Lp* embryos, it is covered over by mesenchymous tissue in the former (Fig. 2)

acoustic ganglionic complex[5] is not clear. Mutants necessary for the elucidation of this point have recently been discovered, and their investigation is in progress.

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ENTOMOLOGY

Plastron Respiration in Marine Insects

The immature stages of more than 150 species of insects appear to be restricted to the intertidal zone. Most of these are flies, and most of the flies belong to the families Chironomidae, Tipulidae, Dolichopodidae and Canaceidae. A fair number of beetles, a few bugs, and at least one caddis-fly also breed between tide marks. The immature stages of most of these insects absorb oxygen from the water directly through their cuticle. However, the respiratory horns of the pupae of apparently all Tipulidae, the species of *Aphrosylus* (Dolichopodidae), and *Canace nasica* Haliday (Canaceidae) are modified to form plastron-bearing spiracular gills. About thirty-five species of Canaceidae are known. All appear to be marine except two species of *Protocanace* that have invaded freshwater streams, one in the Hawaiian Islands and the other in Java. On general grounds I suspect that many of the pupae of the Canaceidae will be found to have plastron-bearing gills.

A plastron is a gas film of constant volume and an extensive water–air interface. Such films are held in position by a system of hydrofuge structures (Fig. 1) and can resist wetting by the hydrostatic pressures to which they are normally subjected in nature. In well-aerated water a plastron enables the insect to remain immersed indefinitely, when it obtains the oxygen it requires from the ambient water. Insects with a plastron are restricted to well-aerated waters because a plastron becomes a very efficient means of extracting oxygen from the tissues if the oxygen pressure of the environment falls below that of the tissues.

A characteristic feature of the environments that contain well-aerated water is that they may be alternately flooded and dry—streams, the littoral of lakes, and intertidal areas. The insects that inhabit them are therefore liable to be exposed above water. The great advantage of a plastron is that it enables the insect to meet the contradictory demands presented to it by such environments: a plastron is adapted both for the extraction of oxygen dissolved in the water and for atmospheric respiration. In water the plastron provides an enormous surface area for the extraction of oxygen, but, when the insect is exposed above water, it provides a direct route for the entry of atmospheric oxygen that does not necessarily involve water-loss over a large surface area because the connexion between the plastron and the internal tissues may be very restricted.

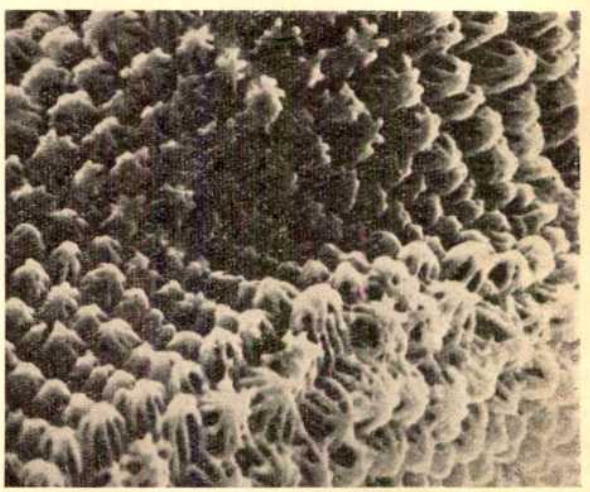


Fig. 1. Surface view of the plastron of the spiracular gill of *Geranomyia unicolor* Haliday taken with a stereoscan electron microscope

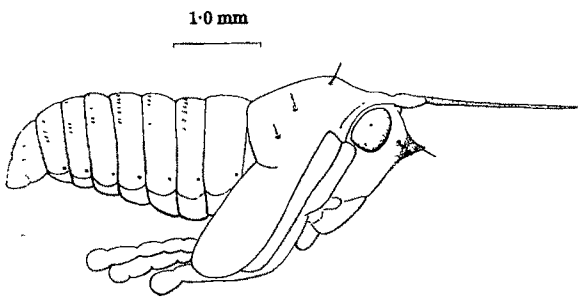


Fig. 2. Right side of the pupa of *Aphrosylus celtiber* Haliday. The plastron-bearing area of the spiracular gill is stippled

In most climates many terrestrial insects are alternately dry and flooded. When they are covered by water, the water is usually well aerated. To be submerged in water for several hours or even days when it rains heavily is no rare or isolated event but is a normal hazard of their environment. It might therefore be expected that many terrestrial insects are adapted for respiration in water in a manner no less complex than are many aquatic insects. Indeed, it has been shown that a wide variety of terrestrial eggs use the plastron method of respiration when they are flooded[1–6].

The intertidal Tipulidae all belong to the tribe Limoniini. It has previously been noted[7,8] that in all or nearly all Limoniini the epidermal cells which secreted the gill are left behind in the lumen of the gill at the time of the pupal–adult moult. During the pupal–adult moult a thin sheet of cuticle, the basal membrane, is secreted across the opening into the gill, with the result that the tissue in the gill is completely isolated from the haemocoele of the living animal not only by the basal membrane but also by a layer of moulting fluid and the cuticle of the adult. In *Geranomyia unicolor* Haliday, and presumably all other intertidal Limoniini, the epidermis that is shed at this time does not degenerate but remains in good condition throughout the pharate adult period, that is, from the pupal–adult moult to the pupal–adult ecdysis. It has been shown[7] that during this period the epidermis isolated in the gills of *Geranomyia unicolor* is competent to repair damage to the walls of the gill by secreting a plug of sclerotin.

In *Aphrosylus* epidermal cells are isolated in the spiracular gill in the same manner as in the Tipulidae. It may be noted here that epidermal cells are also isolated in the lumen of the respiratory horns of a number of terrestrial Dolichopodidae. The isolation of epidermal cells, or both epidermal and blood cells, in the spiracular gills or in the respiratory horns is now known to occur in about ten families of insects. Only a few of those insects which have evolved the habit of discarding tissue in the appendages of a previous stage exploit some of the attributes of the discarded tissue, which then comes to have a selective value it did not originally possess. The function of the isolated tissue in different spiracular gills has been summarized by me[8]: in some, for example the Simuliidae, it is used to expand the gill after the pupal–adult ecdysis[9], whereas in others it is used both to expand the gill and to maintain its turgidity throughout the life of the pharate adult, as in the Tipulid *Taphrophila vitripennis* Meig[7].

I thank the Cambridge Instrument Co., Ltd., for Fig. 1.

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MICROBIOLOGY

Problem of Absolute and Relative Specificity of Intestinal Microfloras based on Investigations on *Bibio marci* (Diptera) Larvae

The question whether in the intestinal tract of arthropoda and worms, living in the soil and feeding on it, a specific microflora, differing from that of the soil, is existing or not has not yet been clarified. According to some authors the intestinal flora is essentially identical with that of the soil[1,2]. Others have found it different and characteristic of the animal species or group[3–5]. We examined this problem on streptomycetes living in the intestine of *Bibio marci* larvae. The larvae are extremely widespread in numerous soil-types of Middle Europe and live in populations containing 400 to 2,000 individuals (from the same egg-laying). Those examined by us were taken from a rendsina soil, where their food consisted of forest litter and the organic material of the soil. More than 500 larvae were dissected for the examination of the intestinal content, and the microbiological analysis of many excrement masses, which had accumulated under the larva-populations, was also carried out.

For the isolation of streptomycetes, glycerol–arginine[6], glucose–asparagine and glucose–casein agar-media were used. From the soil about 3,000 (ref. 7), and from the intestine and excrement more than 1,000 strains were isolated. The taxonomic examination of the strains was based on the internationally accepted methods of Shirling and Gottlieb[8]. The relationships of the great number of *Streptomyces* varieties, isolated from the intestine of the larvae, were tracked down by the use of the principles of numeric taxonomy, the determination of similarity indexes[9]. Isolates were determined on the basis of collective (parallel) examinations with type or authentic cultures of known species. The results can be summarized as follows:

(1) The percentage occurrence of streptomycetes in the total intestinal microflora of *Bibio marci* larvae fluctuated between 20 and 50 per cent. (2) While the number of *Streptomyces* and *Nocardia* species found in the soil was more than 30, the *Streptomyces*-flora of the intestinal tract was extraordinarily monotonous, only a few species being found, and absolute dominance of one or two species was characteristic. (3) In the intestine of individuals belonging to the same larva-population the species-composition of the *Streptomyces*-flora, more precisely the taxonomic identity of the dominant species (or several species), was the same. (4) Although it appears from point 2 that in the intestine a 'specific *Streptomyces*-flora' is formed, differing essentially from that of the soil, this specificity was not absolute, only relative. In the intestine of individuals of different larva-populations the same species did not consistently dominate. In other words, the local circumstances of the soil, the composition of food, etc., have an effect on the selecting processes taking place in the intestine, which in turn result always in the predominance of one or two species. (5) No *Streptomyces* species could be found which could live and propagate exclusively in the intestine. (6) The *Streptomyces* species found to be dominant in the intestinal tract showed remarkable variability and could be isolated in several varieties. (7) The *Streptomyces* species, dominating in the intestinal tract, preserved their predominance also in the fresh excrement of the animal, where one or two species may give 95 per cent of the *Streptomyces*-flora, and 70 per cent of the total microflora. These species are later gradually repressed, relinquishing their place to the natural multispecies microflora of the soil. (8) The most frequently occurring dominant *Streptomyces* species isolated from the intestine of *Bibio marci* larvae were the following: *Str. fimlayi*, *olivaceus*, *antibioticus*, *flaveolus*

and *aureofaciens*. A detailed account of this work will be published elsewhere.

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VIROLOGY

Effect of 'Cephaloridine' on Vaccinia Virus *in vitro*

Experiments in our laboratory have indicated that a new cephalosporin preparation, 'Cephaloridine' (CR), is capable of arresting the development of a trachoma agent at an early stage, when used in concentrations as low as 0·1 μg/ml. of culture medium. In the present communication, some findings on the effect of this antibiotic on the multiplication of vaccinia virus in cell culture will be described.

A dermal strain of vaccinia virus (*V-Led-R*) was used[1]. Details of the preparation and storage of stock virus have been described elsewhere[2]. Cell cultures were prepared from a human kidney line originally obtained from the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada. Growth medium consisted of *M* 199 containing 10 per cent calf serum. As maintenance medium, a 0·5 per cent solution of lact-albumin hydrolysate in Earle's solution supplemented with 2 per cent calf serum was used. Plaque titrations and experiments were carried out in Leighton tubes, in which confluent cell-sheets were obtained 2–3 days following inoculation with 1-ml. portions of growth medium containing 4×10^5 cells. Details of the plaque technique in liquid medium for the assay of virus and infected cells have already been reported[2,3].

In preliminary experiments, the effect of various concentrations of CR on plaque formation was examined. About 100 plaque-forming units (p.f.u.) of the virus were added simultaneously with the drug to cell cultures. As seen in Table 1, complete plaque suppression was obtained at a concentration of 500 μg/ml. A concentration of 300 μg/ml. reduced the plaque count to less than 50 per cent of that in controls, and the average plaque diameter by about 70 per cent. Plaque count and size in cultures treated with 50 μg/ml. were the same as in control cultures.

Control experiments indicated that a concentration of 1,000 μg/ml. of CR was toxic to cultures, causing the cells to slough off the glass. Since the dose causing complete plaque suppression was so close to the toxic dose, experiments were carried out to examine its effect on cells in the monolayer. It was found that exposure of cultures to 500 μg/ml. of the drug for 48 h had no damaging effect on the cells as judged by microscopic examination, uptake of neutral red, and growth on sub-culture. Moreover, following removal of the drug after 48 h, the cultures were as competent as unexposed controls in adsorbing and synthesizing virus, forming plaques of the same number and size and yielding the same amount of virus. All further experiments were therefore carried out with a CR concentration of 500 μg/ml.

Table 1. Effect of Various Concentrations of 'Cephaloridine' on the Development of Vaccinia Plaques in Cultures of a Human Kidney Cell Line*

'Cephaloridine' concentration (μg/ml.)	Average plaque No. experiments 1	2	3	Average plaque diam. (mm)
0	78	112	151	1·5
50	81	110	154	1·5
100	64	84	109	1·5
200	46	65	83	1·0
300	36	50	71	0·5
400	10	12	14	0·5
500	0	0	0	—

* CR was added simultaneously with virus, and the number of plaques and diameter were recorded after 48 h.

In order to determine whether the suppression of plaque formation was due to total or partial inhibition of virus multiplication, the increase in virus yield and infected cell count was determined in cultures treated with CR after they had been allowed to adsorb virus. As seen in Table 2, which summarizes a representative experiment, both values showed an increase in the CR-treated cultures which was, however, much smaller than that in the untreated controls. The virus yield after 24 h was only 1/50, and the infected cell count only a sixth of the corresponding values in control cultures. During the following 24 h, virus yield increased more than sixty-fold in the control, and less than two-fold in the treated cultures.

Table 2 Effect of 'Cephaloridine' on the Yield of Virus and Infected Cells in Cultures of a Human Kidney Cell Line*

Time (h)	Concentration of virus in p.f.u. per Control culture	CR-treated culture	Concentration of infected cells per Control culture	CR-treated culture
0	25	25	25	25
24	$1·2 \times 10^4$	$2·4 \times 10^2$	$1·2 \times 10^3$	2×10^2
48	$7·5 \times 10^5$	$3·5 \times 10^2$	ND†	ND

* Each culture was allowed to adsorb 25 p.f.u. of vaccinia virus, as determined from the average number of plaques in 3 control cultures inoculated at the same time and stained after 48 h. 500 μg CR were added to the appropriate cultures 2 h after the termination of a 1-h adsorption period.
† Not done.

A few experiments were carried out to examine the effect of the drug during various stages of virus-cell interaction. A reduction in plaque count of up to about 50 per cent was obtained when the drug was present during the adsorption period only. A similar reduction was noted when CR was incubated with the virus *in vitro* and the mixture was then diluted beyond an inhibitory drug concentration prior to inoculation into cultures. It seems, therefore, that a direct interaction between virus and drug can occur. In order to obtain information on the effect of the drug at different stages of the growth cycle of the virus, cultures were first inoculated at a multiplicity of 10–20 p.f.u. of virus per cell to ensure simultaneous infection of all the cells. After an adsorption period of 1 h, they were exposed to CR for various time intervals. As seen in Table 3, virus yields decreased with increasing periods of contact between drug and cells. No significant difference in yield was noted when treatment was confined to either the first or the last third of the 24-h period of the experiments.

Table 3. Effect of the Presence of 'Cephaloridine' for Various Time Intervals on the Multiplication of Vaccinia Virus in Cultures of a Human Kidney Cell Line*

'Cephaloridine' present (h)	Virus yield: control (per cent) Experiments 1	2	3
0–24	15	13	12
2–24	21	20	25
4–24	28	30	34
6–24	46	44	41
8–24	51	54	59
0– 8	67	65	68
16–24	74	70	71

* Control and CR-treated cultures were inoculated at a multiplicity of 10–20 p.f.u./cell, and virus yields were determined after 24 h.

On the basis of these findings, it would seem that there are two aspects to the action of CR. When it is present during the adsorption period only, it lowers the efficiency of the virus to adsorb to the cells or to penetrate and initiate infection. When it is added after the termination of the adsorption period, it causes a reduction in the amount of virus synthesized. As a result of this reduction, which does not seem to be related to a particular stage in

virus development, plaque formation is suppressed or impaired, depending on the drug concentration used.

'Cephaloridine' was kindly supplied by Glaxo Laboratories, Greenford, Middlesex.

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PSYCHOLOGY

Behavioural Correlates of the Oestrous Cycle in the Rat

CHANGES in certain behavioural functions including learning and exploratory behaviour during the oestrous cycle of the intact rat have been reported[1,2]. Gray and Levine[3] recently re-opened the question of the extent to which sex differences in such essentially non-sexual behaviour could be determined by the altered behaviour of females during oestrus (heat). They induced oestrus in rats of the Maudsley reactive (*MR*) and non-reactive (*MNR*) strains by injections of oestrogen and progesterone and observed that emotionality as defined by emotional elimination and ambulatory activity in the open-field test was reduced. Our purpose is to repeat this work using natural as opposed to induced oestrus and to extend it both by the use of other inbred strains and measures of learning as well as emotionality.

The oestrous cycle was examined in 62 female rats, commencing at a mean age of 130·1 ± (*S.D.*) 31 days, in three highly inbred strains, *B*, *F* and *MNR* (*LAC* Nos. 163*b*, 163*c* and 163*g* respectively[4]), reared and maintained under carefully controlled and standardized conditions[5]. Mandl's technique[6] of daily vaginal smearing was used, though only four phases were distinguished: early oestrous, oestrous, early dioestrous and dioestrous. After an average of 37 days, 53 subjects which showed regular oestrous cycles were assigned at random to four groups representing the four phases distinguished, and each of these groups began behavioural testing in a different phase of the cycle as shown in Table 1. The lordosis response to manual stimulation of the vulvar region[7] was used throughout to confirm the presence of behavioural oestrus (heat) in rats showing an early oestrous smear.

Experiments were carried out in two phases. In the first, each subject was exposed in the open field[5] on five successive days to the mild stress of constant light (165 ft.-candles) and sound (78 dB above standard reference intensity) for precisely 2 min. Two scores were taken—the number of faecal boluses deposited, giving a measure of defaecation, and the number of compartments marked on the floor and traversed by the subject, giving, on conversion to a metric distance, the ambulation score. In the second phase, 12–36 days later, 44 subjects still showing regular cycles were given 40 trials each on a single day in a fully automated shuttle-box[8]. The rat learns to avoid an unconditioned stimulus shock of 0·2 m.amp by crossing to the other side during a conditioned stimulus (buzzer), the onset of which always preceded shock by 8 sec. The total number of these conditioned avoidance responses (*CAR*), their mean latency and the number of trials required to reach various criteria were all used as measures of learning. All testing was carried out in darkness after 9 p.m. (using red light for identification of subjects) in order to maximize the chances of detecting heat[9].

The results of both tests showed variation attributable to stage of oestrus. Open-field test results for a complete oestrous cycle were analysed separately for each strain because of the strain differences detected in defaecation and ambulation scores, which, incidentally, confirm those previously reported[10]. Because the *B* strain showed very regular four-day cycles, analysis of their ambulation scores was confined to the results for the first four days. The marked oestrual variation found throughout is shown in Table 1, in which the increment for rats in the early oestrous phase, irrespective of the day of its occurrence, is apparent. Analysis of variance of all scores grouped together for each stage, irrespective of day of testing, yielded a statistically significant *F*-ratio (5 per cent level). Subsequent *t*-tests showed that two of the three groups not in heat were significantly less active than the early oestrous group (mean score 6·00 ± 1·45 m). These were early dioestrous (3·93 ± 2·33) and dioestrous (3·84 ± 1·86). The results for the *F* strain were similar, though failing to show statistical significance, nevertheless supporting the view that heat facilitates ambulatory activity in the open field. The results for the third strain, the *MNR*, which had been tested 62·79 ± 5·31 days previously in the open field, suggested that this previous experience abolished both the oestral and daily variation in ambulation scores shown by the *B* strain (Table 1).

Table 1. MEAN AMBULATION (METRES RUN) ± *S.D.* IN OPEN FIELD ACCORDING TO PHASE OF OESTROUS CYCLE (*B* STRAIN)

Oestrous phase on test day 1	No. of subjects	Test day 1	2	3	4	Oestrous phase on test day 4
Oestrous	3	6·26 ± 2·06	1·66 ± 0·54	2·01 ± 1·81	*5·83 ± 1·63*	Early oestrous
Early Di-oestrous	5	6·82 ± 2·08	4·19 ± 1·71	*5·74 ± 1·47*	7·99 ± 2·81	Oestrous
Dioestrous	5	5·27 ± 0·78	*5·44 ± 1·38*	4·02 ± 0·76	2·72 ± 1·31	Early di-oestrous
Early oestrous	5	*6·95 ± 1·40*	3·76 ± 1·12	3·63 ± 0·11	3·15 ± 1·93	Dioes-trous
Total (all phases)	18	6·33 ± 1·63	3·99 ± 1·76	4·05 ± 1·72	4·82 ± 2·95	

Note: Results for groups tested when in early oestrous phase, irrespective of phase on test day 1, are in italics.

The defaecation scores for the *B* and *F* strains showed only a suggestion of a decrement during heat, in contrast with previous findings[3]. The *MNR* strain typically gave zero defaecation scores, having been negatively selected against this trait.

In the shuttle-box, rats of all strains in the early oestrous group consistently gave lower conditioned avoidance responses as compared with the other three groups. Analysis of variance for total number of *CAR*s out of the 40 possible yielded an *F*-ratio for oestrous stage significant at the 5 per cent level. The early oestrous group differed significantly (0·1–5 per cent by the *t*-test) from each of the other three groups. Mean *CAR* scores were 18·80 ± 3·98, for early oestrous group; 25·00 ± 5·97, for oestrous; 24·76 ± 4·77, early dioestrous; and 24·35 ± 5·49, dioestrous. The same significant differentiation of the early oestrous group from the other three groups was revealed in the avoidance latency scores (mean scores (sec): 3·61 ± 1·88, 2·60 ± 2·39, 2·99 ± 1·26, 2·92 ± 2·14 respectively). In addition, four criterial measures, based on the number of trials taken to achieve one, three, five and ten consecutive *CAR*s, lent supportive evidence. These results therefore indicate that during heat *CAR* acquisition was depressed.

It will be noted that the pattern we have detected during the early oestrous phase of the female rat's cycle is one of lowered indices of emotional responsiveness in the open field (increased ambulation and decreased defaecation) together with retarded acquisition of conditioned avoidance responses. This retardation has, however, been characteristically associated with greater emotional responsiveness in comparisons between the two Maudsley strains of rats[8]. On the other hand, the greater adrenal sensitivity to stress known to occur during oestrus[11] may well have occasioned less-efficient learning based on shock while still allowing expression of the gross activity differences which oestrus is known to occasion[2,12]. In the open field these might be seen in

greater ambulation and in the shuttle-box in greater pre-sessional and inter-trial activity—suggestions of which were in fact detected, and which may also have contributed to the poorer learning[13]. But the problem of the mechanism of the decrease in open-field defaecation, so clearly demonstrated by Gray and Levine[3], and which our results to some extent support, remains unresolved.

We thank the Medical Research Council for a studentship awarded to one of us (A. W. B.), and Prof. S. Levine for his advice. This investigation was in part supported by research grant *MH*-08712 from the National Institute of Mental Health, U.S. Public Health Service.

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Locomotor Activity in *Drosophila* as a Function of Food Deprivation

Under conditions of constant temperature, *ad-libitum* food and a 12-h light–dark cycle locomotor activity in *Drosophila* follows a circadian rhythm with a peak of activity soon after light onset. Green[1], working with *Phormia regina*, has recently suggested that spontaneous locomotor activity is controlled by a hormone released from the corpus cardiacum. The release of this factor is thought to be under the control of receptors in the foregut capable of monitoring the presence or absence of food.

The present experiment was designed to investigate the effect of food deprivation on the rhythm of locomotor activity described here. Two groups, an experimental and a control, each containing five male and five female *Drosophila melanogaster* (Pacific strain), were used. Activity was measured in an open field apparatus which consisted essentially of a box made from white 'Perspex', 10 × 10 × 0·5 cm. In one side of the box was a small aperture fitted with a plug through which the flies could be introduced by tapping them from a funnel. The lid of the box, made from clear 'Perspex', was marked off into 1-cm squares. An activity score was obtained by counting the number of squares crossed by an animal in the course of a 5-min test period. All measurements were carried out at a constant temperature of 25° ± 1° C, in constant light conditions. The control group, on an *ad libitum* feeding schedule, were kept individually in vials containing yeasted agar-molasses media, while the experimental, food-deprived group were kept in vials containing cotton wool soaked in distilled water. Measurements were made every hour on each fly by tapping it into the apparatus, allowing 1 min for the animal to settle down and then measuring locomotor activity over 5 min. Measurements were made over an 8-h period from 1 h after onset of light. Fig. 1 shows the activity scores for the two groups. There is no significant change in the scores of the deprived group at the beginning of the experiment compared with their scores at the end, nor are the fluctuations significant. Initially there is no significant difference in activity between the two groups but after 7 h deprivation the difference is highly significant at $<0{\cdot}001$ level (t test). The control animals show a steady decline in activity; however, the deprived animals show no drop in activity and continue to perform around the peak of the circadian rhythm. If the control flies are taken as a baseline, it can be argued that in fact the activity of the deprived flies increases. Fig. 2 shows the percentage change in activity of the deprived animals as the experiment progresses. Flies in the experimental group spent increasingly less time preening as deprivation progressed; no such change was observed in the control animals. The effects of food deprivation in *Drosophila* appear to be similar to those found in rodents by Thompson[2] and Fehrer[3]. Experiments are in progress to investigate the genetic basis of spontaneous activity.

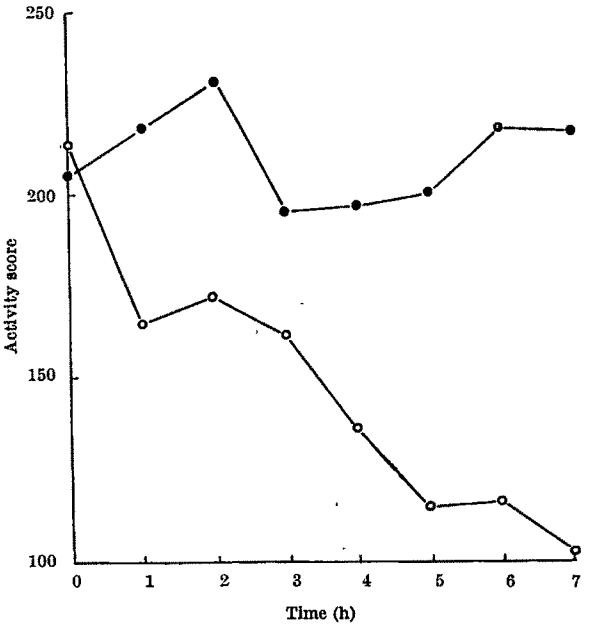


Fig. 1. Activity scores of food-deprived (●—●) and control groups (○—○)

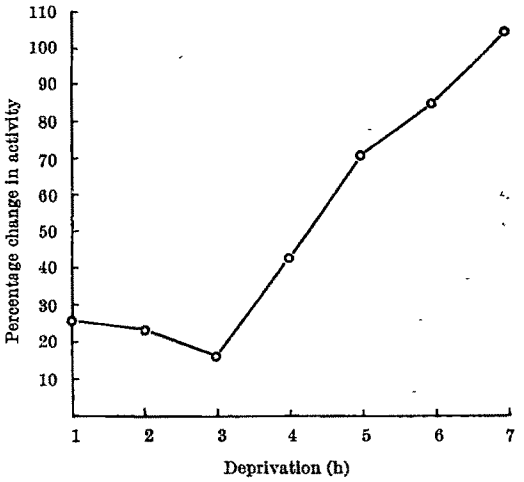


Fig. 2. Percentage increase in activity of food-deprived group

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 10

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Taurus and Tibesti" (colour films of recent expeditions).

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (joint meeting with the London Section of the Oil and Colour Chemists' Association, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mrs S. M. Rybicka and Mr. A. F. Sherwood. "Surface Properties of Titanium Dioxide Pigments".

Tuesday, January 11

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Alpha-Numeric Cathode-Ray-Tube Displays" opened by Mr. H. C. A. Hankins, Dr. A. T. Starr and Mr. L. W. Whittaker.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. S. D. Sutton: "The Purpose and Nature of Management". 7 p.m.—Ir. O. K. F. Bussemaker (Netherlands): "Theory of Tack and Factors Influencing this Property".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. T. L. H. Butterfield: "Design and Development of Fighting Tanks".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Manson Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Discussion Meeting on "State Registration of Technicians".

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Dr. D. E. Steele: "Plastics in Medicine".

Wednesday, January 12

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (at Imperial College, London, S.W.7), at 2.15 p.m.—Symposium on "Gains to Mathematics Teaching from a Decision to go Metric".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the Society for Applied Bacteriology, at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 2.15 p.m.—Meeting on "Microbiology of Desirable Food Flavours".

GEOLOGICAL SOCIETY OF LONDON (at the Shell Centre, London, S.E.1), at 5 p.m.—Prof. J. Tuzo Wilson: "On a Possible Explanation for the Crust's Growth and Movements" (Nineteenth William Smith Lecture).

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.—I.E.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "The Analogue Computer and its Application in Medical Research" opened by Mr. B. Hammond, Mr. J. R. Abrahams and Mr. J. R. L. Read.

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. P. K. M'Pherson, Mr. G. B. Collins, Mr. C. B. Guppy and Mr. A. Sumner: "Dynamics Analysis of a Nuclear Boiler".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Dr. L. C. Payne: "The Impact of the Computer Age on Medicine".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Dr. O. Bjorklund (Stockholm): "Principles Behind Standardized Shock and Bump Testing".

THE ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE, WITH IMPERIAL COLLEGE CAVING CLUB (in the Department of Physics, Imperial College, Prince Consort Road, London, S.W.7), at 7.30 p.m.—Dr. G. Warwick: "The Origins of Caves".

Wednesday, January 12—Thursday, January 13

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 9.30 a.m daily—Conference on "V.H.F. and U.H.F. Mobile Communications Systems and Equipment".

Thursday, January 13

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Liner Trains and Roundabout Trains" introduced by Mr. L. W. Ibbotson.

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Dr. W. Marshall: "Investigations of Magnetic Materials Using Neutron Scattering" (further lectures on January 20 and 27).*

CHEMICAL SOCIETY (in the Lecture Theatre, School of Pharmacy, Brunswick Square, London, W.C.1), at 6 p.m.—Prof. M. C. Whiting. "The Nature and Reactions of Classical Carbonium Ions" (Tilden Lecture).

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Automation of Large Steam Plant".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Dr. F. P. Gloyns: "Problems Connected with the Use of Colour Film for Colour Television".

Friday, January 14

ASSOCIATION OF APPLIED BIOLOGISTS (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 10.50 a.m.—Symposium on "Damp Grain Storage".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.), at 6 p.m.—Discussion on "How Should Engineering Education Take Account of Developments in New Materials?—Fibre Reinforced Materials".

Saturday, January 15

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Brian Moser and Mr. Donald Tayler: "Fiestas and Forests—Two Aspects of Colombia".*

Monday, January 17

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 2.30 p.m. and 5 p.m.—Colloquium on "Active Filters".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Prof. A. J. Birch: "Some Natural Anti-Fungal Agents".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. M. W. Thring: "The Present and Future Uses of Machinery". (First of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Prof. M. G. Say: "Unusual Electrical Machines".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Major P. G. Boxall: "Island of Bliss".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TECHNICIAN (preferably with O.N.C. or equivalent in mechanical engineering, metallurgy or physics) IN THE DEPARTMENT OF PHYSICS, for work on the fabrication, maintenance and operation of crystal growing equipment—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (January 14).

COMPUTER OFFICER (with a suitable university degree and experience in computer programming and operation) at the Tidal Institute and Observatory—The Registrar, The University, Liverpool, quoting Ref. CV/375/N (January 15).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE SCHOOL OF PHYSICS—The Registrar, The University Newcastle upon Tyne, 2 (January 15).

LECTURER IN HYDROLOGY IN THE DEPARTMENT OF FORESTRY AND NATURAL RESOURCES—The Secretary, The University, Edinburgh (January 15).

ASSISTANT LECTURER (preferably with special interests in electrophysiology or entomology and insect physiology) IN ZOOLOGY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (January 17).

RESEARCH ASSISTANT IN PHYSICS—The Registrar, University College of North Wales, Bangor, North Wales (January 20).

ASSISTANT LECTURER IN PSYCHOLOGY (any branch of the subject)—The Registrar, The University, Sheffield (January 21).

ASSISTANT LECTURER (with particular interest in experimental and applied psychology) IN PSYCHOLOGY—The Registrar, University College of Swansea, Singleton Park, Swansea (January 22).

LECTURER OR ASSISTANT LECTURER IN MATHEMATICAL STATISTICS, to work with an existing group of five members of staff—The Registrar, The University, Manchester, 13, quoting Ref. 229/65 (January 22).

LECTURER OR ASSISTANT LECTURER IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Leeds, 2 (January 24).

LECTURER/SENIOR LECTURER IN VETERINARY VIROLOGY IN THE DEPARTMENT OF VETERINARY PATHOLOGY AND BACTERIOLOGY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 28).

ASSISTANT LECTURERS or LECTURERS (with interests in the following: statistics and operational research; pure mathematics, especially geometry and algebra; applied mathematics, especially discrete and numerical systems; mathematical physics, especially nuclear and elementary particle theory) IN THE DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University of Southampton, Southampton (January 29).

LECTURER IN THE SCIENCE AND TECHNOLOGY OF PRIMARY METAL PRODUCTION IN THE DEPARTMENT OF METALLURGY—The Registrar, University College of Swansea, Singleton Park, Swansea (January 29).

LECTURER OR ASSISTANT LECTURER IN APPLIED MATHEMATICS in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (January 29)

EMBRYOLOGIST (scientist with research experience in embryology or developmental genetics)—The Director, The Poultry Research Centre, A.R.C., West Mains Road, Edinburgh, 9 (January 31).

LECTURER IN APPLIED MATHEMATICS; and a LECTURER (with a special qualification and interest in microbial genetics) IN GENETICS—The Secretary, The Queen's University, Belfast, Northern Ireland (January 31).

LECTURER or ASSISTANT LECTURER (preferably with a special interest in either sociological theory, or industrial sociology, or research methodology) IN THE DEPARTMENT OF SOCIOLOGY—The Assistant Registrar, Faculty of Commerce and Social Science, The University of Birmingham, Birmingham 15 (January 31).

LECTURER (preferably with research interest in geophysics) IN PHYSICS at the University College, Dar es Salaam (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, W.C.1 (January 31).

LECTURER (with a good honours degree in agriculture, agricultural botany, agricultural science, rural science or botany, and research experience in the field of plant breeding and/or crop ecology) IN AGRONOMY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 4).

CHAIR OF THEORETICAL CHEMISTRY—The Deputy Secretary, The University, Southampton (February 5).

LECTURER or ASSISTANT LECTURER (with research interests in any branch of physical chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Deputy Secretary, The University, Southampton (February 5).

LECTURER or ASSISTANT LECTURER (with research interests in theoretical organic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Deputy Secretary, The University, Southampton (February 5).

PROFESSOR OF GEOGRAPHY AT RHODES UNIVERSITY, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (South Africa and London, February 7)

LECTURER or SENIOR LECTURER IN MATHEMATICS, and a LECTURER, SENIOR LECTURER or READER IN STATISTICS at the University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 11).

POPULATION GENETICIST (with a Ph D. degree in a relevant field or post-graduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability) IN THE DIVISION OF ANIMAL GENETICS, Commonwealth Scientific and Industrial Research Organization, North Ryde, Sydney, New South Wales, to work in the field of selection genetics—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W C.2, quoting Appointment No. 675/162 (February 12).

TURNER AND NEWALL RESEARCH FELLOWS IN ENGINEERING, INORGANIC CHEMISTRY, PHYSICS AND ALLIED SUBJECTS—The Registrar, The University, Manchester, 13 (February 14).

ASSISTANT DIRECTOR (with a degree in biological science, preferably in nutrition, physiology, zoology or biochemistry, experience in research in nutrition, and preferably a reading knowledge of languages and editorial aptitude) OF THE COMMONWEALTH BUREAU OF ANIMAL NUTRITION, Rowett Research Institute, Aberdeen, Scotland—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (February 15).

DEPARTMENTAL DEMONSTRATOR IN BIOCHEMISTRY—The Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford (February 15).

CHAIR OF PURE MATHEMATICS at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar of the University (New Zealand and London, February 28).

LECTURER/SENIOR LECTURER (with a degree in veterinary, agricultural or general science with special training or experience in physiology, and preferably some experience in teaching at university level) IN ANIMAL PHYSIOLOGY IN THE VETERINARY SCIENCE DEPARTMENT, Lincoln College (University of Canterbury), New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 28).

SENIOR LECTURER or LECTURER IN GEOMORPHOLOGY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 28).

WILLIAM JULIEN COURTAULD CHAIR OF HELMINTHOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C 1 (March 11).

ASSISTANT PROFESSOR OF CHEMISTRY—Head, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

BASIC GRADE BIOCHEMIST to work in a Metabolic Ward under Prof. Dent (previous experience in a metabolic field is not essential)—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

FOREST PATHOLOGIST (national of the United Kingdom or the Republic of Ireland, with a degree in forestry and relevant postgraduate experience) in Trinidad and Tobago, to carry out a survey of forest pathogens—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W 1, quoting Ref 324/178/03.

HISTOPATHOLOGIST (with either a veterinary or medical qualification); and a TOXICOLOGIST or PHARMACOLOGIST (recent graduate in pharmacology, pharmacy or zoology) in the APPLIED PHARMACOLOGY DIVISION, to be concerned primarily with the pathology and toxicology of drug induced lesions in experimental animals—The Personnel Manager, Arthur D. Little, Ltd., Inveresk Gate, Musselburgh, Midlothian, Scotland.

ORGANIC CHEMISTS and PHYSICAL CHEMISTS (with a Ph.D., a good honours degree, or H.N C qualification, and preferably under 30 years of age). The work is in many cases of a fundamental nature and every encouragement is given to publish the results and study for a higher degree—The Director, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian, Scotland

POST-DOCTORAL BIOCHEMIST (Lecturer grade) for a research group working on isoenzymes in normal and malignant tissues—Prof. D N. Baron, Department of Chemical Pathology, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

RESEARCH ASSISTANT (preferably with experience of immunology or bacteriology) FOR A RESEARCH PROJECT—The Secretary, Institute of Clinical Research, Middlesex Hospital Medical School, 40 Hanson Street, London, W.1.

RESEARCH ASSISTANT (with a good honours degree) to work on bacterial genetics—Prof. G. G. Meynell, Guinness-Lister Research Unit, Lister Institute, Chelsea Bridge Road, London, S.W.1.

STUDENT TECHNICIAN IN THE DEPARTMENT OF FORENSIC MEDICINE, to assist (in a technical capacity) in a programme of research into the development of rapid methods of toxicological analysis—Dr. Harold V. Street, Department of Forensic Medicine, University New Buildings, Teviot Place, Edinburgh, 8.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Drug Houses, Ltd. 'Union' Carbide Molecular Sieves for Selective Adsorption. Third edition Pp. 43. Ion Exchange Resins. Fifth edition. Pp. 80. (Poole: British Drug Houses, Ltd., 1965.) [1011

Royal Observatory Bulletins. No. 100 Studies of the Globular Cluster ω Centauri. III. Proper Motions. By C. A. Murray, D. H. P. Jones, and M. P. Candy. Pp. E81–E98. 3s. net. No. 101: Studies of the Globular Cluster ω Centauri. IV. Photometry of RR Lyrae Variables. By R. J. Dickens and Jennifer Saunders. Pp. E101–E123. 4s. net. (London: H.M. Stationery Office, 1965.) [1011

The Cotton Silk and Man-Made Fibres Research Association. Report of the Council and Statement of Accounts for the year ended 31st March, 1965. Pp. 40. (Didsbury, Manchester: The Cotton Silk and Man-Made Fibres Research Association, 1965.) [1011

Other Countries

Conseil Permanent International pour l'Exploration de la Mer, Service Hydrographique, Charlottenlund Slot, Danemark. ICES Oceanographic Data Lists. 1958, No. 8. Pp. xii+286. 35 kr 1959, No. 3. Pp. xiii+214. 30 kr (Copenhague: Andr. Fred. Høst et Fils, 1965.) [911

Berichte des Deutschen Wetterdienstes. Nr. 98 (Band 14). Studien zur Phänologischen Agrarklimatologie Europas. Von Erika Freitag. Pp. 59 mit 13 Tabellen im Text und 16 Abbildungen im Anhang Bibliographien des Deutschen Wetterdienstes. Nr. 18. Beobachtungsergebnisse der Erdbodentemperatur. Bearbeitet von Rudolf Aniol Pp. 15. (Offenbach a M.: Selbstverlag des Deutschen Wetterdienstes, 1965) [911

South Australia Report of the Museum Board, 1st July, 1963, to 30th June, 1964. Pp. 11. (Adelaide. Government Printer, 1965.) [911

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions ANARE Interim Reports, Series A. Publication No. 76: List of Publications Resulting from the Work of Australian National Antarctic Research Expeditions. Compiled by M. R. O. Millett. Pp. 48. (Melbourne: Antarctic Division, Department of External Affairs, 1965) [911

Royal Society of Tasmania. Special Publication No. 2. A Glacial Map of Tasmania. By E. D. Derbyshire, M. R. Banks, J. L. Davies and J. N. Jennings. Pp. 11+map. (Hobart: Royal Society of Tasmania, 1965.) [911

World Health Organization. Public Health Papers, No. 28: Aspects of Family Mental Health in Europe. Contributed by D. Buckle *et al.* Pp. 123. (Geneva: World Health Organization, London. H.M. Stationery Office, 1965) 5 Sw. francs; 8s. 6d., 1.75 dollars. [911

Comité International des Poids et Mesures Comité Consultatif pour les Étalons de Mesure des Radiations Ionisantes, 5e Session, 1964 (28–29 Septembre). Pp. 106. (Paris. Gauthier-Villars et Cie., 1965.) [911

Tokyo Astronomical Observatory. Tokyo Astronomical Bulletin, Second Series. No. 167 (January 5, 1965): Discussions of Occultations, Observed in Japan with the Photoelectric Equipments from 1960–1962. By S. Aoki, M. Takahashi and Y. Endo. Pp. 2053–2059 No. 168 (March 31, 1965). Photographic Observation of Comets. By H Hirose, K. Tomita and H. Kosai. Pp. 2061–2065. No. 169 (March 31, 1965): Data of Time Observations and Variation of the Rotation Speed of the Earth By M Torao, S. Okazaki and S. Fujii. Pp. 2067–2077. No. 170 (March 31, 1965): Polar Tube Observations During 1964. By N. Sekiguchi, J. Matsumoto and F. Nemichi Pp. 2079–2080. Annals of the Tokyo Astronomical Observatory, Second Series. Vol. 9, No 2: A Method of Numerical Solution of Integral Equations Whose Kernels Have Almost Diagonal Forms. By N Sekiguchi. Pp. 111–121. Vol. 9, No. 3: Spectral Classification and Three-Color Photometry of A-Type Peculiar Stars By L. Osawa. Pp. 123–144. Mitaka, Tokyo: Tokyo Astronomical Observatory, 1965.) [911

Republic of Cyprus Annual Report of the Geological Survey Department for the year 1964. Prepared by Y. Hji Stavrinou. Pp. 53. (Nicosia: Geological Survey Department, Ministry of Commerce and Industry, 1965.) 150 mils. [911

International Journal of Cancer, Vol. 1, No. 1 (January 1966). Annual subscription (6 numbers): 180s.; 25 dollars; 110 Sw. frans; 120 Fr. francs. (Geneva: International Union Against Cancer, 1966. Subscription orders to Munksgaard Publishers, 47, Prags Boulevard, Copenhagen, S., Denmark.) [1011

United States Department of the Interior: Fish and Wildlife Service. Bureau of Commercial Fisheries. Fishery Bulletin, Vol. 65, No. 1: Marine Decapod Crustaceans of the Carolinas. By Austin B. Williams. Pp. xi+298. (Washington, D.C.: Government Printing Office, 1965.) 2 dollars. [1011

American Geographical Society. Serial Atlas of the Marine Environment. Folio 8. Zooplankton Indicator Species in the North Sea. By James H. Fraser. Pp. 2+3 plates. The Trace Elements. By Robert Johnston. Pp. 4+2 plates. Folio 9 Meteorology of the North Sea. By Frank E. Lumb. Pp. 3+9 plates. (New York, N.Y.: American Geographical Society, 1965.) [1011

Coconut Research Institute, Ceylon. Bulletin No. 21: Biological Control of Coconut Leaf Caterpillar (*Nephantis serinopa* Meyrick) in Ceylon. By Edwin Dharmaraju. Pp. 46. Bulletin No. 22: Coconut Withering Disease of Ceylon—a Report on the Survey Made of the Affected Areas in December 1962. By Prof. T. A. Davis. Pp. 29. Bulletin No. 23 Studies on the Nutrient Status of Some Coconut Soils in Ceylon. 4: The "Lateritic Soils" on Ratmalagara Estate. By K. Santhirasegaram. Pp. 23. (Lunuwila, Ceylon: Coconut Research Institute, 1962, 1963 and 1965.) [2611

United States Department of the Interior: Geological Survey. Bulletin 1140–C. Exchange Adsorption of Strontium on Clay Minerals. By J. S. Wahlberg, J. H. Baker, R. W. Vernon and R. S. Dewar. Pp. iii+26. 15 cents. Bulletin 1182–E: Investigations of Molybdenum Deposits in the Conterminous United States, 1942–60. By Harold Kirkemo, C. A. Anderson and S C. Creasey. Pp vi+90+plates 1–7. Bulletin 1189: Geology and Ground-Water Resources of the Pica Area, Tarapaca Province, Chile. By Robert J Dingman and Carlos Galli O. Pp v+113+plates 1 and 2. 1.25 dollars. Bulletin 1191: The Black Canyon of the Gunnison, Today and Yesterday. By Wallace R. Hansen. Pp. vii+76. 50 cents. Bulletin 1198–B: Geochemical Prospecting Investigations in the Copper Belt of Vermont. By F. C. Canney. Pp. iv+28. 15 cents. Bulletin 1198–D: Element Content of Bryophytes. By Hansford T. Shacklette. Pp. vii+21. 45 cents. Bulletin 1199–I: Bauxite Deposits of the Warm Springs District, Meriwether County, Georgia. By Walter S. White. Pp. iii+15+plate 1. Bulletin 1199–J: Bauxite and Kaolin Deposits of the Irwinton District, Georgia. By Walter B. Lang, Walter C. Warren, Raymond M. Thompson and Elizabeth F. Overstreet. Pp. iv+26+plate 1. (Washington, D.C . Government Printing Office, 1965.) [2611

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SCIENCE AND THE NATIONAL PLAN

*THE National Plan**, covering all aspects of Britain's economic development for the next 5 years, was prepared in full consultation with industry, as well as in close association with the National Economic Development Council. It analyses the problems to be overcome in order to achieve a 25 per cent growth in the national product between 1964 and 1970, and in his foreword the Secretary of State for Economic Affairs, Mr. G. Brown, claims that the Plan is both a statement of Government policy and a commitment to action by the Government. It means also that industry itself must plan investment, manning and training to achieve this 25 per cent increase in output and, above all, it requires an acceptance of change from all concerned.

The Plan is outlined in an introductory chapter, following which the basis of growth is discussed in seven consecutive chapters, which deal with output and productivity; manpower; industrial efficiency; investment; prices and incomes; balance of payments; and regional planning. Six chapters on industrial sectors follow, dealing with engineering and allied industries; construction; energy; transport; agriculture; and with other industries. Under "The Use of Resources", there are eight further chapters on the use of resources generally; consumers' expenditure; housing; public expenditure; defence; health and welfare services; education; and benefits and assistance. Although the Plan was published in September, it was first presented to the House of Commons by Mr. G. Brown on November 3, when it was welcomed without division. It was discussed in the House of Lords on November 24, and it is in the light of these two debates that its features of scientific interest are appropriately considered.

On November 3, Mr. Brown said that the Plan was designed to achieve four main objectives. Besides accelerating the rate of growth it was to rectify the balance of payments. Thirdly, it was to secure a better regional balance of development, and fourthly, it was to ensure that the fruits of future growth were used in accordance with the needs of a civilized and just society. An effective policy for productivity, prices and incomes was an essential key to these four objectives, and, although the Government hoped that voluntary effort would suffice, it would reinforce voluntary effort by legislative power, if necessary. Mr. Brown recognized that much more information was required, but he hoped that by the middle of 1966 the councils and boards would be able to offer preliminary advice on how their regional resources could be better used, and on the broad policy objectives and strategy of development towards which regional planning should be directed. He referred to the large increase in population which could be expected between now and the end of the century, and emphasized the importance of changes in the distribution of Britain's industrial effort which could, meanwhile, accelerate economic growth.

This would involve co-operation at all levels, including greater mobility of labour, and in this connexion he referred to the manpower situation. The Plan itself forecasts an increase of 390,000 in the working population between 1964 and 1970, apart from any special measures. It is estimated, however, that by such measures, including a reduction of 50,000 in the number of unemployed, it should be possible to achieve an increase of about 600,000. Nevertheless, Mr. Brown said that consultations with industry had indicated that there would be a demand for some 400,000 people more than were expected to be available in 1970. Regional policies might reduce this deficit by half, but there would be a need to make much better use of manpower, particularly in those industries where, at present, over-manning and wasteful use of people existed. This was essentially a problem for industry itself.

Mr. Brown did not refer specifically to trained manpower, although in its preliminary report (issued in November) the Willis Jackson Committee on Scientific and Technological Manpower indicated its close concern with the efficient use of such resources. The National Plan itself has three short paragraphs on scientific and technological manpower, and it estimates the number of scientists and technologists employed in manufacturing industry in 1965 to have been about 300,000. This figure does not include about 1·2 million technicians and other technical supporting staff. The present gross annual increase in the number of scientists and technologists of about 20,000 is expected to rise to nearly 28,000 by 1970 and, here again, emphasis is placed on improved deployment and more effective use of such manpower; however, it is also anticipated that the Government's defence policy will release some technically qualified people for civil work.

Here the Plan refers specifically to the work of the Willis Jackson Committee on Manpower Resources for Science and Technology, and to the important role of the technician. Besides an enquiry into training requirements for graduate engineers in the manufacturing industries, the Ministry of Technology is sponsoring a study of factors affecting the status of engineers in society, and is also planning a survey, in conjunction with the Engineering Institutions' Joint Council, into the ways in which such personnel are employed. Clearly the long-term increase in productivity in Britain will depend heavily on the greater skill and technical proficiency of the labour force. There is a further reference to the manpower situation in the chapter dealing with education, where it is claimed that a great effort is being made to develop courses for technologists, technicians and others.

The Plan envisages an increase in current expenditure on further education from the 1964–65 figure of £125·9 million to £189·5 million by 1969–70, and in capital expenditure from £23 million to £46 million. It is expected that recurrent grants to the universities will rise, over this period, from £140 million to £209 million, and that non-recurrent grants will reach £356 million; building programmes in the period will be concentrated almost entirely on provision for extra students. These figures contemplate more than 70,000 places in technical colleges and other institutions of higher education in 1969–70 and about 199,000 university places, with 98,000 places in teacher training colleges.

This expansion itself represents a large demand for manpower, estimated at just more than 100,000 full-time

* *The National Plan.* (Cmnd. 2764.) Pp. xviii+474. (London: H.M.S.O., 1965.) 30*s.* net.

staff between 1964 and 1970, or a 25 per cent increase on that in 1964. This would represent nearly two-fifths of the increase during the period in the number of those with full-time higher education, apart from the substantial increase in non-teaching staff, which, in the universities alone, could be almost 20,000.

It is recognized that the manpower situation may involve some limitation on the expansion that is planned, but this was not mentioned by Mr. Brown, nor was the question of trained manpower considered in either debate, although there were several references to the general manpower situation. This is the more surprising in that the manpower situation, and especially the supply of trained manpower, is the key to the whole Plan—as appeared to be implicit in some of the criticisms of the Government's educational policy. Even if the forecasts for manpower are accurate and the net shortage is no more than 200,000, it should be remembered that this assumes both an industrial and a regional mobility of labour which has not yet been witnessed in Britain. It also assumes a geographical mobility of expanding industries to which serious obstacles exist, and also abstention from serious industrial disputes even by small minorities or groups. Mr. A. Albu, who wound up the debate for the Government in the House of Commons, admitted that the shortage of skilled workers was a much more serious problem than the total shortage of workers revealed by the projections of the Plan. He was content, however, to leave the remedying of this situation mainly to the Industrial Training Boards, with assistance from the Government in expanding training centres. He did not otherwise discuss the educational issue.

This issue was more prominent in the debate in the House of Lords. Lord Winterbottom from the start questioned the adequacy of the proposed increase in total expenditure on the universities, particularly in the light of the contemplated expenditure on drink and on entertainments and other services. Such priorities were also questioned by other speakers, such as Lord Drumalbyn and Lord Wade, but Lord Brown, the Minister of State at the Board of Trade, restricted himself largely to export questions, though admitting that the educational system had failed to bring home to young people the central importance of British industrial effort. In consequence, the ablest sixth-formers were still not sufficiently attracted to industrial careers, nor was this always corrected in the universities. This has had a considerable effect on the leadership of industry, from which Britain is still suffering.

This is indeed specifically recognized in the Plan, which states that the most intangible, and at the same time the most important, factor in improving industrial efficiency is the quality of industrial management. With the growing interest in management education, it is expected that expansion in this field, as well as improvements in quality, will make an important contribution in raising standards of management; the increased mobility of managers and the wider use of professionally qualified consultants should also help. The Economic Development Committees must be concerned with all aspects of effective management, if they are to achieve their objectives. They will need to examine the adequacy and effective use of research and development, the training and recruitment of staff, design, control of stock and quality, and marketing. Some of the Committees have already started to examine these problems and the Government's insistence on fuller disclosure in company accounts is stated to be aimed at stimulating greater awareness of the scope for improving the performance of management.

Besides the two new graduate Business Schools at London and Manchester, and the similar facilities now being provided or planned at other universities or institutions of higher education, it is suggested that more needs to be done for the young non-graduate, for the older manager, and for certain specialized requirements, such as small firms in the export market. A wider dissemination of the many special techniques, such as operational research, network analysis, value analysis, systems engineering, linear programming and other more advanced methods of appraising investment could also help. Interest in management questions within the Trade Unions is also growing, but while the Plan notes the two-week courses on production and management held each year at the Trades Union Congress Training College, and the other training arrangements in this field, it overlooks the effect on recruitment of the expansion of higher education which can significantly affect the quality of leadership unless officials are recruited to a larger extent from the universities.

The rationalization of the industrial structure increases the need and opportunity for specialized management expertise, and the Government is justifiably anxious to do what it can to increase the numbers of professionally trained managers in control of industry and commerce. This applies equally to the administration of the Trade Unions, and the opportunities for efficient management can be restricted unless the leadership of the Unions is also effective and efficient and the implications of the growing opportunities in higher education are fully appreciated. British professional standards are respected throughout the world, but if, in the past, industrial management as such has tended to remain somewhat outside the mainstream of professional life, an improvement in the efficiency and standing of management could do something to ease the whole question of labour relations and the reform of the Trade Unions themselves.

This point was firmly pressed by Lord Peddie in the House of Lords on November 24, particularly in reference to the removal of restrictive practices. He pointed out that the responsibility here did not rest solely either on labour or on management. Nevertheless, the initiative for reform rested fundamentally with management, which must increasingly take labour into its confidence and seek to convey some understanding of the basic ideas behind policy. This was essential to secure full co-operation, and Lord Hanley fully supported him in this emphasis on co-operation and on the importance of management education. Lord Cohen, also, emphasized the importance of education, as did the Earl of Dundee and Lord Shepherd (the latter replying for the Government in the debate), but here the criticism was directed rather at the curtailment, even if temporary, of university building expenditure and the criticism was not effectively answered by Lord Shepherd. Earlier, Lord Eccles had also questioned the soundness of this pause, but he had been much more concerned to refute any suggestion that education was not an end in itself, but an equipment for life, rather than, as the protagonists of the Plan seemed to imply, a tool for economic growth.

Two other points of some scientific or technical interest in the National Plan were raised in the House of Commons debate. A section dealing with Government action, in the chapter on industrial efficiency, refers at some length to the work of the Ministry of Technology. After a reference to the effect of heavier competition in establishing a more dynamic and efficient economy, to the stimulation of

technological innovation and to increasing the efficiency of the public sector, the Plan refers also to the way in which mergers may assist technological development and increase the scope for co-operation in scientific and technical research. Where Government initiative can promote such developments, the Ministry of Technology is its chosen instrument, and, especially in the application of the results of research, the National Research Development Corporation. The powers of the Atomic Energy Authority to undertake research and development have also been extended with a like end in view.

The application of technology depends largely on engineers, and many weaknesses of British industries and their failure to meet foreign competition adequately can be attributed to a shortage of engineers and to insufficient attention to engineering design. There is evidence that both the profession of engineering and design activity generally are failing to attract sufficient of the ablest school-leavers and university graduates. In this connexion, also, the Ministry of Technology has been assigned the difficult long-term task of co-ordinating activities which will help to raise the status of engineers and improve their training and deployment in industry. Here the Ministry's programme involves implementing the recommendations of the Fielden Committee on Engineering Design in co-operation with other Departments and organizations; in co-operation with the Confederation of British Industry, much has already been done to increase the awareness by management of the importance of design. The formation of the Engineering Institutions' Joint Council is a further important and constructive step, and the Ministry is discussing with several universities and colleges the establishment of institutes of advanced engineering studies. The Science Research Council is also assisting by sponsoring advanced design work at the Universities of Cambridge, Glasgow and Liverpool.

Besides this, the Ministry of Technology has been given responsibility for sponsoring the machine tool, electronics, communications and computer industries, and this responsibility has since been extended to the mechanical and electrical engineering industries as a whole. While, in some areas of industrial automation, British invention is to the forefront, it is urgent that the present position should be improved and automation technology developed and applied more generally. It is a major objective of the Ministry of Technology in the field of development to support projects which will demonstrate the technical and economic benefits of automatic operation and process control. Measures were also announced in March 1965 to give greater assistance to the computer industry in developing both hardware and software, to support university research, and to provide advice on computer procurement in the public sector and elsewhere. A short section of the Plan discusses some of the measures being taken to this end in the public sector generally.

It should not be forgotten that the policy outlined in this chapter of *The National Plan* and the organization chosen to achieve that end rests on some assumptions regarding research and development that, far from being generally accepted, are still the object of informed debate. Much of the structure established for civil research and development has as yet, to say the least, to prove itself. Nor are misgivings confined to scientific and technological circles. In its third report for the session 1964–65 the Estimates Committee was highly critical of the structure and competence of the Ministry of Technology (see *Nature*, 207, 1113; 1965). It is difficult to point to concrete evidence that the position has improved since the report was published in July, nor has it as yet been discussed in Parliament.

In this context, Sir Edward Boyle was fully entitled to ask in the House of Commons on November 3 if the Minister of State for Economic Affairs was really satisfied with what the Minister of Technology was doing, or should be doing, to further the application in industry of scientific and technical knowledge. Sir Edward questioned whether the new Ministry was any closer to industry than the Department of Scientific and Industrial Research had been, finding support for his view in that section of *The National Plan* which has been summarized here. He agreed that the Ministry should be striving to make the whole of industry more 'science minded', but it was obvious that Sir Edward thought that the Minister, if not the Ministry, lacked drive and, possibly, confidence. Nothing has since been said either in debate or public speeches to remove such doubts.

The second point relates to fuel policy. This is considered in the chapter of the Plan devoted to Energy, which was written in advance of the White Paper on Fuel Policy, published in October. So far as policy is concerned, it does no more than emphasize the importance of freedom of choice for the ultimate consumer in helping to keep down fuel costs and of the endeavour, in recent years, to moderate the impact on coal of competition from imported fuels. It admits that, in the long term, a major uncertainty is the part that natural gas will play and that further imports on a large scale or the discovery of large quantities in the British part of the North Sea Continental Shelf could radically change the energy position in Britain. Nothing in this chapter, however, gives any hint that any more vision and determination have gone into the National Plan in this vital sector than in the subsequent White Paper, and Mr. Ian MacLeod's criticism on this point in the House of Commons debate was largely justified. It is not simply that the assumptions made by successive Governments in the field of fuel policy for many years have been consistently wrong. There has also been a persistent failure to face the difficulties and implications arising out of the clash of interests between Departments and the nationalized fuel industries.

This is a criticism of all recent Governments and, in that sense, this is not a matter of party politics. It illustrates, however, the extent to which the issues arising in national planning are technical and factual and are not to be resolved by means shaped primarily to suit a political ideology. Fuel policy, in fact, brings us back to the real gravamen of the criticism which has been made of the Ministry of Technology. It is not simply that there are good reasons for questioning whether the soundest possible structure has been established for Britain's effort in civil research and development. The structure might work, even if imperfectly, provided due regard were paid to the vital criteria for assessing the balance of the effort, the choice of projects and the conditions of effective scientific work. These have been admirably set forth in papers by G. B. Kistiakowsky and Dr. A. M. Weinberg, which have been discussed recently in these columns (*Nature*, 209, 1; 1966).

What is disturbing is the lack of evidence in the Plan, or elsewhere, that these conditions are appreciated by the Government, or that it is prepared to make the continuous allocation of resources, free from limitations or 'stop–go' restraints, which are essential for Britain's continued

advancement. Mr. Brown will find that the real test of the National Plan will lie in the way in which it channels the national effort, and not the least the way in which it affects the civil research and development effort.

PANGLOSS OR PLOTINUS

The Uniqueness of Biological Materials

By A. E. Needham. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 25.) Pp. xix + 593. (London and New York: Pergamon Press, 1965.) 100*s*. net.

A FEW years ago those who followed in the footsteps of L. J. Henderson and argued that the properties of elements and compounds were adapted, to an extent that was unlikely to be accidental, to the requirements of life and that therefore our form of life was likely to be the only one, had little reason to fear refutation. It takes rather more self-confidence to argue this case now, for space travel could produce contradictory evidence. Those who maintain that Henderson paid insufficient attention to the probability that, in evolving here in the way it did, life exploited the existing situation opportunistically, and could be different in a different environment, are still on reasonably safe ground; they can 'have it both ways'. If organisms should be found on another planet that depend on a basic biochemistry similar to that used on Earth, it may mean no more than that both have come (or just conceivably have been sent) from a common source. If an alien form of life should be found, our case will be proved. The two (or more) systems might be called chthonobiology and xenobiology, and the investigation of the extent to which each is adapted to local conditions will make a fascinating study.

While waiting for the possible start of this extreme form of comparative biochemistry, it is as well to survey the extent to which organisms have adapted themselves to local conditions and exploited all chemical potentialities. Needham attempts this comprehensibly and, on the whole, successfully. *The Uniqueness of Biological Materials* is not without faults: there is a great deal of matter in it with no obvious relevance to the theme, there are several factual errors, and the citing of references is capricious. Too many references are to secondary or even popular sources, and often relate to matter that is dealt with in text-books, whereas interesting, and sometimes improbable, statements slip in with no authority. Nevertheless, it is a serious and original attempt to deal with an important subject and represents the fruit of a vast amount of reading.

Three-quarters of the book is devoted to carbon compounds. Those aspects of biochemistry that are nearly universal are outlined and some of the more intriguing by-ways are mentioned. Needham not only discusses the molecules that are used but also those that are not—or that are used rarely. This is fundamental to the proposition implied by his title. The frequent reappearance of the same molecules could arise because these are the only ones able to bring about the required reactions effectively; because the dependence of organisms on one another in food chains forces the later links in the chain to comply with the pattern defined by the first member; or because, once a molecule is established in a well-integrated enzyme system, natural selection will tend to eliminate a variant, even if it uses a potentially better molecule, during the phase in which enzymes adapted to the new molecule are evolving. These points can be explored experimentally. Thus the diversity of phosphagens used in different phyla shows that different molecules can perform a similar function, but it is still by no means clear what advantage, if any, each has in the phylum in which it occurs.

The general use of L-amino-acids in proteins is reasonably satisfactorily explained by invoking food chains and the greater instability of polymers containing both isomers compared to those containing only one. It is more difficult to explain the preference organisms show for the same twenty amino-acids. It would be interesting to know whether polymers containing some of the excluded amino-acids, for example, L-α-amino-butyric acid, have any anomalous properties *in vitro* that could justify the exclusion. It is well known that an amino-acid substitution, azetidine carboxylic acid for proline, or valine for glutamic acid, gives rise to a significantly different protein —but still a protein. Before we regard the usual amino-acids as unique we should know whether a polymer containing a significant proportion of the excluded amino-acids would be a protein at all.

The elements other than carbon are, naturally, considered in less detail. The manner in which the properties of such elements as iron and calcium adapt them for their observed biological roles is outlined. These are common elements, so their general use is comprehensible; the rare use of such elements as barium, strontium and vanadium is more difficult to understand. Is this rarity simply a consequence of their patchy distribution on Earth's surface; or are these elements, in spite of the antiquity of the species that use them, intrinsically less suitable? This book gives no answer but, by presenting the evidence, it usefully calls attention to the existence of the problem. In the course of this discussion the conclusion is reached that biological processes do not alter the properties of the elements. That will be generally agreed, but it is surprising to find the issue discussed at all; one had thought the possibility was mooted only by those who think that the fluoride ion has different properties when coming directly from underground rather than from a bag, or that 'fixed' nitrogen differs in some way from that in dung.

The biochemical aspects of biology depend, in essence, on the interplay between morphology and enzyme action. If we bear in mind the observed rate of dimensional change (4 per cent or more per million years), the plasticity and flexibility of proteins (exemplified by the ability of antibodies to conform to an immense range of antigens), and the 2,000 million years that have apparently been available for evolution, the observed congruence between chemical potentialities and the needs of organisms seems to raise no difficulties. The number of possible proteins is so vast that it is reasonable to expect them to cope with any chemical reactions that have possible 'survival value'. On this view our present state may not yet be "the best in the best of all possible worlds", but we are heading there. The rival view is that the congruence is improbably good; it leads to the concept of design, or to Plotinus's thesis that organisms and the basic properties of the environment act reciprocally on one another. The observations made so far do not seem to force on us so radical an overthrow of accepted scientific canons. N. W. Pirie

LUNG CIRCULATION

The Lung Circulation

By Prof. Domingo M. Aviado. Vol. 1: Physiology and Pharmacology. Pp. xxiv + 1–590. Vol. 2: Pathologic Physiology and Therapy of Diseases. Pp. xix + 591–1405. (London and New York: Pergamon Press, 1965.) 300*s*. net, per set.

UNTIL recent years the pulmonary circulation was not a popular subject of investigation among either physiologists or clinicians. This was due, in part at least, to a lack of interest and the belief, generally fostered by text-books of physiology, that the pulmonary circulation is effectively independent of the nervous system or of any humoral control. It had been repeatedly shown from the late nineteenth century onwards that pulmonary vasomotor responses could be excited by both nervous and hormonal stimuli, but such evidence was generally

dismissed on the grounds that the responses were too small to be significant and that, in any case, since all blood must flow through the lungs, imposed changes in the resistance of the lung vessels would serve no useful purpose. This is still a difficult argument to meet, but, as readers of *The Lung Circulation* may see for themselves, it is possibly no longer of great importance. Now it is not so much a question of whether control exists but by what mechanism it is made effective. For this new attitude a great deal of credit must be given to both Liljestrand and Cournand, the former because of the post-war investigations initiated in his department on the pulmonary vasomotor effects of ventilation hypoxia and hypercapnia, the latter because of his influence and example in developing techniques for the examination of the pulmonary circulation in man. The great improvement in technical facilities and instrumentation has undoubtedly also contributed to the increase in research on the circulation of the lungs. How accelerated this has been may perhaps be appreciated from the fact that the author of this book has quoted from about 7,000 papers, 80 per cent of which have appeared in the past 15 years. Those who know how sparse was the pre-war literature on this subject will appreciate the contrast.

The author of these volumes, Dr. Domingo Aviado, with his colleagues at the University of Pennsylvania, has himself made a distinguished contribution to the pharmacology of the pulmonary circulation during the past 15 years and, from his knowledge of the problems involved, both technical and theoretical, is well qualified to write about this subject. It is remarkable, however, that he should also have managed to write so informatively about many other aspects of the lung circulation in disease as well as in health.

Containing more than 1,200 pages of text, the book is divided into two volumes comprising five sections. The first section (*A*) deals with the effects of ventilation hypoxia, the evidence for baro- and chemo-receptors in the lungs, the bronchial circulation and the evidence for the release of certain chemicals, 5-hydroxytryptamine and the like, from lung tissues. Section *B* deals with the action of drugs which either imitate or alter the action of autonomic nerve stimulation. Section *C* takes up the actions of a comprehensive list of vaso-active drugs of clinical and scientific interest. Next (*D*) comes a discussion of acute pulmonary diseases and (*E*) of chronic pulmonary hypertension. The discussion throughout the five sections is linked together by the emphasis placed in each on the idea that the blood vessels of the lungs behave as any other blood vessels in health and disease and are under similar controls.

As a reference work, teachers and clinicians cannot help but find it a useful text. The information about the action of drugs must alone be considered a valuable contribution; but the account of the clinical observations made on the human lung in the past 15 years is equally valuable. For those who want to find out more about the use of such observations, and the techniques utilized, the book provides plenty of references.

Physiologists who have studied the problems treated in the first two sections may not agree with all the author's ideas. One cannot help but feel that there has been too great a desire to bring all information under one theoretical umbrella. There are large areas of theory which have very little experiment to reinforce them. But whatever one may think about individual arguments and interpretations, the total structure built up is impressive and cannot fail to interest and stimulate other investigators in the field.

A welcome subject of discussion in the book is the bronchial circulation. This adds greatly to its value. Except in some specialized anatomical treatises there are few accounts of this system of vessels, although, because of its nutritive function and because of its connexions with the pulmonary vessels, it has great functional importance. As Daly showed over thirty years ago, for experimental investigations of the innervation of the lungs the bronchial circulation must be maintained or the nerves will die. Extensive communications between pulmonary and bronchial circuits also mean that haemodynamic events within either circuit may affect the other; this factor must be borne in mind when pulmonary vasomotor responses to any stimulus are under test. There are obviously good reasons why account must be taken of the bronchial circulation when planning experiments on the pulmonary circuit, but a knowledge of the bronchial circulation is also of clinical importance. Its reactions and growth changes in pulmonary disease constitute one aspect of its clinical relevance; another is the significance of the bronchial circulation as a nutritive system of vessels to a number of lung structures. The author should also be commended for giving a fair and balanced account of broncho-pulmonary anastomoses. It is sometimes a temptation to think that one function of pulmonary vasomotor control may be in the opening and closing of precapillary anastomoses between the two systems; maybe it will prove to be so; but, as the author emphasizes, the evidence that these connexions exist is still controversial.

It is a disappointment that the chapter on the autonomic control of the pulmonary circulation is largely a discussion of drugs and that the direct evidence from experiments on the effects of stimulating the nerve supply of the lungs is given such cursory treatment. There is an extensive literature dealing with this question which goes back to the nineteenth century, and one cannot but wonder a little whether the author does not lack a sense of proportion when, as evidence that sympathetic nerve stimulation increases pulmonary blood flow and pressure and pulmonary vascular resistance, he quotes a paper of his own written in 1961 and another by a Japanese author written in 1962. It is here that one would expect I. de B. Daly to get honourable mention, but at this stage his work is quoted only in support of some minor points in the exposition. In any event, the title of the chapter is misleading, since the author evidently had no intention of discussing the innervation of the vessels as such. What is more serious is the superficial treatment of the pharmacological evidence. The author concludes that the simplest concept is to regard acetylcholine as having a dilator action on all blood vessels, pulmonary and systemic, even though most of the classical evidence, including some quoted by him, shows that it can be both pulmonary vasoconstrictor and vasodilator in a number of species. Such theoretical treatment is not very helpful.

In certain respects the presentation is confusing. The reader will very often find it difficult to know which evidence has been obtained by Dr. Aviado himself and which is being quoted from others. Statements which seem to be theory only are made as if they were authenticated experimental fact. When, as I suppose will happen, this book comes to a second edition, these points might well be rectified. Similarly, it would be helpful if, under figures taken from journals, the authors' names were quoted. Presumably most of those used were from Dr. Aviado's own papers; even so, it would be helpful to know. The figures would also be easier to understand if the tracings, such as those of Fig. 1.5, were always labelled.

Line drawings appear in many parts of the text to illustrate or summarize methods or ideas. Some, such as Fig. 7.1, are moderately successful but it must be admitted that many are less successful. In some cases this is simply because the lettering is insufficient or insufficiently explained (see Fig. 1.9) and the diagram itself is not inspired enough to help the reader over the difficulty. Other figures such as 5.9 seemed pointless to me; this one simply indicates a few of the well-known actions of histamine, suggests that it can be released in three parts of the circulation and from any one is likely to be carried

to either of the others. An elaborate diagram to make these points seems unnecessary.

Fig. 6.3 deserves a special comment. It is a good idea to carry the same pressure-flow diagram through the book and to explain its meaning at the beginning. The idea would be even better, however, if it were made clear there and then that the symbols explained, but not used in it (Fig. 6.3), are the ones which will appear in succeeding diagrams of which it is the prototype. Otherwise, readers who like to understand each stage of an argument before going on to the next may spend more time than they should looking for the lost symbols on or around p. 330. Nevertheless this is a useful idea and should be commended.

Chapter headings have not always been well chosen. The one beginning "The Release of Chemical Substances" (Chapter 5) has comparatively little evidence about the actual release of the various substances discussed. It affords a lot of evidence suggesting that, if such agents as histamine, 5-hydroxytryptamine and others are released, then they would act on the pulmonary vasculature in various ways. Under certain circumstances some evidence of the release of histamine can be obtained, but it remains to be shown whether this normally happens in such a way as to produce responses of the lung muscle. Even less is known about the normal functions of the other substances discussed, but the tone of the discussion here seems to imply that the various agents considered are in fact concerned in the regulation of the lung circulation. Far more evidence will be needed before these views can possibly be considered seriously.

There are some inaccuracies, and signs that some of the papers referred to have not been read very carefully. For instance, it is implied on page 9 that Beyne was the first to test the effects of anoxia uncomplicated by asphyxia on the lungs. In fact, the credit for this should go to Plumier, whose experiments were published nearly forty years earlier. Again, on page 156, Duke *et al.* are quoted inaccurately and the point in their paper that excess CO_2 is not itself an adequate stimulus for the postulated chemoreceptors of the pulmonary artery is missed. It is a pity, too, that in dealing with the effects of anoxia the author fails to make clear to the reader the essential differences between experiments done on the completely isolated perfused organ and those done on lobes or single lungs perfused *in situ*. In the latter, not only the innervation of the tissue but its bronchial blood supply is intact, and the influence of the heart on the venous pressure and drainage may introduce other haemodynamic variables.

In the context of the total achievement of the book these criticisms are minor ones. It has to be remembered that this is the first book of the kind to be written about the pulmonary circulation and, whatever one may feel about some of his individual judgments, there is no gainsaying that the author has managed to present a coherent and logical account of the subject and that he is in full command of his material throughout. Moreover, the discussion is generally lively as well as informative. Those who are interested in learning about the lesser circulation will find a great deal to the purpose in this book; others who are already indoctrinated will be grateful to Dr. Aviado for his extensive analysis of the recent literature and will find *The Lung Circulation* a useful addition to their bookshelves.

Catherine Hebb

VITAMIN ASSAY METHODS

Vitamin Assay

Tested Methods. By Rolf Strohecker and Heinz M. Henning. Translated by D. D. Libman. Pp. 360. (Weinheim/Bergstr.: Verlag Chemie, G.m.b.H., 1965.) 48 D.M. ; 12 dollars.

THE subject of *Vitamin Assay : Tested Methods* must be of importance to many workers in laboratories, both industrial and academic, throughout the world. The number of methods and modifications of methods advocated in the literature has reached a forbidding total, and many workers have their own favoured procedures which are often not generally available. It is useful, therefore, that tested methods in collected form should be made available to English readers, the more so since books on this topic appear to be surprisingly few.

An accurate knowledge of the content of vitamins in drugs, foods and feeding-stuffs has assumed great importance at the present time. The assay of vitamins in drugs is relatively simple, since the free vitamin is normally present and the separation of concomitant and possibly interfering substances is not usually unduly complicated. In foods and feeding-stuffs the preparation of an extract containing essentially the free vitamin is often a much more protracted procedure than the actual estimation itself.

Biological methods—the direct appeal to the animal—are probably the best in theory; but they have serious disadvantages in respect of time, space and cost, without concomitant compensations of precision and accuracy. Nevertheless, in some cases, notably that of vitamin D, it is still necessary to resort to biological methods. Chemical and physico-chemical methods are more speedy and precise, and microbiological methods for the B group of vitamins are likely to be at least as precise and of higher specificity. Owing to the exacting requirements of microbiological assay, only a limited number of organisms is available, but it is desirable, if possible, to use those which have similar nutritional requirements to those of man and animals. The increasing use of protozoa (for example, *Ochromonas malhamensis* for B_{12}) is a step in the right direction.

The limits of error in any of these methods are such as to cause some surprise to the academic chemist as they are often between $\pm$ 5 per cent and $\pm$ 10 per cent, and may range as high as $\pm$ 20 per cent. Indeed, in a recent collaborative report the 'between laboratories' limits were no better than $\pm$ 35 per cent.

The authors describe in detail methods for the assay of all the vitamins likely to be encountered. Preliminary separations are given in full, and valuable recent techniques—spectrophotometric, polarographic, fluorimetric, chromatographic (column, paper and thin layer)—are called in to the aid of the analyst. An interesting variation —'streak' chromatography on paper—is described in connexion with nicotinic acid, vitamin C and vitamin D, whereby larger volumes can be applied than in the case of the more usual 'spots', with better separation and more rapid working.

The determination of the content of a given vitamin, adequate in the case of pharmaceutical preparations, can be subtly misleading where the real nutritional value of foods or feeding-stuffs in respect of vitamins is in question. For example, in the case of vitamin E, the assessment of α-tocopherol or of total tocopherols is scarcely adequate since to obtain a complete picture of vitamin E activity it is desirable to know the proportions of some seven or eight tocopherols, a sizeable analytical problem which has by no means reached a final solution.

Example calculations, often regrettably omitted in original papers, are a valuable feature throughout the volume, although, somewhat surprisingly, there appear to be no indications of the importance of design of microbiological assays, and subsequent statistical treatment of results. It is also surprising that there is no description of cup-plate techniques which have been widely used for routine determinations in Britain.

Principal references are given at the foot of the appropriate page and there is a short bibliography in the appendix. This also includes a comprehensive list of reagents and stock solutions required in microbiological assays and the composition of media in tabular form.

The usefulness of this volume of tested methods, under the sponsorship of Merck AG, is not in any doubt and it

should find a ready welcome wherever vitamin assay is practised. It is finely printed and produced and includes clear diagrams, together with photo-reproductions in black-and-white, and good colour, notably of column and thin-layer chromatography. F. W. NORRIS

DEVELOPMENT AT THE CELLULAR AND MOLECULAR LEVEL

Molecular and Cellular Aspects of Development

Edited by Dr. Eugene Bell. Pp. xi+525. (New York and London: Harper and Row, 1965.) n.p.

THERE must be many who have been disappointed at the past failure of embryologists and geneticists to establish a common front in the battle for an understanding of the role of the gene in ontology. It looks now as if neither, but rather the molecular biologists, are going to be in the front line when the day of the breakthrough dawns. We may have been foolishly impatient in our expectations, overlooking the methodological hurdles in the way of such a straightforward synthesis of genetics and embryology.

The minute analysis, both of the synthetic processes in the development and growth of a complex organism and of the chemical nature, spatial exposition and behaviour of the 'gene' had to await new and dramatic developments in biophysical and biochemical instrumentation, including the advent of the electron microscope. Only then could the stage be set for bridge-building. When this finally happened the molecular biologists naturally had to take the lead.

The collection of fourteen chapters on the theme of *Molecular and Cellular Aspects of Development*, collected and edited by Dr. Eugene Bell of the Massachusetts Institute of Technology, represents an attempt at a synopsis by no fewer than eighty contributors of progress in the following fields: the study of cell association, induction, cyto-differentiation and cell division, the role of the nucleus, chromosome differentiation, genetic control of differentiation, isozymes, enzyme regulation, RNA synthesis, the synthesis of cell-specific products, macromolecules as the basis of structure, hormones, growth substances and cell-virus interaction. The central topic of the book is cell differentiation, although a number of articles deal also with tissue and organ development. Each of the chapters is introduced by a résumé by the editor covering the problems which form its main theme. The papers themselves are reprints of published articles by outstanding representatives in the field in question.

It is obviously impossible in a short review of such a complex work to deal with any of the individual contributions. Nobody can deny the great difficulties in the dissemination of scientific information, and any attempt to ease access to it ought to be welcomed. The alternatives are a comprehensive review by a single author from a central vantage point, or a herding together of systematically related papers into a work like the present synopsis.

The editor states that he has used this collection of papers for a first course in developmental biology at the Massachusetts Institute of Technology. This is remarkable in view of the rather advanced nature of most of the articles. In browsing through the various chapters, one becomes deeply conscious of the ebullient sense of adventure in present-day biology and of how good it is to be able to witness all this. The challenge of attempting the near impossible that has to be accepted in experimental ventures into the realm of molecular happenings, the unfolding of undreamt-of complexities in the form of interlocking self-regulatory systems, the satisfaction to be gained in the design of ingenious experimental and intellectual stratagems, the continuous call for self-criticism in the evaluation of inherently ambiguous observational and statistical results against the backcloth of the great fundamental generalizations governing our theorizing about gene action in the synthesis of proteins and other 'bio' materials, the ordered chaos of tissue and organ determination in embryonic development—of all this the reader of this book is constantly and excitingly made aware.

Extensive lists of references and 234 well-produced illustrations add to the value of the book, but, apart from a list of contributors, there is no index. The editorial prefaces to the individual chapters read in sequence give coherence to what could otherwise appear to be a rather haphazard collection of reprints. O. LOWENSTEIN

COMPUTERS IN MEDICAL RESEARCH

Computers in Biomedical Research

Vol. 1. Edited by Ralph W. Stacy and Bruce D. Waxman. Pp. xxii+562. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 160*s*.

"IN the space of a few years biomedical computing and automatic data processing have become a significant part of the biomedical scientific effort . . ." This assertion, carried on the dust-cover, is just. An increasing number of important problems in molecular biology, physiology, cardiology and other branches of medicine and its allied sciences are soluble only by extensive mathematical or numerical analysis. Many questions in clinical, social and administrative medicine can be answered only by large-scale data processing. Within the past half-decade computers have become not only more powerful and more numerous, but, through the introduction of automatic programming languages, more easy to use. A few pioneers have shown the potentialities of computer science in a wide range of medical applications and have thereby kindled a flame which must grow rapidly in the years to come.

Much of this work has been difficult to follow for those not centrally engaged on it. The publications are scattered and often assume considerable prior knowledge of computer science or the particular field of application. A number of symposia have appeared recently, particularly from the United States, but the presentations have often been too sketchy. *Computers in Biomedical Research*, Volume 1, and its forthcoming companion, Volume 2, appear to provide the best set of expository papers so far. The range covered is wide and most of the topics are discussed in some depth. Even here, however, a reader like myself, on the fringe of the subject, is likely to have difficulty in entering fully into such diverse topics as enzyme systems, protein structure, statistical analysis, personality assessment and neural processes. The specialist in one of these topics, on the other hand, is likely to ask for more detail, particularly about computing procedures, than can be given in these chapters. Perhaps we now need a series of monographs, each treating one field of medical computation in full detail.

An excellent introduction by the editors is followed by 22 chapters arranged in sections devoted to the life sciences generally, the simulation of biological processes, the analysis of specific systems, and applications to neurophysiology, clinical medicine, psychology and psychiatry. It may be useful here to classify the chapters according to the use to which the computer is put. Three chapters describe extensive data-processing systems: in laboratory medicine (B. G. Lamson), in pre-symptomatic screening for many diseases simultaneously (M. F. Collen, L. Rubin and L. Davis) and for automatic personality assessment (H. P. Rome *et al.*). Several chapters are concerned with complex calculations for diverse purposes: genetics (K-I. Kojima), protein structure analysis (C. L. Coulter),

statistical analysis (W. J. Dixon—it should be mentioned that the BMD programmes described here are written for the IBM 7090), and calculation of radiation dosages (T. D. Sterling and H. Perry). A group of chapters is concerned with the investigation, by calculation and simulation, of models of biological systems; mathematical aspects are discussed by G. B. Dantzig, simulation by D. Garfinkel, analogue computers by J. E. Randall, hybrid computers by W. Siler, neurotic systems by K. M. Colby, neural networks by B. G. Farley, and perceptrons by J. A. Daly, R. D. Joseph and D. M. Ramsey, and L. Uhr has a general chapter on model building. Two papers are concerned with cybernetics (F. S. Grodins) and computer-controlled experiments in psychology (G. A. Miller, A. S. Bregman and D. A. Norman). A group of chapters describes the use of computers for the processing of bio-electric signals: in neurophysiology (W. R. Adey, M. A. B. Brazier), cardiology (H. V. Pipberger), and foetal cardiology (E. H. Hon). Finally, L. B. Lusted has an excellent chapter on medical diagnosis. Many contributions are, of course, broader in scope than is indicated by the above summary.

This book is a stimulating guide to present-day American work on medical computing and the second volume will be awaited with interest. P. Armitage

CHROMOSOMES OLD AND NEW

Cytology

By Prof. C. D. Darlington. Part 1: A Reprint of *Recent Advances in Cytology*, Second Edition, 1937. Part 2: Recent Advances in Cytology, 1937–1964. Pp. xvi+768+16 plates. (London: J. and A. Churchill, Ltd., 1965.) 60*s*.

THE two pre-war editions of Darlington's *Recent Advances in Cytology* are undoubtedly classic texts, which were outstandingly successful in achieving what they were intended to do. This was the provision of a detailed account of chromosome form and behaviour, both normal and abnormal, in plants and in animals. They incorporated major syntheses of the literature of the field, built into a framework of interpretations and ideas developed by the author. They combined, in Prof. Darlington's unique style of writing, information with interest, wit with wisdom. The essential uniformity of the chromosome mechanism in both plant and animal kingdoms was emphasized throughout and an eye was always kept on the genetic roles of the chromosomes. The chromosomes contained the genes, indeed they were the genes, and were in turn subject "*as individuals to the laws they enact as a body*".

It is a measure of the stature of *Recent Advances*, and the value of the author's approach, that, despite its second edition being almost thirty years old—a great age for a book in a subject advancing as rapidly as cytology—it is a volume which must still be consulted by a practising cytologist as an authoritative basis for chromosome study. This continued demand has now been met, under the abridged title of *Cytology*, with a reprint of the second edition, originally published in 1937, supplemented by an appendix in which Prof. Darlington provides an assessment of the advances which have taken place in the field between 1937 and 1964. This very much shorter second section is not meant to have the detail and approach of the first section. Rather, it is a guide to those developments during the past twenty-seven years which have been of particular interest to the author himself and which support, qualify or, occasionally, disprove information or predictions outlined in the first section of the book. This personal approach lends coherence to the two sections and yields a result which is in many ways preferable to an attempt to combine the two in an entirely re-written edition. For this is not really a book for the beginner in cytology: the treatment of some topics is too detailed for this purpose and a number of introductory books, including some by the author himself, are more appropriate for this purpose. No, this is a book for the more serious student of chromosome cytology, who will be able to trace within its pages the development both of this important branch of biology and of this important biologist. I feel sure that all who fall within this category, including many who already possess one of the earlier editions, will wish to avail themselves of this new opportunity to survey the field from so respected a point of view.

S. A. Henderson

METEOROLOGY IN THE TROPICAL PACIFIC

Cloud Structure and Distributions over the Tropical Pacific Ocean

By J. S. Malkus and H. Riehl. Pp. ix+229. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 60*s*.

THE wide expanse of the central Pacific is a fruitful region for research in tropical meteorology, unhindered by the often overpowering effect of local orographic features. The two authors are well known for their published papers on the trade-wind regions. Whether they were wise to attempt a more detailed presentation in book form is questionable. For *Cloud Structure and Distributions over the Tropical Pacific Ocean* is simply a detailed account of observations made on three flights in 1957; it has taken much longer to appear, and will, one suspects, attract fewer readers than would a briefer account in a scientific journal. The flights were made at low level in a single aircraft, and observations were accordingly limited. Synoptic scale analyses must be considered subjective, and it is the meso-scale features that carry most interest.

The three tracks chosen were in a generally east–west direction just inside the Tropics; a north–south direction across the equator would have yielded results even more intriguing and difficult to interpret. However, the situations described are full of interesting detail. For example, the familiar 'lean' of trade-wind cumuli was found to be indicative of weak convection at the shear level; a change to more intense convection occurred due to the building of 1 in 4 of the cumulus lines at the expense of others; the cloud streets were at times related to the upper shear rather than the low-level wind, indicating that cloud observations from satellites should not be used in wind analyses.

On the larger scale, a remarkable feature of tropical Pacific meteorology is the high variability of flow pattern above the settled trade-wind flow. Data presented here seem inadequate for the theories advanced—Doppler winds from high-level aircraft were needed to supplement the scattered radar-wind reports. Nevertheless, the authors present a good case for relating the 250–150 mbar thickness tendency to large-scale development. I have noted that, on occasion, disturbances at 200 mbar are accompanied by unsettled weather, but more frequently there appears to be little association. More investigations of wind in the upper troposphere are needed in these regions.

All the data are well presented with full documentation of observations, including cloud photographs, together with appropriate synoptic charts and radio-sonde reports. It is a relief to find that the stream-lines illustrated do not reproduce the rather fanciful patterns to be found in other studies of tropical meteorology. After reading of these three flights one realizes the wealth of meteorological knowledge which must be acquired by airline pilots and which so rarely seems to be channelled through to the meteorologist. P. Graystone

Science in Nineteenth-Century America

A Documentary History. Edited and with an Introduction by Nathan Reingold. (American Century Series.) Pp. xii+339. (New York: Hill and Wang, 1964.) Cloth 5.95 dollars; Paper 2.45 dollars.

THIS collection of documents from nineteenth-century America is designed to help us understand the "present American eminence in the sciences".

The documents are, for the most part, private letters and personal records not intended for public consumption. They begin with the writings of Benjamin Silliman, sen., and Nathaniel Bowditch—both essentially popularizers with an eye to the practical application of science.

A majority of the American scientists during the nineteenth century were natural historians and explorers. Their work was inspired by the needs of a new continent and of a young country. Geophysics and meteorology thus became established as separate disciplines. With the advent of Darwinian evolution, however, the natural historians turned into biologists. With the rise of modern physics towards the end of the nineteenth century in Europe, the American explorers changed their interest from the physical world to the world of physics.

The founding of the National Academy of Sciences in the middle of the Civil War was undoubtedly a main event in the scientific life of the country. The Academy never played as important a part as the Royal Society did in Britain; its difficult origins may have held back the growth of the Academy.

Apart from Agassiz and Asa Gray, few of the American scientists were of international stature. It is only at the end of the century that native genius made itself felt. The physicists Newcomb, Rowland and Michelson were undoubted leaders in their field. Charles Sanders Peirce and J. Willard Gibbs are the two scientists who are outstanding in the originality and universality of their minds. Their genius has become recognized only in recent years. Peirce's contribution to a wide range of knowledge is not yet sufficiently appreciated, at least in Europe.

There was a prevalence of practical and a dearth of theoretical scientists during the earlier period. The flowering of American science at the turn of this century—and ever since then—was caused by the fertilizing contact with European science. It would be wrong to be blind to the fact that science is, and always has been, international. The present success of American scientists is built on, and impossible to divorce from, the achievements of the scientists of Western Europe and, indeed, of every part of the globe.

E. H. HUTTEN

Recent Advances in Selenium Physics

Edited by European Selenium–Tellurium Committee. (A Symposium on Solid and Liquid State Selenium Physics arranged by the European Selenium–Tellurium Committee and held at the Chemical Society, London, June, 1964.) Pp. vii+160. (London and New York: Pergamon Press, 1965.) 80*s*. net.

SELENIUM is the oldest of semiconductors and is still widely used as a rectifier material, in spite of the success of silicon and germanium diodes. One would imagine that the physical properties of selenium would have, by now, been fully understood but, in fact, they are still being actively investigated and it appears that much work remains to be done. The symposium which is reported in this book allowed a large proportion of those who are engaged in selenium research to meet one another, relatively few of the participants having less than a direct interest in the material. Not unexpectedly, the discussion was particularly lively, and forms a most valuable part of the report.

Most solid-state physicists nowadays are unhappy if they cannot work with single crystals, and four of the papers are devoted to the growth of crystals of hexagonal selenium; the work of D. E. Harrison in this field has been exceptionally successful. Most of the remaining papers discuss transport and optical measurements and their interpretation. It is quite clear that many of the established semiconductor concepts must be discarded for selenium, because of its very low carrier mobility. However, it is probably an example of a wide class of materials, and, being an element, may make an excellent prototype for testing theoretical ideas. In summarizing the symposium, H. Gobrecht points out some notable deficiencies in the work to date. Most important, the selenium used in the experiments has often been of doubtful purity. Surprisingly also, the atomic weight of selenium is known only to two decimal places.

Although the sub-title of the book mentions liquid selenium, there is only one paper devoted specifically to it (other than the consideration of its properties as relating to the growth of crystals). Only two of the papers discuss applications of selenium (in xerography and thermoelectricity, and in the latter field selenium is important only in compound form). It would not be unexpected, however, if the commendable encouragement of selenium research by the European Selenium–Tellurium Committee were to lead to new applications of a fascinating substance.

H. J. GOLDSMID

Evolution of Mathematical Thought

By Prof. Herbert Meschkowski. (The Mathesis Series.) Pp. 157. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1965.) 6.55 dollars.

THIS translation of *Evolution of Mathematical Thought* adds to the number of books which attempt to explain the spirit and aims of modern mathematics to the intelligent layman.

The Greeks began the search for a discipline which should be abstract and general, but Prof. Meschkowski takes a long stride from Greek mathematics to the next significant stage, the discovery, early in the nineteenth century, of the plurality of geometries by Gauss, Lobachevski and the Bolyais. Curiously, he scarcely notices the sequential but equally important work of Grassmann, Hamilton and Boole, on the plurality of algebras—so seminal for modern formalist developments. The antinomies and paradoxes of Cantor's theory of sets, and Brouwer's dismissal of the law of the excluded middle, lead to problems of mathematical logic, which Prof. Meschkowski approaches from the geometrical side by a study of Hilbert's axiomatic construction. The formalism which dominates so much of present-day thinking about mathematics demands discussion of the consistency and completeness of an axiomatic system, and presents the 'undecidable' problems of mathematical logic, with Gödel's proof that the consistency of the formal number system cannot be established by means of the system alone, and Skolem's theorem that a finite system of axioms cannot uniquely characterize the set of natural numbers. The account of this rugged domain is as clear as we could hope for; many readers will appreciate the quotation from André Weil: "God exists since mathematics is consistent, and the Devil exists since we cannot prove it".

A further chapter on operative mathematics, based on papers by P. Lorenzen, will prove a severe test of the sophistication of even the most intelligent layman. In an appendix, the author suggests that there are educational possibilities in modern mathematics which were only latent in the classical nineteenth century developments, and that these could be made to lead to a new unity in our educational system. He remarks that the liberal arts might be better served if students in technical institutes, instead of being compelled to attend lectures on literature, were encouraged to reflect seriously on the fundamentals of their own subjects. Thus they would be led to see that poets, philosophers and scientists may indeed all have the same goal.

T. A. A. BROADBENT

RADIONUCLIDE FRACTIONATION IN AIR-BURST DEBRIS

By Dr. E. C. FREILING and M. A. KAY

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WHEN one considers world-wide fall-out, air bursts are of much greater significance than surface bursts for two reasons. First, because a much greater quantity of fission products has been produced by air bursts than by surface detonations and, secondly, because the entire amount of debris from an air burst goes into world-wide fall-out, as compared with some small but unknown fraction for a surface burst.

Although a large amount of information has been collected on air-burst debris, relatively little has appeared in the unclassified literature. A few remarks on the nature of air-burst debris are therefore in order by way of background. The particles from air bursts are almost exclusively spherical, although occasionally particles are found which consist of two spheres stuck together. The particles are small, rarely occurring larger than 20μ in diameter. Lapple has estimated that these particles can only grow to 0·3μ by condensation and are probably no smaller than 0·03μ (ref. 1). Therefore either the larger particles must have been formed by the coalescence of small particles when the debris was still in the molten state, or they were formed from debris which was never completely vaporized. In either case they probably consist predominantly of particles formed at the earlier times. The size-frequency distribution has been measured down to 0·05μ diameter and found to be approximately lognormal with a modal diameter less than 1μ (ref. 2). The densities of the particles vary from 3 to 4·3 g/c.c. (ref. 3). They may be colourless, gold, yellow, orange, red, brown, green or black. Their chemical composition is usually a mixture of the oxides of iron, aluminium, uranium and plutonium. Radiochemically, their specific activity is much greater than that found in surface burst debris, as would be expected. Thus, while the more active particles from surface bursts contain of the order of 10^{14} ^{95}Zr-equivalent fissions per gram*, air burst particles may have specific activities from 10^4 to 10^7 times higher. Benson *et al.* have recently determined that in larger particles from air burst debris ^{95}Zr content varies as the 3·1–3·4 power of the diameter, while ^{140}Ba–^{140}La content varies as the 2·3–2·6 power (ref. 4).

Most of the samples investigated have shown a relatively small degree of fractionation. However, since most samples were collected with precautions to obtain as representative a collection as possible, one should not leap to the conclusion that air-burst debris is fractionated to only a small extent. A large number of samples have also been obtained which showed large departures from the representative composition. The analytical data from these samples provide a wealth of material for analysing fractionation in air-burst debris.

It is helpful to draw a distinction between potential and actual fractionation when considering the extent to which air-burst debris is fractionated. By 'potentially fractionated debris' we mean debris for which the radiochemical composition varies among particles, whether because of their size, density, macroscopic composition or history in the fireball, even though these particles are intimately mixed so as to provide a representative composition for any given cubic foot of cloud. For example, the work of Benson *et al.* already cited (ref. 4) clearly shows that the debris from an air burst is potentially fractionated, even though the debris taken as a whole is obviously representative. In order to actualize this potentiality, a particle separation process must occur. If the separation is the result of unequal rates of sedimentation through the atmosphere, the process could extend over long periods of time, and the degree of actualization would increase as time went on.

The correlation of radiochemical data from fractionated samples of air-burst debris throws light on the fundamental nature of the processes involved in debris formation: the nuclear processes of the primary fission products; the dispersion and subsequent nucleation, condensation and agglomeration of the carrier material; the incorporation of the primary fission products in the particle or their deposition on the particle surface. The correlative parameters show the influence of matrix material on the character of the fractionation and provide input data for prediction schemes, such as the radial-distribution model[6]. A search of available radiochemical data on air-burst debris revealed thirteen detonations in which factors of ten or more were observed between the same radionuclide ratios in different samples from the same detonation. Two additional shots were included to extend the range of weapon yields. These detonations ranged in total yield over a factor of about 300. All the radiochemical data available from these fifteen detonations were used in this work. For each of these detonations, the investigators in charge of the analyses had estimated representative (that is, unfractionated) values of the radionuclide ratios obtained. Therefore, the ratios of fractionated radionuclides were converted to fractionation ratios ($r_{i,j}$) by simply dividing them by the representative values, as described in ref. 6.

Standard statistical methods were used to analyse the correlations between ratios, to test the variation of the correlation parameters with total weapon yield, and to test the hypotheses that nuclides of certain groups do not fractionate from one another.

To illustrate the range of fractionation observed in air-burst debris, Table 1 lists for each shot the ratio of the largest $r_{89,95}$ value observed to the smallest $r_{89,95}$ value observed. The shot numbers are assigned in order of increasing yield. The data show that air-burst debris can be extremely fractionated. There is no evident trend of the ratio with yield. The wide variation in the ratio probably manifests nothing more than its dependence on the sampling conditions. The observed $r_{89,95}$ values ranged from 0·002 to 5·3, although few fell outside the range of 0·1–3·0.

The next property considered was the relative ability of logarithmic and linear correlations to fit the data. The measure used for goodness-of-fit was the coefficient of determination (that is, the square of the coefficient of

* In any given event, an equivalent fission of a fission product or induced activity is the ratio of the total quantity of product to the total number of fissions and thus represents the average quantity of product formed by one fission.

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Table 1. RANGE OF OBSERVED $r_{89,95}$ VALUES FOR AIR BURSTS

Shot No.	$(r_{89,95})$ max/$(r_{89,95})$ min
1	7
2	22
3	19
4	28
5	26
6	919
7	43
8	64
9	70
10	449
11	10
12	90
13	19
14	503
15	5

correlation)[7]. These coefficients indicate the fraction of variance in the dependent variable accounted for by the best fit of the type indicated. Individual shots varied considerably and were about equally divided in their preference. Table 2 shows the cumulative coefficients (that is, coefficients obtained from treating all the data from all shots as belonging to a single population) for several important radionuclides which fractionate from ^{95}Zr to varying degrees. With the single exception of ^{141}Ce, which shows no significant difference, the cumulative coefficients indicate a preference for the logarithmic correlations. Unfortunately, this preference is influenced to some unknown extent by the type of scatter in the data. Data which have about the same 'relative' uncertainty (as these do) will tend to follow a logarithmic correlation, while data which have about the same 'absolute' uncertainty will tend to follow a linear correlation[8].

Table 2. CUMULATIVE COEFFICIENTS OF DETERMINATION FOR IMPORTANT FRACTIONATION-SENSITIVE NUCLIDES

Radionuclide	Coefficient of determination: Logarithmic fit	Linear fit
^{90}Sr	0 71	0·51
^{91}Y	0·85	0·60
^{137}Cs	0 69	0·60
^{140}Ba	(0·76)*	(0 65)*
^{141}Ce	0·71	0·72
^{147}Nd	0·37	0·05
^{239}Np	0·78	0·64

* Slopes are yield dependent.

The yield dependence of the slopes of the logarithmic correlation lines were calculated for the nuclides ^{90}Sr, ^{91}Y, ^{97}Zr, ^{99}Mo, ^{137}Cs, ^{140}Ba, ^{141}Ce, ^{147}Nd and ^{239}Np. This was done for both total device yield W and for log W. In most cases the regression slope for this dependence was equal to or less than the 95 per cent confidence limit. The results for those cases where the regression slopes exceeded the confidence limits are shown in Table 3. The ^{140}Ba slopes definitely appear to be dependent on yield, the dependence is in the expected direction, and the slopes themselves varied from 0·41 to 0·92. The ^{99}Mo slopes also appear to be dependent on yield. These ranged from 0·10 to 0·59. The cases for the other nuclides appear much less definite.

Table 3. REGRESSION SLOPES FOR STATISTICALLY SIGNIFICANT CASES OF YIELD DEPENDENCE

Radionuclide	Correlation with W (kt^{-1})	Correlation with Log w
^{91}Y	0·00016 ± 0·00015	———*
^{99}Mo	0 00019 ± 0 00011	0 066 ± 0 039
^{140}Ba	0·00016 ± 0 00009	0·053 ± 0 035
^{141}Ce	0 00011 ± 0 00007	———*
^{147}Nd	−0 00013 ± 0 00010	———*

* Slopes did not exceed confidence limits.

We come now to the values of the regression slopes for logarithmic correlations of the data from all shots treated as a single population. Because of our basis of presentation, these slopes are measures of the volatility of behaviour of the various mass chains. The cumulative slopes for the logarithmic correlations, their 95 per cent confidence limits, and the number of data points involved, are shown for various important radionuclides in Table 4. Nuclides are listed in order of decreasing volatility (decreasing slope) for air bursts. Although not strictly admissible, because of yield dependence, ^{99}Mo and ^{140}Ba have been included to indicate typical values for these cases. Listed for comparison are the cumulative results from high-yield surface bursts which have been taken from ref. 6 and converted to the present basis (that is, ratios are here based on ^{95}Zr instead of ^{89}Sr).

Table 4 CUMULATIVE SLOPES FOR VARIOUS IMPORTANT RADIONUCLIDES

Radionuclide	No. of data points	Cumulative slope	High-yield surface bursts
^{89}Sr	by definition	1·000	1·000
^{90}Sr	209	0·92 ± 0 08	0 69 ∓ 0 08
^{137}Cs	190	0·90 ± 0·09	1·07 ± 0 17
^{239}Np	280	0·80 ± 0 05	−0 05 ± 0·09
^{136}Cs	194	0·67 ± 0 08	—
^{140}Ba	201	(0·62 ± 0·04)*	0·57 ± 0 09
^{91}Y	192	0 56 ± 0 03	—
^{141}Ce	232	0·40 ± 0 03	—
^{99}Mo	200	(0·30 ± 0·05)*	−0·10 ± 00 9
^{147}Nd	193	0·22 ± 0·04	—
^{95}Zr	By definition	0·000	0 000

* Yield dependent.

Table 5 shows the slopes of log $r_{i,j}$ versus log $r_{89,95}$ for various nuclide pairs of similar behaviour.

Table 5. SLOPES OF LOG $r_{i,j}$ VERSUS LOG $r_{89,95}$ FOR CASES OF SIMILAR BEHAVIOUR

Nuclide i	Nuclide j	No. of points	Slope
^{90}Sr	^{89}Sr	209	−0 08 ± 0 08
^{137}Cs		190	−0·10 ± 0·10
^{137}Cs	^{90}Sr	150	0 00 ± 0·11
^{112}Ag		132	−0 05 ± 0·16
^{240}U	^{239}Np	185	0·00 ± 0·02
^{237}U		279	0·00 ± 0·17
^{111}Ag		250	0 00 ± 0·02
^{115}Cd		229	0 00 ± 0·04
^{115m}Cd		214	−0 09 ± 0·08
^{136}Cs	^{140}Ba	190	0 04 ± 0·08
^{238}Pu	^{99}Mo	85	−0·01 ± 0·12
^{238}Pu	^{147}Nd	70	−0·09 ± 0·09
^{144}Ce		164	−0 05 ± 0·20
^{143}Pr		188	−0·10 ± 0·03
^{161}Tb	^{95}Zr	156	0 04 ± 0·05
^{153}Sm		159	0·03 ± 0 05
^{156}Eu		175	0 00 ± 0 05
^{97}Zr		239	−0 03 ± 0 03

The simplified picture of fall-out formation has visualized a yield-dependent time of condensation or solidification of debris, at which refractory elements were largely incorporated in the particle matrix while volatile elements had either to cool further or to decay to refractory elements before they could condense on the available surfaces. Some of the results in the preceding section are unexpected from such a model, but it cannot be universally stated whether this disagreement lies with the fault of the model, of the basic nuclear data and its application, or in the accuracy and treatment of the radiochemical data. Each anomaly must be treated individually, compared with other correlations, and the relative contribution of all four possible causes evaluated.

The insensitivity of correlation parameters to yield, in all cases except ^{99}Mo and ^{140}Ba, simplifies the prediction of debris properties but is difficult to reconcile with the simplified model described here. Equally difficult to explain on a physical basis is why these particular nuclides should show the greatest yield dependence. Plots of correlation parameter versus yield show a great deal of scatter and the results here may simply reflect a larger number of data points (compare Table 4) and more

reliable results for these nuclides, rather than signifying a real insensitivity for the others.

The observed volatility of ^{239}Np, on the other hand, appears more real. Besides the magnitude of the volatility, the results are well supported by independent data. Thus, ^{239}Np, ^{237}U and ^{240}U are all present as uranium at condensation time and, as Table 5 shows, the data from these three nuclides are all in excellent agreement. Moreover, a similar, though less marked, behaviour has been reported for the silicate surface bursts Small Boy and Johnie Boy in ref. 9.

In using Table 5 to determine whether two nuclides do not fractionate from each other, the slope, confidence limits, and the chemical and nuclear properties of the precursors must all be considered. The following groups of nuclides fulfil well all these qualifications: ^{237}U, ^{239}Np and ^{240}U; ^{111}Ag and ^{115}Cd; ^{95}Zr and ^{97}Zr; and ^{153}Sm, ^{156}Eu and ^{161}Tb. The data also indicate that there is no reason for excluding ^{112}Ag from the silver group, that the silver group does not fractionate sensibly from the uranium group and that the zirconium group does not fractionate from the heavy rare earth group. As would be expected, ^{136}Cs and ^{140}Ba behave very similarly. The volatile nuclides ^{89}Sr, ^{90}Sr and ^{137}Cs may also follow one another in air-burst debris and ^{115m}Cd may follow ^{115}Cd, but the indication that they do is marginal, to say the least. The data of Tables 4 and 5 indicate that ^{143}Pr is more volatilely behaving than the heavy rare earths and that ^{147}Nd behaves more volatilely than ^{143}Pr. The confidence limits for ^{144}Ce are too wide to establish its position among the other rare earths.

Fig. 1 presents a visual comparison of the chain volatility inferred from fractionation behaviour with the parent thermodynamic volatility shown by Fig. 2 of ref. 10. In the latter, Bedford and Jackson show the predominant form of the condensed phase and its equivalent pressure at 2,500° K and 1 atm. O_2 for the fission-product elements and uranium. The correspondence between the two sets of data indicates that the thermodynamic equilibrium model for nuclear debris formation proposed by Miller[11] may be applicable to air bursts and this possibility should be investigated further.

Some of the results here were compared with those for silicate surface bursts in ref. 9. The comparison with results from high-yield surface bursts in Table 4 shows ^{239}Np to be the nuclide the behaviour of which differs most in these environments. The difference between the values for the 140 chain in the two cases is well within the 95 per cent confidence limits as is the difference between values for the 137 chain. Discussion of the differences for ^{90}Sr and ^{99}Mo are best postponed until work in progress on additional correlations from high-yield surface bursts in the Pacific area is complete.

In conclusion, it is evident that our understanding of the situations prevailing in the formation of air-burst debris is rudimentary in all phases: the basic theory is lacking; the models are crude and it is somewhat surprising that they work as well as they do; fundamental thermodynamic and nuclear data are unavailable; the environmental conditions during debris formation are inadequately defined; the particles are formed and grow and coagulate at different times, places and temperatures, the larger ones being probably the first formed; and the subtlety of some of the effects sought appears to be beyond the accuracy of the data and the methods of correlation. For these reasons, no more detailed treatment has appeared to be warranted at this time. As correlation data accumulate from other burst types, as fundamental data become available, and as basic processes are better understood, more sophisticated statistical treatment and interpretation may prove fruitful.

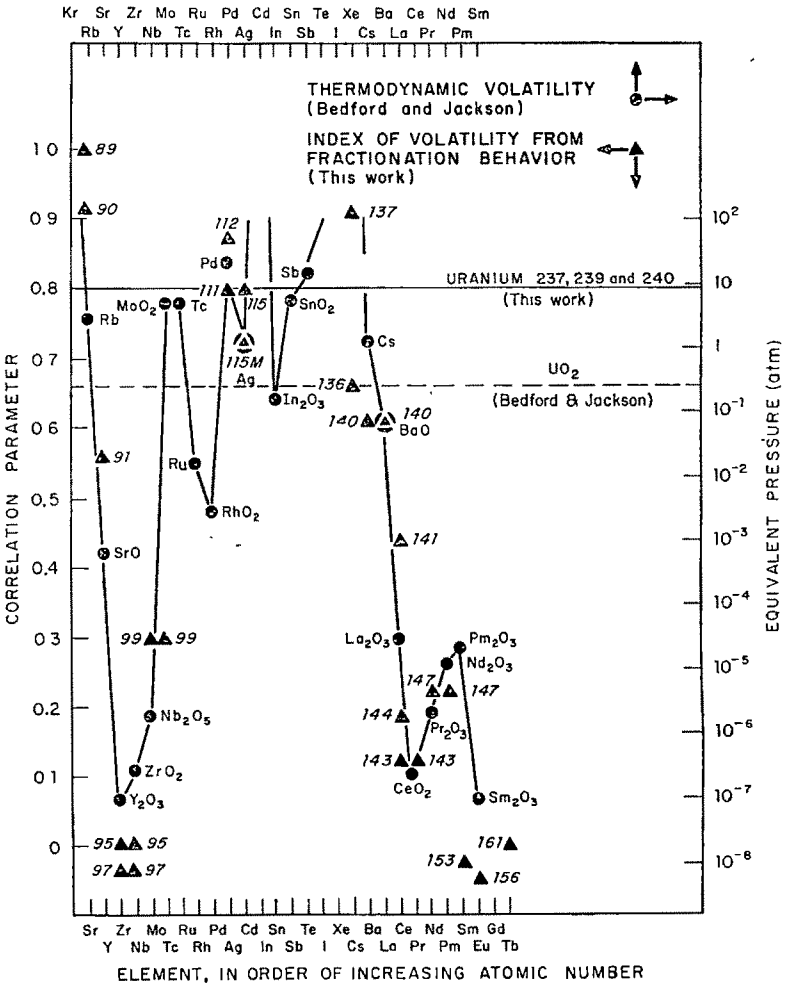


Fig. 1. A comparison of chain volatility inferred from fractionation behaviour with thermodynamic volatility of the parent

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HIGHLY FRACTIONATED NUCLEAR DEBRIS RESULTING FROM THE VENTING OF A SOVIET UNDERGROUND NUCLEAR TEST

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THREE months after the first Chinese atomic bomb test the U.S.S.R. conducted a large-scale underground nuclear test on January 15, 1965, at Semipalatinsk in central Asia. The total yield of this explosion was roughly estimated to be 0·1–1·0 megaton of TNT. The high radioactivity was detected in rain-water collected at Niigata on January 21. The total β-activity of the rain sample decreased with an apparent half-life of 23 days, whereas the total γ-activity of the rain sample indicated an apparent half-life of 13 days, as shown in Fig. 2. It was reported by Murayama *et al.*[1] that Wakkanai (Fig. 1) was also subject to radioactive rainfall on January 21 and 22, and that the apparent half-lives of total β-activity of the rain samples were about 27 days, similar to the Niigata rain sample. On the other hand, highly radioactive dry fall-outs were observed at Fukuoka, Osaka and Sapporo meteorological observatories (Fig. 1) during the period of January 21–23, where no rainfall occurred[1]. Their apparent half-lives of total β-activity were all 15 days, shorter than that of the Niigata rain sample. Such a long apparent half-life of total fission-product β-activity has not previously been observed within a week of explosion, from the point of view of fresh nuclear debris from an atmospheric or ground-level nuclear weapon testing.

From the γ-ray scintillation spectra for the Niigata rain sample, it will be seen that the principal γ-emitting nuclide was barium-140 (–lanthanum-140) and that the peak at 0·23 MeV showed the presence of a small fraction of tellurium-132 (–iodine-132), relevant to an underground nuclear test. The γ-ray spectra, however, indicated no separate peak at 0·36 MeV of iodine-131—perhaps one of the most interesting fission products resulting from an underground test[2]. This lack of iodine-131 in the γ-ray spectra may be explained by the differences in the following mass-131 and mass-132 fission-product decay chains:

$$^{131}\mathrm{Sn}(3{\cdot}4\ \mathrm{min}) \rightarrow {}^{131}\mathrm{Sb}(23\ \mathrm{min}) \begin{cases} \xrightarrow{5\%} {}^{131m}\mathrm{Te}(30\ \mathrm{h}) \\ \xrightarrow{95\%} {}^{131}\mathrm{Te}(25\ \mathrm{min}) \end{cases} \rightarrow {}^{131}\mathrm{I}(8\ \mathrm{day})$$

$$^{132}\mathrm{Sn}(2{\cdot}2\ \mathrm{min}) \rightarrow {}^{132}\mathrm{Sb}(2{\cdot}1\ \mathrm{min}) \rightarrow {}^{132}\mathrm{Te}(78\ \mathrm{h}) \rightarrow {}^{132}\mathrm{I}(2{\cdot}3\ \mathrm{h})$$

That is, the selective deposition of fission products from an underground nuclear test is greatly affected by their physical and radiological properties, including those of their precursors. The half-lives of tin-131 and tin-132 are comparable with each other and hence the activities of both nuclides decrease with a similar rate. The half-life of antimony-131 is more than ten times longer than that of antimony-132. Consequently, when only a half of the antimony-131 is converted into tellurium-131, the conversion of antimony-132 into tellurium-132 is almost entirely completed. The boiling-point of elemental antimony (1,380°) is higher than that of elemental tellurium (990°), and, in addition, the specific activity of tellurium-132 is a few times larger than that of iodine-131. From such radiological properties of these nuclides and, further, the distance from Semipalatinsk to Niigata (5,000 km), it can be plausibly explained that the peak of iodine-131 was not identified in the γ-ray spectra of the rain sample. However, it seems reasonable to associate

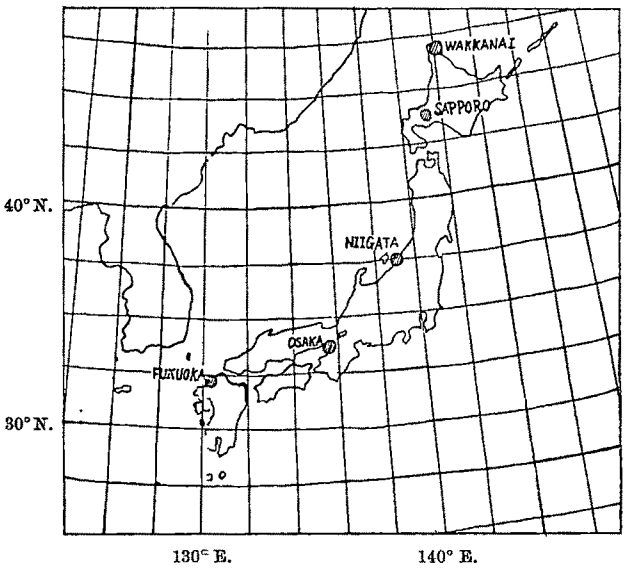


Fig. 1. Locations where wet or dry fall-out from the Soviet underground test was observed during January 21–23, 1965

the source of such a highly radioactive rain or dry fall-out with an accidental venting of the large Soviet underground test.

As the testing date and site of this test explosion are clearly known, the debris is of interest as it demonstrates the radiochemical composition of nuclear debris from a distant underground test and the duration of atmospheric contamination caused by such a test.

A rain-water sample collected on January 21 (sample volume, 6·0 l.) was submitted to radiochemical analysis, and the results are summarized in Table 1. The 'measured' values are shown by the activity contribution from each nuclide on January 30, 1965. The 'expected' values are also given by the contribution to be expected 15 days after the fission of uranium-235 by means of slow neutrons. The first striking feature of the results shown in Table 1 is that nuclear debris from the underground test indicated the occurrence of high fractionation during escape, transport or deposition, as demonstrated above by γ-ray spectrometry. The main components of the rain-water sample were strontium-89 and barium-140 (–lanthanum-140) and they represented 95 per cent of the total activity on January 30, 15 days after the explosion. The contribution from the remaining components, such as tellurium-132 (–iodine-132), ruthenium-103 (–rhodium-103) and rare earths, made up only 5 per cent of the total

Table 1. Radiochemical Composition of Rain-water Sample collected on January 21, 1965

Nuclides	Contribution to total activity (per cent) 'Measured' on January 30, 1965	'Expected' 15 days after fission
Strontium-89	60	4
Strontium-90	0·6	0·03
Barium-140	16	12
Lanthanum-140	18	14
Caesium-137	0·2	0·03
Remainder	5	70

activity. It is considered that the selective release of strontium-89 and barium-140 into the atmosphere results from the fact that their precursors in the fission-product decay chains include inert gas elements, such as krypton and xenon, which have a half-life of more or less than 1 min. Nuclides with very short-lived precursors were not enriched in fall-out samples, although their precursors were inert gas elements. From this selective enrichment of some nuclides it is possibly to be expected that the contribution from strontium-90 and caesium-137 exceeded, by a factor of 10, that calculated in the case of thermal neutron fission of uranium-235. The ratio of caesium-137 to strontium-90 was 0·5 and less than one-third of an averaged value of the ratios which have been reported by other investigators in Japan[3,4]. It should be noted that the debris from the underground test was enriched with strontium-90 in great excess as compared with caesium-137.

A decay curve of the mixture sample plotted on semi-logarithmic paper is shown in Fig. 2. This assumes an artificial mixture sample containing strontium-89 and barium-140 (–lanthanum-140) with an activity ratio, strontium-89/(barium-140+lanthanum-140), of 60/34 on January 30. From this graph it was assessed that the total β-activity of the mixture sample had an apparent half-life of 24 days during the period January 21–February 10: its half-life was in good agreement with that of our rain-water sample of January 21. This agreement suggests that the rain samples collected at Wakkanai would also consist mainly of strontium-89 and barium-140 (–lanthanum-140).

The activity concentration of strontium-89 and the ratio of strontium-89 to strontium-90 during January 21–24 are presented in Table 2, together with their average values for the period January 21–31. All activity values in Table 2 have been corrected for radioactive decay to mid-period of the collection. For the first half of January the average activity concentration of strontium-89 due to the first Chinese test had been reduced, on the average, to the very low level of about 1 pc./l. The rain on January 21, however, showed the surprisingly high activity concentration of strontium-89 of 9,970 pc./l. Afterwards the activity concentration decreased rapidly, and on January 24 it was but a fiftieth of its value of January 21. The atomic and activity ratios of barium-140 to strontium-89 are also listed in Table 2, together with the fractionation coefficient. The fractionation coefficient is equal to the measured barium-140/strontium-89 ratio divided by the calculated barium-140/strontium-89 ratio for uranium-235 thermal neutron fission. The activity ratio of barium-140/strontium-89 was only 0·46 on January 21, and the ratio rose abruptly to 4·66 in the afternoon of January 22; however, its mean value over the period January 21–31 was 2·12 because the individual values were averaged by pooling individual samples for 10-day periods. The strontium-89 activity was substantially enriched in the first sample collected. The barium-140 activity clearly indicated a reverse trend of the enrichment. However, the average value of the fractionation coefficient was about 1 for the period January 21–31. From the results described here, it seems probable that the radioactive cloud of strontium-89-rich nuclear debris, which escaped from the underground test, was carried on an easterly course,

Table 2. Concentration of Strontium-89, Strontium-89/-90, and Barium-140/Strontium-89

Period of collection January, 1965	Sample volume (l.)	^{89}Sr concentration (pc./l.)	Ratio $^{89}Sr/^{90}Sr$	$^{140}Ba/^{89}Sr$ Atomic	$^{140}Ba/^{89}Sr$ Activity	Fractionation coeff.
1400, 21–1800, 21	5 8	9,970	100	0·13	0·46	0·2
1800, 21–1200, 22	21·0	2,480	98	0·70	2·72	1·0
1200, 22–1800, 22	19·0	430	71	1·20	4·66	1·7
1800, 22–900, 23	20 5	372	48			
1000, 24–1400, 24	19 5	195	20			
21–31	46·0	378	34	0·55	2·12	0·9

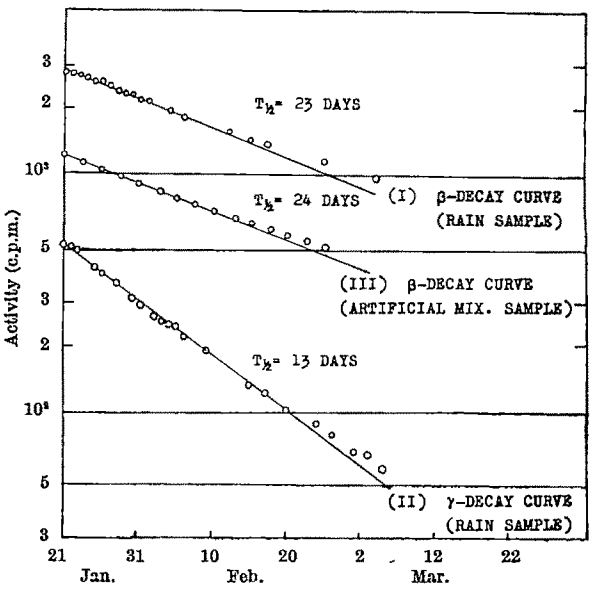


Fig. 2. β- and γ- decay curves of rain samples (I and II) collected on January 21, 1965, and the β-decay curve of an artificial mixture sample (III)

at the level of the rain-forming layers, and reached Japan within 5 days; moreover, in transit the strontium-89 was scavenged by the rain clouds. On the other hand, it is reasonable to assume that the cloud of barium-140-rich nuclear debris travelled towards Japan along a low-level course in the troposphere and that the cloud was brought down near the Earth surface by descending air flows associated with surface anticyclone.

Table 3. Atmospheric Mean Time of Residence of Strontium-89 produced by the Underground Test

Period of collection January, 1965	Composite removal constant ($\lambda r + \lambda_{Sr\text{-}89}$) By ^{89}Sr concentration	By ratio $^{89}Sr/^{90}Sr$	Mean time of residence From concentration	From ratio
1400, 21–1200, 22	2·48		(0·4)	
1800, 21–1800, 22	2·48	0·67	(0·4)	1·5
1200, 22–0900, 23	0·79	0·41	1·3	2·5
1800, 23–1400, 24	0·65	0·81	1·5	1·2
		Average	1·4±0·1	1·7±0·5

From the ratio of two consecutive values of strontium-89 concentration, the nominal composite removal constant ($\lambda r + \lambda_{Sr\text{-}89}$) and the mean residence time of strontium-89 in the lowest troposphere were estimated on the assumption that removal of strontium-89 activity from the atmosphere was an exponential function. The results obtained are presented in Table 3, where λr is the nominal removal constant for the depletion process of strontium-89. The composite removal constant, which is listed in the column designated as 'by ratio', was also determined by means of two consecutive values of the ratio (Sr-89/Sr-90) in the same way as by the strontium-89 concentration. In this case, the strontium-89/strontium-90 ratio is regarded as the 'amount of deposition of strontium-89 activity' corrected for an additional increase arising from the meteorological conditions. The mean time of residence of strontium-89 in the lowest troposphere, below the rain-forming layer, was estimated by the two different methods to be, on the average, 1·5 days. This value may suggest a measure of the duration of atmospheric contamination with downwind fall-out due to a distant underground test. It can be stated that the substantial air contamination with the fission-product activity continued for less than a week.

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SYMPTOMS AS AN INDEX OF BIOLOGICAL BEHAVIOUR AND PROGNOSIS IN HUMAN CANCER

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MOST concepts of the biological behaviour of human cancer depend on the morphology of the tumour. The evidence obtained by physical, surgical, roentgenographic and microscopic examination is used to identify the site, size, cellular type, regional invasion and distant dissemination of the tumour. These identifications then form the basis for decisions about rates of growth, prognosis and treatment for the cancers of individual patients.

From these morphological assessments, physicians have evolved the therapeutic concept that a cancer is most curable when discovered early and removed completely. In this concept, a tumour is 'early' if found before it has invaded any important regional structures or spread to distant sites; and the tumour is removed 'completely' if its anatomical situation permits excision of all the visible lesion together with a wide margin of normal tissue.

These morphological principles of therapy have been repeatedly confirmed by the many cases in which prompt surgical resection has cured a patient of his or her cancer. Yet the rule has also been violated by many exceptions. Some patients have died quickly despite a prompt and apparently 'complete' excision of a cancer discovered 'early'. Other patients have had long survival with cancers that were not removed either because the patient refused operation, or because the surgeon decided not to attempt resection.

An explanation for this capricious behaviour of cancer has been proposed in a hypothesis called "biologic predeterminism"[1]. According to this hypothesis, tumours with similar morphological appearance may nevertheless grow differently, and those with favourable biological growth are those most likely to be discovered 'early' in a state suitable for surgical cure. Although this hypothesis provides an attractive explanation for the observed discrepancies in behaviour of cancer, verification of the hypothesis has been difficult because of inadequate methods for identifying 'favourable' features of growth of the tumour.

Perhaps the main reason for the inadequacy of existing methods is that they identify form without identifying function. The conventional morphological data describe the appearance of the tumour and the structural damage it has produced. But morphology does not provide direct evidence of the functional effects of the tumour on structures or systems that are not anatomically invaded, nor does the morphological appearance indicate how long the tumour has been present. The term 'early', used so often in discussions of cancer therapy, is generally applied inappropriately. Although 'early' refers to a dimension in time, the usual evidence assessed in the designation of 'early' comes mainly from anatomy, not chronometry. The evidence depends on size, lymph nodes and metastatic dissemination, not on the length of time the tumour has been present.

Additional evidence of the functional and temporal growth of a tumour is available in the symptoms the tumour produces in its human host. Yet symptoms are seldom formally analysed or correlated in the therapeutic evaluation of human cancers. Although regularly noted in diagnostic decisions, and in the informal reasoning of 'clinical judgment', symptoms are usually not specifically cited, categorized, and analysed in the evaluations of prognosis and therapy. One main reason for the neglect of symptoms has been their variability. Because patients with the same morphological properties may have different symptoms, physicians have often concluded that symptoms are too psychically subjective to merit scientific consideration. A second reason for ignoring symptoms has been their lack of a taxonomy. No formal system of classification has been available for dividing symptomatic patients into sub-groups whose categories are consistent and reproducible.

The purpose of this article is to demonstrate that symptoms can be classified, that variations in symptoms often represent functional variations in the tumour rather than mere psychic reactions of the host, and that symptoms are an important index of biological behaviour, prognosis, and therapy of human cancers. The concepts will be illustrated by data obtained from surveys of large numbers of patients with cancers of the lung and of the rectum.

METHODS

(I) Classification of Symptoms

A disease (or pathological lesion) either does or does not produce symptoms in its human host, and the symptomatic host either reacts or does not react to the symptoms by seeking medical attention. These clinical humanistic aspects of a disease are the basis for classifying symptoms in a recently described system of clinical taxonomy[2,3]. The basic features of classification are the types of symptoms and the manner in which the diseased patient came to clinical detection.

A cancer can produce three main types of symptoms:

(*a*) *Primary symptoms.* These symptoms can be attributed to the tumour at its primary locus or to inflammation surrounding the tumour; none of the symptoms *per se* implies dissemination of the tumour. For example, in cancer of the lung, the primary symptoms would be: a recent cough; haemoptysis; chills, fever or sweats of an associated pneumonia; recent dyspnoea; or appropriate types of chest pain. In cancer of the rectum, the primary symptoms would be: bleeding; a significant recent change in stool appearance or in bowel habits; ano-rectal discomfort; or appropriate types of abdominal pain.

(*b*) *Systemic symptoms.* These symptoms arise as systemic effects remote from the primary site of the tumour, but the systemic symptom does not *per se* imply that the tumour has spread beyond that primary site. For example, anorexia, significant loss in weight and fatigue are systemic symptoms in cancer of either lung or rectum. The joint pains of hypertrophic pulmonary osteoarthropathy are systemic symptoms in cancer of the lung.

(*c*) *Metastatic symptoms.* These symptoms imply *per se* that the cancer has spread beyond its primary locus. For example, in cancer of the lung, invasion of the mediastinum is implied by dysphagia, by hoarseness due to a non-moving vocal cord, or by the manifestations of the superior vena cava syndrome; dissemination of tumour beyond the thorax is implied by bone pain, by cutaneous nodules, or by neurological manifestations. In cancer of

the rectum, metastatic symptoms would be bone pain, ascites, jaundice, or an unequivocally enlarged, nodular, hard liver.

(2) Attribution of Symptoms

Two main problems in the categorization of symptoms arise from decisions about the sources of symptoms. The first problem is the decision whether a particular symptom is due to the tumour or to some other disease that may co-exist in the same patient. For example, if a patient has both chronic emphysema and a cancer of the lung, should his recent dyspnoea be attributed to the emphysema, to the cancer, or to both? Our classification policy in these situations has been to attribute a symptom to the cancer when the symptom could not clearly be attributed to some other disease. For example, the symptom of visual difficulty in a patient with cancer might have been caused by an ocular or cerebral metastasis of the cancer. These neoplastic causes of visual difficulty could be excluded, however, if the patient had a cataract or some other local condition that was clearly the cause of the ocular symptoms. In making these 'attribution' decisions, we ascribed a symptom to some other cause if the other disease existed and seemed unequivocally responsible for the symptom. If a symptom could not be clearly excluded as definitely due to some cause other than the cancer, we included and classified that symptom among those attributable to the cancer.

A second type of problem is the decision whether certain symptoms attributable to the cancer should be regarded as primary or metastatic. For example, the symptomatic entity of pleuritic chest pain could arise from inflammation of pleura in a pneumonia secondary to the obstruction by the tumour of its primary bronchial site. Alternatively, the symptom might be due to neoplastic invasion of muscle, bone or pleura. In these situations, the symptoms are due to the cancer, and the problem is one of classification, rather than attribution. Any reasonable policy of categorization can be applied, so long as it is used consistently. Our policy has been to use the term 'metastatic', as defined earlier, for symptoms that were unequivocally associated with metastasis. When the symptom did not *per se* indicate metastasis, it was arbitrarily assigned to the 'primary' category for these analyses. In more detailed analyses to be reported elsewhere at a later date, such symptoms have been further sub-divided according to the concomitant presence or absence of morphological evidence of metastasis.

(3) Spectral Combination of Symptoms

Of all the symptoms present in a particular patient, certain symptoms can thus be noted as attributable (or possibly attributable) to the cancer. Of those attributable (or possibly attributable) to the cancer, some symptoms will be classified as primary, some as systemic and others as metastatic.

At the time a patient is first discovered to have a cancer, he may have none, one, two or all three types of these symptoms. Because the symptoms can occur alone, or in various combinations, the Venn diagrams of symbolic logic and set theory are a convenient way of illustrating the different components of the symptomatic spectrum. In the population of patients with a cancer, the seven possible types of sub-sets with different combinations of symptoms are shown in the Venn diagram of Fig. 1, which represents the complete clinical spectrum of a diagnosed cancer. An additional sub-set is provided by the patients whose tumour is discovered by accident while the patient is still asymptomatic. The circumstances in which such asymptomatic accidental discoveries occur have been described elsewhere[2,3]. (The remainder of the 'universe' of cancer of the lung, beyond these eight sub-sets, contains patients in whom the disease is present but not diagnostically detected.)

(4) Prognostic Implications of Symptomatic Stages

Of the eight sub-sets of patients just described, the four with metastatic symptoms (sub-sets 5–8) have a manifestly poor prognosis, because the presence of the metastatic symptoms indicates anatomic spread of the tumour beyond the primary site. In the remaining four sub-sets—who have no metastatic symptoms—the presence of systemic symptoms in sub-sets 3 and 4 suggests a situation less favourable than in the patients of sub-sets 1 and 2, who have neither metastatic nor systemic symptoms.

In sub-sets 1 and 2, whose symptoms *per se* have no unfavourable morphological or functional connotations, the duration of the symptoms can next be considered. For patients with primary symptoms only (sub-set 2), the duration of symptoms indicates a minimal time for the duration of the tumour—since the tumour has been present at least as long as its symptoms. If the symptoms are of short duration, no conclusion can be drawn about the rate of growth of the tumour. The symptoms may be short because the tumour has grown rapidly, or the tumour might have grown slowly with an eventual 'iceberg effect' in which symptoms did not appear until quite late in the course of the tumour. If the symptoms are of long duration, however, the tumour is likely to be slow-growing. The only way a patient could continue to have primary symptoms only, for a long time, without developing systemic or metastatic symptoms, would be to have a slow-growing tumour.

In the asymptomatic patients of sub-set 1, whose disease is discovered by accident during an examination performed routinely or for a complaint unrelated to the cancer, the duration of symptoms cannot be assessed since no symptoms exist. In such patients, the tumour has been present for unknown lengths of time and might have remained symptomatically silent for even longer lengths had the tumour not been accidentally discovered.

With these considerations, the eight sub-sets of Fig. 1 can be rearranged to create five main symptomatic groups or stages. In the new arrangement, which is shown in Fig. 2, sub-set 1 remains intact as the 'asymptomatic' group (Stage I). Sub-set 2 has been divided into two parts, according to whether the duration of symptoms was less than 6 months or at least 6 months: the 'long primary' group (Stage II) contains patients with primary symptoms only, of duration 6 months or more; and the 'short primary' group (Stage III) contains patients with primary symptoms only, of duration less than 6 months. Sub-sets 3 and 4 have been combined to form the systemic group (Stage IV), and sub-sets 5, 6, 7 and 8 have been combined to form the 'metastatic' group (Stage V).

From the foregoing discussion and from the hypothesis of "biologic predeterminism", we can anticipate a prognostic gradient among these five symptomatic states. The survival rates should be best in Stages I and II,

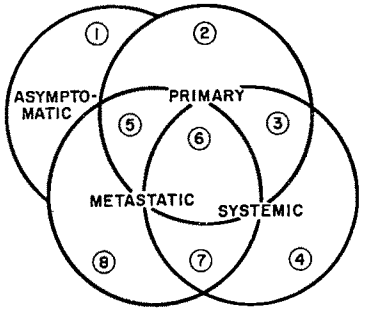


Fig 1. The clinical spectrum of a diagnosed cancer. This Venn diagram shows the 'intersections' of sets of patients with primary, systemic, or metastatic symptoms, occurring alone or in the various illustrated combinations. The asymptomatic group—with none of these symptoms—is distinguished from the remainder of the 'universe' of patients whose cancer has not been diagnosed. The eight sub-groups (or sub-sets) are further described in the text

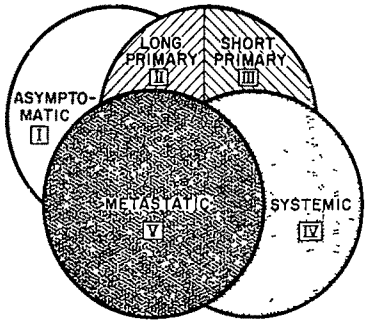


Fig. 2. Symptomatic stages of a cancer. The eight sub-sets of Fig. 1 are combined or divided to form the five mutually exclusive symptomatic stages shown here. For further details, see text

declining through Stages III and IV to the worst results in Stage V. Yet these prognostic distinctions—particularly in Stages I–IV—would not be discernible from morphological data, and could be detected only from analysis of symptoms.

(5) Selection of Patients and Methods of Survey

The medical record files of both the Yale–New Haven and West Haven Veterans Administration Hospitals were searched for the names of all patients who had received the diagnosis of primary cancer of the lung or of the rectum during the years 1953–58. The latter boundary was selected to ensure at least a 5-year follow-up period for the survey, which began in 1963; the opening year coincided with the opening of the VA Hospital. The survey was restricted to patients in whom histological proof of the cancer had been obtained during life or after death. Patients with cancer of the lung had had various cellular types of tumour; in cancer of the rectum, the type was exclusively adenocarcinoma. Records of the individual patients were then reviewed by methods described elsewhere[4,5].

For each patient, a complete chronology of the neoplastic illness was constructed, including all details pertinent to its earliest manifestations and extending for at least 5 years beyond the index date. For this chronology, additional data were obtained as needed from the Yale–New Haven Tumor Registry and from communication with patients, relatives, physicians and other hospitals. These investigations provided a complete description of the entire subsequent course, for at least 5 years beyond the index date, for each patient in this survey.

The index date selected as a common 'zero time' for all patients was the date of the first treatment. The 'zero time' served as the point at which the clinical state of each patient was classified, and from which the antecedent duration of symptoms and other manifestations, and the subsequent duration of survival, could be measured. Treatment was defined as surgery originally intended to remove the primary tumour (regardless of whether the tumour was actually removed), or radiotherapy directed at the primary tumour, or the administration of a cytotoxic chemotherapeutic agent. Patients who received only analgesics, sedatives, antibiotics or therapeutic measures other than one or more of the procedures just cited were regarded as having received no treatment. For such patients, 'zero time' was the date on which the physician (or patient) had decided against specific anti-neoplastic therapy. In patients who died before any therapeutic decision could be made, 'zero time' was the date of diagnosis.

RESULTS

(1) Overall Survival

Fig. 3 shows the 5-year survival rates for all patients in this survey, regardless of mode of therapy. In the 596 patients with lung cancer, the total 5-year survival was 9 per cent, but the rates ranged from 32 per cent in symptomatic Stage I to 18 per cent in II, 10 per cent in III, 8 per cent in IV, and 0·6 per cent in V. In the 201 patients with cancer of the rectum, the total 5-year survival was 27 per cent, with rates in the different symptomatic sub-groups ranging downward from 67 per cent in Stage I, to 49 per cent in II, 29 per cent in III, 16 per cent in IV, and 0 per cent in V. Thus, the prognostic gradient anticipated from symptomatic considerations was clearly evident in patients with two different cancers. In each cancer, the survival rates were highest in Stage I and became progressively lower to a nadir in Stage V. The distinctions among patients in Stages I–IV are particularly important, since the metastatic aspects of patients in Stage V are usually evident morphologically as well as symptomatically, but the differences among Stages I–IV can be discerned only from symptoms.

For simplicity in the subsequent statistical analyses presented here, we have combined the results of Stages I and II, and of Stages III and IV, thus reducing to two categories the four symptomatic sub-groups of Stages I–IV. With these consolidations, the survival rate in cancer of the lung was 22 per cent (28/127) for Stages I and II and 9 per cent (27/300) for Stages III and IV [$\chi^2 = 12{\cdot}4$; $P < 0{\cdot}001$]. In a similar comparison for the combined symptomatic sub-groups of cancer of the rectum, the rates are 50 per cent (31/62) in Stages I and II as compared with 19 per cent (24/124) in Stages III and IV [$\chi^2 = 17{\cdot}2$; $P < 0{\cdot}001$]. Both differences are highly significant statistically.

(2) Five-year Survival after Surgical Resection

Table 1 shows the five-year survival rates for patients whose tumours were surgically resected. The patients are classified according to the conventional notation of regional lymph node involvement, and are simultaneously categorized by symptomatic stages. The prognostic gradient previously noted for Stages I and II compared with Stages III and IV is still significantly present after surgery. The 5-year survival rate in these two symptomatic categories after resection of lung cancer was 37 per cent compared with 22 per cent [$\chi^2 = 4{\cdot}6$; $P < 0{\cdot}05$]; for rectal cancer, the corresponding data were 55 per cent compared with 28 per cent [$\chi^2 = 9{\cdot}3$; $P < 0{\cdot}01$].

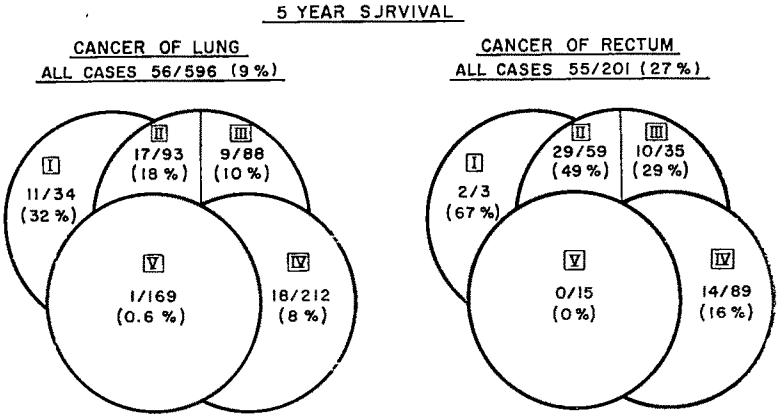


Fig. 3. Five-year survival, regardless of therapy, for the symptomatic sub-groups shown in Fig 2. Denominators represent the number of patients initially in each symptomatic stage, and numerators are the number who survived 5 years or more after therapeutic decisions Although the rates for cancer of the rectum are consistently higher than those for cancer of the lung, the same descending prognostic gradient is present from symptomatic Stages I–V for both cancers

As expected, the patients without neoplastic involvement of regional lymph nodes had better results than those whose nodes contained tumour. In the total results for cancer of the rectum, the patients with 'negative nodes' had a survival rate of 51 per cent, compared with 19 per cent in those with 'positive nodes'. This difference is statistically significant at $P < 0{\cdot}001$ [$\chi^2 = 15{\cdot}0$]. The corresponding values in lung cancer were 31 per cent and 22 per cent—a difference of uncertain significance [$\chi^2 = 1{\cdot}9$; $P > 0{\cdot}2$'.

In each of the two lymph node categories for each of the two tumours, the patients in symptomatic Stages I and II had better survival results than those in Stages III and IV. Although most of the numerical values in these sub-categories are too small for statistical significance, a significant distinction is present in the group with rectal cancer and 'negative nodes'. In patients of this morphological category, the 5-year survival was 68 per cent in symptomatic Stages I and II, and 40 per cent in Stages III and IV. This difference is significant at $P < 0{\cdot}05$ [$\chi^2 = 5{\cdot}2$].

(3) 5-year Survival and Cellular Type in Lung Cancer

All the rectal cancers in this survey were adenocarcinomata. The cancers of the lung, however, had various cellular types. Table 2 shows the 5-year survival rates according to the symptomatic stage and cytological differentiation of the tumours. As expected, the survival rates were better in well-differentiated than in not-well-differentiated tumours. For the total series, regardless of therapy, the cellular distinction in survival rate was 14 per cent compared with 4 per cent—a highly significant difference [$\chi^2 = 17{\cdot}1$; $P < 0{\cdot}001$]. Within the same cytological categories, however, patients in Stages I and II had higher survival rates than those in Stages III and IV. In the well-differentiated group, the two comparative percentages—29 per cent and 13 per cent—were significantly distinct at $P < 0{\cdot}01$ [$\chi^2 = 8{\cdot}5$].

In patients whose tumours were surgically resected, the survival rate for the well-differentiated tumours, 29 per cent, was not significantly higher than the 20 per cent found with undifferentiated tumours [$\chi^2 = 1{\cdot}0$; $P > 0{\cdot}3$]. Yet, in each cellular category, the results of patients in Stages I and II were similar, and higher than those of patients in Stages III and IV. The differences in post-operative survival between these two symptomatic categories—regardless of cellular type—were previously noted in Table 1 to be statistically significant at $P < 0{\cdot}05$. Consequently, as a harbinger of post-operative prognosis in cancer of the lung, the symptomatic stage distinctions were more significant than the cytological appearance of the tumours, and also, as noted earlier, than the invasion or non-invasion of lymph nodes.

Table 1. Five-year Survival after Surgical Resection of Cancers of Lung or Rectum

Symptomatic stages	Cancer of lung* Regional lymph nodes negative for tumour	Regional lymph nodes positive for tumour	Total	Cancer of rectum* Regional lymph nodes negative for tumour	Regional lymph nodes positive for tumour	Total
I and II; (asymptomatic and long primary)	21/51 (41)	7/24 (29)	28/75 (37)	23/34 (68)	7/21 (33)	30/55 (55)
III and IV; (short primary and systemic)	15/60 (25)	11/58 (19)	26/118 (22)	19/48 (40)	5/39 (13)	24/87 (28)
V; (metastatic)	0/4 (0)	1/6 (17)	1/10 (10)	—	0/4 (0)	0/4 (0)
Total	36/115 (31)	19/88 (22)	55/203 (27)	42/82 (51)	12/64 (19)	54/146 (37)

* Values in parentheses are percentages.

Table 2. Five-year Survival and Cellular Type of Cancer of Lung

Symptomatic stages	All types of therapy* Well-differentiated	Not well-differentiated	After surgical resection* Well-differentiated	Not well-differentiated
I and II; (asymptomatic and long primary)	23/79 (29)	5/48 (10)	23/59 (39)	5/16 (31)
III and IV; (short primary and systemic)	22/170 (13)	5/130 (4)	22/91 (24)	4/17 (24)
V; (metastatic)	1/79 (1)	0/90 (0)	1/8 (13)	0/2 (0)
Total	46/328 (14)	10/268 (4)	46/158 (29)	9/45 (20)

* Values in parentheses are percentages.

DISCUSSION

These results confirm the expectations of traditional clinical logic that have not previously been possible to demonstrate because the symptomatic components of clinical reasoning had not been given a formal structure that would permit quantified analysis. With the aid of Venn diagrams, the identified symptomatic ingredients can be arranged into a distinctive and suitable 'taxonomy'.

It is apparent from the data that symptoms indicate aspects of the function and rate of growth of a cancer that cannot be discerned morphologically. Conversely, the morphological properties of a cancer denote anatomical features of growth that cannot be discerned from symptomatology. To understand the biological behaviour of a tumour, and to appraise its prognosis and therapy effectively, both the morphological and symptomatical features must be appropriately classified and analysed.

That patients with a long duration of symptoms often have a paradoxically good prognosis, despite 'late' treatment, has been repetitively noted in many therapeutic surveys of cancer. Among the most recent reports have been the results cited for Hodgkin's disease and for cancer of the stomach[6–8]. Although the existence of a good prognosis for many such patients prompted MacDonald[1] to form his hypothesis of "biologic predeterminism", the hypothesis has seldom been adequately tested and has hitherto remained generally unconfirmed. In many surveys, the significance of the duration of certain distinctive symptoms has been obliterated because the investigators calculated duration as an average for all types of symptoms, instead of restricting the delineation to patients with primary symptoms only. In other investigations the data demonstrating paradoxically good prognosis for many patients discovered after a long 'delay' were usually dismissed as isolated peculiarities of the behaviour of cancer and man, and the biological significance of the data was not further considered.

A patient's decision to seek medical attention is obviously prompted by many different features of man or of disease. Different people, developing the same types of symptoms, will respond to those symptoms in diverse ways. Some people will seek medical attention promptly; others may wait. Nevertheless, few people will long ignore symptoms that are rapidly progressive, frightening, severe or incapacitating. On the other hand, many patients may not rapidly seek medical help when the early primary symptoms of the tumour are neither inconveniencing, dramatic, nor debilitating. Since slowly growing tumours produce symptoms slowly, such patients are often not motivated to visit a doctor until later in the illness when more significant symptoms have developed. In this way, many 'favourable' tumours are not detected until a long duration of symptoms has elapsed. If the tumour is discovered by accident before it has produced any symptoms, it has an even greater likelihood of being slow-growing.

This morphologic-functional interaction of tumour and host in human cancer has several other important implications:

(1) In selecting treatment for individual patients, physicians should not rely on morphology alone. A slow-growing tumour may be resectable for 'cure' even though it invades lymph nodes and is attached to contiguous structures. In many patients of the series surveyed here such tumours, left unresected because these features

made the tumours seem 'inoperable', eventually caused death not by subsequent widespread metastases but by their effects as 'foreign bodies' in the chest or abdomen. Death often resulted from a lung abscess that developed behind the intact tumour, from uraemia due to the compression of ureters by the tumour, or from massive haemorrhage as the tumour later invaded a blood vessel. Conversely, other patients with tumours that were clearly of rapid and unfavourable progression were subjected to fruitless operations and other therapy that increased the agony of the illness without affecting survival.

(2) In the design of therapeutic trials, the clinical population should be stratified according to symptomatic as well as morphological stages. Otherwise the base populations will not be reproducible, and the results will contain inconsistencies that will add to controversy and dilemma in statistics of cancer therapy[8]. Suppose, for example, that two treatments *A* and *B* are equally ineffective, but that treatment *A* is given to a population consisting predominantly of patients from symptomatic Stages I and II, while the population receiving treatment *B* consists mainly of patients from symptomatic Stages III and IV. If the patients have not been stratified for these symptomatic distinctions, the investigators will be unaware of these deceptive differences in two groups of patients that seem the same because they are morphologically similar. When treatment *A* is followed by results that are significantly better than those of treatment *B*, the difference may be erroneously ascribed to the treatment instead of to the disease.

(3) In the research at present devoted to the aetiology and pathogenesis of cancer, investigators may apply many biochemical, immunological, chromosomal and other tests to human neoplastic tissue. The results may show a wide range of variation that appears to have no particular correlation with the morphology of the tumour. Yet, if the neoplasms were categorized by function as well as by form, many of the apparent 'discrepancies' might be explained. Laboratory research at present dismissed as valueless might actually provide important new revelations if the neoplasms were more clearly and specifically defined for correlation.

The complexity of man increases the difficulty of studying human disease, but also enables a diseased man to talk. His description of his symptoms gives crucial information about function of the diseased structures under investigation.

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NEWS and VIEWS

Charles-Leopold Mayer Prize: Dame Honor Fell, F.R.S.

DAME HONOR FELL, director of the Strangeways Research Laboratory, Cambridge, has been awarded the Charles-Leopold Mayer Prize (valued at 100,000 francs) by the Academie des Sciences de l'Institut de France for her contributions to cell biology. These range from experimental embryological studies on limb bone rudiments to the analysis of the mechanism of action of vitamin A and other compounds on cell components and, in particular, the lysosomal apparatus. Dame Honor has been director of the Strangeways Research Laboratory since 1929. In 1964 she received the honorary degree of doctor of science from the University of Oxford and from Harvard University; she was elected a foreign member of the Royal Netherlands Academy of Science, and an honorary Fellow of Somerville College, Oxford. She is also an honorary doctor of science of Smith College, Massachusetts, an honorary LL.D. of Edinburgh, and a Fellow of Girton College, Cambridge.

Deputy Director and Keeper of the Herbarium and Library of the Royal Botanic Gardens, Kew: Dr. C. E. Hubbard, C.B.E.

DR. C. E. HUBBARD retired on November 30 from the post of keeper of the Herbarium and Library and deputy director of the Royal Botanic Gardens, Kew. Dr. Hubbard was born in 1900 at Appleton, Norfolk, and in 1916 started work in the Royal Gardens at Sandringham where, except for a spell in the Royal Air Force during 1918–19, he remained until he joined the Gardens staff at Kew in April 1920. He entered the Herbarium as a temporary technical assistant in 1923 and soon afterwards, under the guidance of Dr. O. Stapf, he started to specialize on the grasses (*Gramineae*), on which family he later became a leading world authority. In 1924 he made a plant-collecting journey in southern and central Spain with the late Rev. E. Ellman, and in 1930 he spent a year in Australia studying grasses and travelling extensively in Queensland, where he made important botanical collections. In 1954 he was awarded the O.B.E. for his outstanding work on the classification of grasses. On October 1, 1957, he succeeded the late Dr. W. B. Turrill as keeper of the Herbarium and Library and assumed the additional responsibility of the deputy directorship of Kew in 1959. In 1960 he was awarded an honorary doctorate of science by the University of Reading. He was advanced to C.B.E. in 1965. Dr. Hubbard's first major published contribution to the study of *Gramineae* was Part 5 of Volume 9 of the *Flora of Tropical Africa* (1930), written jointly with Dr. O. Stapf, including such difficult genera as *Setaria* and *Pennisetum*. In 1937 he alone wrote Part 1 of Volume 10. He contributed the treatment of the grasses to both editions of Dr. J. Hutchinson's *Flowering Plants* (1934, 1959). He also contributed the key to the tribes and genera in Hutchinson and Dalziel's *Flora of West Tropical Africa* (1936). In 1954 his *Grasses*, an admirably illustrated pocket-book dealing with those species occurring in Britain, was published by Penguin Books. In addition, he wrote numerous shorter papers, and many of them were published in the *Kew Bulletin*, including no less than twenty-eight in the series entitled "Notes on African Grasses". Dr. Hubbard's advice on the identification and classification of grasses has been sought by botanists and agriculturalists in many parts of the world, and his specialized knowledge of grasses has also enabled him to give useful service on the Advisory Committee for Research on Sea Defence.

Mr. J. P. M. Brenan

Dr. Hubbard has been succeeded as deputy director and keeper of the Herbarium and Library by Mr. J. P. M. Brenan. Mr. Brenan was born at Chislehurst, Kent. He was educated at Tonbridge School and Brasenose College, Oxford, where he gained first-class honours in the Final Honour School of Natural Science (Botany) in 1940, later becoming M.A. He worked as a departmental demonstrator and then as a University demonstrator in the Imperial Forestry Institute. During 1947–48 he travelled in Northern Rhodesia and Tanganyika, studying their woody plants, and was also a member of the Cambridge Botanical Expedition to Nigeria and the Cameroons, under the leadership of Prof. P. W. Richards. He joined the staff of the Herbarium at the Royal Botanic Gardens, Kew, in 1958, working in the tropical African section, of which he was in charge from 1959 to 1965. In 1954 he was awarded a B.Sc. by the University of Oxford for work on the plant-geography of tropical Africa. In 1965 he was elected botanical secretary of the Linnean Society of London. Mr. Brenan has specialized on the flora of tropical Africa, especially the families *Leguminosae*, *Commelinaceae*, *Sterculiaceae* and *Chenopodiaceae*. His first major work was, in conjunction with Dr. P. J. Greenway, *Check Lists of the Forest Trees and Shrubs of the British Empire, No. 5, Part 2, Tanganyika Territory* (1949). He was responsible, with help from collaborators, for elaborating the plants collected by the Vernay Nyasaland Expedition of 1946 (published in the *Memoirs of the New York Botanical Garden*, 1953–54). He has contributed various families to the *Flora of Tropical East Africa*, including *Leguminosae* Subfamily *Mimosoideae* (1959). He has also written numerous shorter papers on the flora of Africa, and also some on the flora of Britain. Mr. Brenan translated into English Prof. P. Jaeger's book *Le Vie Étrange des Fleurs*, published in 1961 as *The Wonderful Life of Flowers*.

Director of the Tropical Products Institute, London: Mr. E. S. Hiscocks, C.B.E.

Mr. E. S. Hiscocks, director of the Tropical Products Institute of the Ministry of Overseas Development, will retire from the public service on March 31, 1966. After studying chemistry in the University of Wales, where he worked with J. A. V. Butler on physico-chemical properties of solutions, Mr. Hiscocks joined the Civil Service in 1926 in the then Department of the Government Chemist and for several years collaborated with Sir Robert Robertson and Sir John Fox in researches on infra-red absorption spectra of gases and the preliminary work on the structure of diamond. He later did work on ultra-violet absorption spectra of organic compounds and, after serving in various of the revenue sections of the Department, transferred in 1939 to the Raw Materials Department, Ministry of Supply. Here he set up, and ran, the technical branch of that Department. In 1944 he was appointed secretary of the National Physical Laboratory and remained there until 1957, when he became director of the United Kingdom Scientific Mission (North America) and Scientific Attaché to the British Embassy, Washington, D.C. He returned from the United States at the end of 1960 on his appointment as director of the Tropical Products Institute. During his period at the National Physical Laboratory he was closely concerned with the adjustment of the Laboratory's organization and work to the requirements of the post-war period. During this time he wrote numerous papers on various aspects of the organization and administration of scientific work. He also organized an international symposium on the direction of research establishments, held at the Laboratory in 1956. In that year his book *Laboratory Administration* was published. During the five years he has spent at the Tropical Products Institute, Mr. Hiscocks has been concerned not only with reorganization of the Institute's work, to meet the changed requirements of the developing countries of the Commonwealth, but also in extending its scope of operation to other developing countries in the Middle East, South America and the Pacific, during visits to those areas. He has also furthered collaboration between the Institute and various agencies of the United Nations Organisation. Mr. Hiscocks was a United Kingdom delegate to the Pacific Science Congress in 1961 and was United Kingdom delegate to the United Nations Committee for Industrial Development in 1964.

Dr. P. C. Spensley

Mr. E. S. Hiscocks will be succeeded as director of the Tropical Products Institute by the present deputy director, Dr. P. C. Spensley. Dr. Spensley was a foundation scholar of St. Paul's School and of Keble College, Oxford, where he studied chemistry. His research after the Second World War in the Dyson Perrins Laboratory at Oxford was mainly concerned with the synthesis of organic compounds of possible therapeutic interest. Between 1940 and 1945, Dr. Spensley was on the staff of the Ministry of Supply, Royal Ordnance Factories, where he was concerned with explosives and ammunition production, smoke weapons development and standards. In 1950 Dr. Spensley joined the staff of the National Institute for Medical Research and, in the course of a working visit to East Africa, discovered a method by which a valuable, but minor, component of the sisal plant—hecogenin—could be extracted economically as a by-product of the sisal fibre industry. This process he developed further with the National Research Development Corporation and it is now operated commercially in Tanzania and Kenya to provide hecogenin for the production of steroid drugs. Dr. Spensley received a major inventor's award for this work in 1963. In 1954, Dr. Spensley was appointed to the scientific staff of the Colonial Office in the Imperial Institute and became Scientific Secretary of the Colonial Products Council. With the then director he was closely concerned with the planning which led to the evolution in 1958 of the Tropical Products Institute and soon afterwards he was made assistant director in charge of administration and development. In this post he has travelled widely and attended a number of international meetings. He assisted Sir William Slater in the organization of the British contribution to the major United Nations Conference on the Application of Science and Technology for the benefit of the Less Developed Areas, held in Geneva in 1963.

Plant Pathology in Queen's University, Belfast: Prof. A. E. Muskett, O.B.E.

Prof. A. E. Muskett retired on December 31, 1965, from his chair in the Department of Mycology and Plant Pathology at Queen's University, Belfast, and from his position as head of the Plant Pathology Division of the Ministry of Agriculture for Northern Ireland. A native of Norfolk, he graduated from the Imperial College of Science and Technology, where he studied under Sir John Farmer, and went to Belfast in 1923 on his appointment as a research assistant in the Seed Testing and Plant Disease Division of the Ministry and as an assistant in agricultural botany at Queen's. He obtained his D.Sc. from London in 1938. His continued successes and promotion led to the formation in 1938 of a separate Plant Pathology Division of the Ministry, of which he became head, and led, in 1945, to his appointment to the newly created chair in the University. He was president of the British Mycological Society in 1948. In 1957 he was awarded the O.B.E. Prof. Muskett's early publications on the control of American gooseberry mildew and apple scab led to the adoption of regular spraying programmes suitable for local conditions. The toxicity of lime sulphur sprays to apple trees and the efficiency of an excess-lime Bordeaux mixture were demonstrated. Further research naturally centred chiefly around the potato, oat and flax crops, with special reference to tuber disinfection with organic mer-

curials and to seed disinfection with organo-mercury and thiram-based chemicals. The value of thiram as a satisfactory dressing for flax seed was first shown in 1939 and was a most timely discovery, as it was used on all flax seed saved in the United Kingdom during the Second World War. Following his work on the epidemiology and control of the *Helminthosporium* disease of oats, Prof. Muskett led research in the development of techniques for the examination of seeds for the presence of seed-borne fungi, work which formed a basis for the investigation of biological techniques for the evaluation of fungicides. He also devoted much time to the health of the seed potato crop of Northern Ireland, special facilities being available for work on virus diseases at the Plant Pathology Field Station, erected in 1954. In 1947 he published, with Prof. J. Colhoun, *The Diseases of the Flax Plant*, and in 1964 Dr. J. P. Malone and he published *Seed-borne Fungi*, two books which quickly found a wide circulation. Prof. Muskett was not only an enthusiastic and inspiring lecturer, an energetic administrator during his term as Dean in 1950–57, a useful member of the Academic Council and University Committees and an apt adviser on plant pathology problems, but also a friend and counsellor to many students and members of staff who sought his advice and now wish him a happy retirement.

University Grants

In a written answer in the House of Commons on December 20, the Secretary of State for Education and Science, Mr. A. Crosland, stated that the Government was reviewing the quinquennial system of recurrent grants to the universities. In view of the difficulties which arose because of the fact that under the present system the universities did not know their recurrent grants for the new quinquennium until near the end of the last year of the old quinquennium, the Government, after consulting the University Grants Committee, had decided to make a provisional allocation of recurrent grant for the first year of the next quinquennium (1967–68) well in advance of the final settlement for the quinquennium as a whole. He hoped to be able to announce this allocation towards the end of 1966 and the University Grants Committee had assured him that this change in procedure would be acceptable to the universities. In a written answer on December 22, Mr. Crosland announced that he was authorizing, for starting in 1966–67, a university building programme (in terms of existing cost figures) of £40 million; for 1967–68, £30 million; for 1968–69, £25 million; and for 1969–70, £25 million. He hoped that in future this could be made a rolling programme. The programme allowed a higher level of building starts in 1966–67 and 1967–68 than that at present sanctioned, and it took account of deferment from the present year of some £15 million starts following the Government's measures of July 1965.

Welsh College of Advanced Technology

Mr. Crosland also announced in the House of Commons on December 20 that, after considering the advice of the University Grants Committee, the Government had concluded that it could not support the establishment of the Welsh College of Advanced Technology as a separate degree-giving university. The invitation which the College had received from the University of Wales to become a full constituent member of the University offered an alternative road to university status and the Government was confident that such an arrangement would contribute to the development of the College as a centre of higher technological education. It also believed that the idea of an association between the College, University College, Cardiff, and the Welsh National School of Medicine could well be worked out within the framework of the University of Wales. He understood that the University would shortly be petitioning for the amendments to its Charter necessary to deal with problems arising from its federal constitution.

South-east England : Water Resources

In reply to questions in the House of Commons on December 20, the Minister of Land and Natural Resources, Mr. Willey, said that he hoped to have the advice of the Water Resources Board on the consultant's report on the water resources of the Great Ouse Basin fairly early in 1966, and would then consider the next steps. He would certainly consult the East Anglian Regional Economic Planning Council before putting a feasibility study on the Wash barrage in hand. The Minister of Housing and Local Government had received the report of the consulting engineers, Messrs. Binnie and Partners, on the water resources of the Great Ouse Basin. This report recommended further study of a number of possible conservation schemes, including the use of the Wash as a reservoir. The Water Resources Board had been asked to consider the report in the context of their studies of the South-East as a whole and to advise in due course what action should be taken. The situation was being considered as expeditiously as possible but the advice of the Water Resources Board had first to be awaited.

University of Birmingham—Royal Radar Establishment, Malvern: Working Association

The University of Birmingham has recently concluded an agreement for a working association with the Royal Radar Establishment, Malvern. The agreement, which comes into effect early this year, provides for the co-option of up to six senior scientists from the Royal Radar Establishment on to the University staff; they will have honorary University titles and will spend an average of 1 day a week in the University, taking part in undergraduate and postgraduate teaching, research, departmental policy discussions, and the general life of the University. In the opposite direction, a corresponding number of members of the University staff will be co-opted on to the staff of the Royal Radar Establishment to spend an average of 1 day a week at Malvern, taking part in research and in research policy decisions. The arrangement will not extend to the whole of the Radar Research Establishment, part of which is engaged on work of military importance. On the University side, it will involve the following Departments: Electronic and Electrical Engineering, Electron Physics, Physics (especially solid-state physics), Mathematical Physics, Physical Metallurgy. On the Malvern side the corresponding divisions of the Royal Radar Establishment will be involved.

The Barwell Meteorite

A large meteorite fell at about 16.20 G.M.T. on December 24, 1965, at the village of Barwell in Leicestershire. It is possible that a wider area was involved, as material has now been reported from farther afield, but as yet this has not been examined. The Barwell meteorite has been identified as an olivine bronzite chondrite. So far, 35–40 lb. of this material has been collected, and this is being collated at the British Museum (Natural History). *Bona fide* scientists wishing to investigate material from this meteorite should apply, in the first instance, to Prof. P. C. Silvester Bradley, Department of Geology, University of Leicester.

In-service Training for Science Teachers

Increasing awareness of the need for teachers of science and mathematics to be kept in touch through in-service training (or refresher courses) with modern advances in science moved the Royal Society to hold a conference of representatives of the interests concerned, on September 16, 1965. A report of that conference is now available (The Royal Society. *In-Service Training for School Teachers of Science and Mathematics in England*. Report of

a Conference convened by the Royal Society on 16 September, 1965. Pp. 14. London: The Royal Society, 1965). Prof. H. W. Thompson was in the chair, and in the proceedings sixty-two people took part. The chief conclusions and recommendations which emerged were that a very considerable increase in in-service training for teachers of science and mathematics was urgent. The Department of Education and Science together with Local Education Authorities were urged to provide the necessary financial assistance; in-service training and the development of the science curriculum should go hand in hand; and further links should be established between the learned societies and professional institutes concerned and school teachers of science, to strengthen a sense of community. Those organizations already active in the field should be encouraged to continue, with co-ordination when necessary, perhaps through the Schools Council—in this respect, what was already being done by the Schools Council met with general approval. The recommendations of the Joint Mathematical Council were endorsed. Local Education Authorities, it was felt, should act generously in supporting teachers who wished to attend courses; and the Royal Society should bring the report of the meeting to the attention of a wide range of public bodies, universities and other interested groups. Hesitation about these conclusions came mainly from K. S. Tetlow, representing the teachers' point of view. His enquiries had shown that no teachers were interested in courses of one term's duration or more; there was no enthusiasm for courses held during the holidays; and the pupils would suffer if courses were held during term time. There was, however, a strong desire for more information about modern advances in science, as well as for courses of one or a few days' duration, held at strategic times during the school year.

Registrar General's Quarterly Return

The Registrar General's Quarterly Return for the June quarter of 1965 shows that births in the first two quarters of 1965 were fractionally lower than in the corresponding quarters of the previous year (439,000 as against 449,000) (General Register Office. *The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, Quarter ended 30th June, 1965*. Pp. 40. London: H.M.S.O., 1965. 2*s*. 6*d*.). The seasonally adjusted birth-rate has also fallen slightly. This is the first time during the past ten years that the figures have failed to show an increase, but it is difficult to draw any conclusion from this short period. The infant mortality rates continue to decline, and have fallen by 25 per cent during the past ten years. Marriages tend to be stable in number. The first provisional analysis of death in 1964 by cause, age and sex appears in the *Return*: deaths from lung cancer were 3·5 per cent up on the 1963 figures among men and nearly 6 per cent up among women; the increase in deaths due to motor vehicle accidents was nearly 15 per cent among both sexes. Nearly 45 per cent of the deaths of males aged 15–24 were attributable to this cause. The expectation of life at birth in 1962–64 was 68·1 years for men and 74·2 for women.

Fuels for the Future

"Man's use of energy has progressed from wood and dung to coal and oil, is now taking a large contribution from gas and moving towards uranium, plutonium, and ultimately sea-water." This challenging statement appears towards the end of an entertaining and prophetic article by Keith Richardson entitled "Fuels for the Future" (*Catalyst*, 15; Autumn 1965. London: Shell Chemical Co., Ltd., Plastics and Rubbers and Industrial Chemicals Divisions). Mr. Richardson surveys the present highly competitive fields of coal, oil, electricity and gas; and the future impact of nuclear energy. Of the coal industry, he says that the main trouble is its great inflexibility. The over-riding question is, what is the future of the coal industry in our economic scheme of things? At present, it produces 66 million tons a year from loss-making pits, mainly in Scotland, Wales and the North-East. Arguments in favour of keeping a strategic reserve of coal capacity—pits that can be opened if ever needed—are attractive, but impracticable; once a coal mine is closed it is closed for ever, because of rock-pressure and water flooding; if we are to be able to call on 200 million tons of home-produced coal in the future, that same amount must be bought now to keep the pits in being. Before coal is ever likely 'to get into balance', uneconomic pits must be abandoned. "The tragedy is that business which could profitably be won by Midlands coal at £2 a ton is lost to oil because the price structure is all wrong." It is the oil industry that has damaged coal, but it has also spurred it to greater efficiency. Mr. Richardson considers that the oil supply situation looks as though it will become tougher between 10 and 30 years from now, because costs of exploration and of extraction will rise; oil-bearing countries may grow reluctant to let so much of their resources be shipped to Britain as they now do; this, it is argued, is why the present North Sea explorations are so tremendously important. In the latter context, gas is now better envisaged as an offshoot of the oil industry, because the British Gas Council can make its own gas just as cheaply from oil as from other sources; ". . . as surely as oil has won fuel business from coal, in time it will lose it back to electricity". But the golden future thus predicted for electricity depends neither on coal nor on oil (including gas), but on atomic power. The advanced gas-cooled reactor now offers the cheapest means of generating electricity. By the 1980's fast breeder reactors should produce more fuel than they use. Two other intriguing possibilities for future fuels are briefly discussed in this article: one is the prospect of the fuel cell gradually replacing the petrol and diesel engine; the other is thermonuclear fusion. If the latter is a practicable source, it means power from sea-water "in virtually unlimited quantities". Finally, there is that devastating machine called the 'Thetatron', ". . . which in six-millionths of a second can develop 12 million amps of current, and this has achieved momentary bursts of 10 million degrees". As Mr. Richardson naively says, at least it points the way. This is an extremely able essay into the complex problems of fuels for the future.

Balancing Capacity and Sales

Achieving Balance between Capacity and Sales, a booklet containing papers given at a conference on this theme at Birmingham during May 1965, is now available (Pp. 31. London: British Productivity Council, 1965). The conference was organized by the British Productivity Council, in co-operation with the British Institute of Management, the Centre for Inter-firm Comparison and the Birmingham and Coventry Productivity Associations. The introductory paper, by H. Ingham, director of the Centre for Inter-firm Comparison, stated that there are a few frequently occurring and typical patterns of co-ordination between sales and capacity, each of which can be described as a basic marketing objective with resultant typical opportunities and risks. Companies of a similar type, although making different products, face broadly similar problems of balance, so that a firm can improve its methods of co-ordination by comparing them with those of other firms of its type. The co-ordination outlined in the type descriptions given can be used as 'blueprints'. Ingham also discussed the influences of co-ordination practices on profitability. Three case studies were also presented to show typical major patterns of co-ordination: the first by H. A. Hollingsworth, chief production controller of Wilmot Breeden, Ltd.; the second by J. W. Fewless, director of the Heath Street Division of G.K.N. Screws and Fasteners, Ltd.; and the third by L. A. Williams.

head of the Operational Research Unit of Ferranti, Ltd. (Transformer Division).

Brucellosis

Food and Agriculture Organization Agricultural Studies No. 66 embodies the report of the joint World Health Organization/Food and Agriculture Organization Expert Committee on Brucellosis (Food and Agriculture Organization of the United Nations. *Joint FAO/WHO Expert Committee on Brucellosis—Fourth Report.* Pp. 65. Rome: Food and Agriculture Organization of the United Nations. London: H.M.S.O., 1964. 6*s.* 8*d.*; 1.25 dollars). Its fourteen sections discuss the epidemiology of brucellosis; speciation in the genus *Brucella*; media and methods for the primary isolation and further cultivation of the organism; the standardization and interpretation of the serum agglutination test; human, bovine, caprine and porcine brucellosis and the disease in other animals; the United Nations brucellosis centres and future research needs. Nine annexes deal with various aspects of the immunology of *Brucella* infections.

Engineering Aspects of Lubrication

An intensive course on "Engineering Aspects of Lubrication", organized by Dr. A. Cameron, will be held in the Department of Mechanical Engineering, Imperial College of Science and Technology, during May 31–June 17. The various processes underlying the operation of bearings are often extremely complex and the aim of the course is to enable engineers and industrialists to have a better understanding of them. In this way, they will be better able to design equipment and understand failures. The content of the course will be as follows: First, classical lubrication theory will be developed and compared with the practice of journal and thrust bearings, the importance of temperature being emphasized and a method produced for analysing its effect. The combined influence of pressure on viscosity and deformation of surfaces will then be discussed and the method investigated by which ball and roller bearings and gears are lubricated. The various theories of oil film break-down will be considered and a reasonable theory of scuffing put forward. A number of specialist lectures will be given by recognized experts. Full bound sets of lecture notes will be issued to every student attending the course. Further information can be obtained from the Registrar, Imperial College, London, S.W.7. The fee for the course will be 45 guineas.

Post Office : Postgraduate Awards

The Post Office is arranging to make each year a number of postgraduate awards to selected staff. These awards will be tenable at universities which have facilities for research or advanced studies relevant to Post Office problems, initially in telecommunications science and engineering. In appropriate cases the awards will enable the holders to qualify for M.Sc. or Ph.D. degrees. The primary objective, however, is to further research and develop expertise of special value to the Post Office.

Prof. G. Macdonald, C.M.G. : The Ross Institute, and Schistosomiasis

The Editor has received the following communication from Prof. G. Macdonald: "I am happy to correct the statement on p. 30 of your January 1 issue that I have given up my directorship of the Ross Institute, a post which I retain and hope to enjoy for some years. The Ross Institute, under my direction, has been very interested in schistosomiasis for several years, and in 1961 it received a substantial grant from the Rockefeller Foundation to study the medical and public health significance of this infection, the grant being further supported by the Ministry of Overseas Development and the World Health Organization. With this aid, a Schistosomiasis Research Unit under Dr. Forsyth has been working in East Africa since 1962, originally based on the East African Medical Research Laboratory at Mwanza and now in Zanzibar. The World Health Organization* also sponsors work on this disease, and much research has been carried out by the staff of the Research Laboratory at Mwanza".

*WHO Technical Report Series No. 299 (price 5*s.* from H.M.S.O., P.O. Box 569, London, S.E.1) and *Snail Control in the Prevention of Bilharziasis* (price 30*s.*, from the same address).

University News:

Hull

Dr. R. R. Baldwin has been appointed to the second chair of chemistry as from January 1.

Leeds

Dr. E. Irving has been appointed to a chair of geophysics in the Department of Earth Sciences.

London

The following appointments have been made: *Professorships*, Dr. E. W. Horton (pharmacology, tenable at the School of Pharmacy); Dr. J. T. Stuart (theoretical fluid mechanics, tenable at the Imperial College of Science and Technology); *Readerships*, Dr. B. E. Plunkett (botany, tenable at Birkbeck College); Dr. E. Cohen (mineral technology, tenable at the Imperial College of Science and Technology). The following titles have also been conferred: *Professor*, Dr. J. Joseph (anatomy, in respect of his post at Guy's Hospital Medical School); *Reader*, Dr. T. H. Lambert (mechanical engineering, in respect of his post at University College).

Swansea

Prof. J. A. Beardmore, professor of genetics in the University of Groningen, has been appointed to the newly established chair of genetics.

Announcements

A series of courses on subjects in the field of natural sciences will be held at the Scottish Field Studies Association's Field Centre at Kindrogan, Perthshire, during 1966. Further information can be obtained from the Scottish Field Studies Association, Ltd., 141 Bath Street, Glasgow, C.2.

A general discussion of the Faraday Society on "The Role of the Absorbed State in Heterogeneous Catalysis" will be held in the University of Liverpool during April 4–6. Further information can be obtained from the Assistant Secretary, the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

A meeting of the East Midlands Branch of the Institute of Mathematics and its Applications will be held at Loughborough College of Technology on January 18. A lecture on "The Mathematical Theory of Multi-variable Control Systems" will be delivered by Prof. H. H. Rosenbrock. Further information can be obtained from the Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex.

A series of seminars for senior production executives will be held at the Northampton College of Advanced Technology during the first six months of 1966. Subjects under discussion will be as follows: the implications of economic and technological change for industrial relations (January 19); PERT costing as a tool for higher management control of projects (February 16); financial analysis of projects involving capital expenditure (March 23); optimal replacement decisions: alternative approaches to the replacement of machines (April 20); the effects of organization on budgetary control in a manufacturing enterprise (May 13); operations research as an aid to factory location (June 22). Further information can be obtained from the Secretary, Department of Management and Social Science, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

SCIENCE IN PARLIAMENT

University Accounts

IN moving, on December 7, that the House of Commons take note of the first, second and third reports from the Committee of Public Accounts in the Session 1964–65 and of the Treasury minute on the reports, Mr. Boyd-Carpenter referred, more particularly, to the paragraphs in the reports which related to the denial of access by the Comptroller and Auditor-General to the books of the universities. While the Committee had before it much material in respect of the universities, that material was based mainly on information received from the Department of Education and Science and the University Grants Committee. Mr. Boyd-Carpenter questioned whether, with the increasing scale of public provision for the universities and the changed position of the University Grants Committee, which now came under the Department of Education and Science and not directly under the Treasury, this exclusion could be justified and whether its removal would really involve interference with academic freedom. He pointed out that in recent years the Comptroller and Auditor-General had had access to the books and records of the Colleges of Advanced Technology and of the Scottish central institutions. This access would cease when these institutions attain university status. The Committee of Public Accounts suggested, accordingly, that it might be appropriate to reconsider whether the exemption of this large and growing area of expenditure from accountability to Parliament was still justified. Mr. Boyd-Carpenter merely suggested that the issue should be re-examined with an open-minded and clear realization of the implications and that the universities should have the opportunity to express their views. In this he was supported by Mr. T. Dalyell, who also raised the question of the costs of research, suggesting the appointment, apparently under the University Grants Committee, of a small executive body of scientists, seconded for 2 or 3 years to deal with expenditure on physics, engineering, chemistry and biology on a national basis.

The Financial Secretary to the Treasury, Mr. N. MacDermot, in replying on the debate, welcomed the Committee's proposal to look further into this matter, but emphasized the importance of ensuring that academic freedom was maintained, while we obtained the greatest possible value for money. It did not follow that use of the most up-to-date techniques of control and management involved making the University Grants Committee responsible to Parliament and, without prejudicing the question, he seemed more inclined to accept the advice and recommendation of the Robbins Committee.

Manufacture of Enriched Uranium

In a statement in the House of Commons on December 9, the Minister of Technology, Mr. F. Cousins, said that the Government had decided to modernize and reactivate the Capenhurst plant of the Atomic Energy Authority, so that it might supply enriched uranium for the manufacture of fuel for the second nuclear power programme. Owing to a decrease in the demand for enriched uranium for military purposes, and because the civil demand was then very small, it was decided, in 1962, that production of highly enriched material for military purposes should cease, and that of slightly enriched material for civil use be reduced to the minimum level needed to maintain gaseous diffusion technology. This decision was implemented by the end of 1963. For a nuclear power programme of 8,000 MW by 1975, using the advanced gas-cooled reactor design adopted for the Dungeness '*B*' station, several hundred tons of slightly enriched uranium oxide would be required each year. The Authority had designed modifications to the Capenhurst plant that should greatly increase the efficiency of production and had advised him that the gap between Capenhurst prices and those of the alternative United States source should narrow progressively during the 1970s. Other factors to be taken into account included the obvious disadvantages of complete dependence on an overseas supplier, since nuclear power would meet an increasing proportion of the total demand for electricity. Production in the United Kingdom would save imports costing some millions of pounds, and ultimately some tens of millions of pounds per annum. Closure of Capenhurst would make it impossible for Britain to maintain its expertise in this field, while manufacture in Britain would provide the Authority with opportunities to export slightly enriched uranium fuel.

The Government had approved in principle plans involving a capital expenditure of about £13·5 million, the first phase of which, to be started immediately, would cost about £7·5 million. These plans were expected to meet the initial requirements of the new nuclear power programme, and it was envisaged that the plant could be further expanded as requirements increased, and decisions would be taken as necessary. Work would start immediately and the modernized plant would be in operation in time to supply fuel for the first advanced gas-cooled reactor of the second nuclear power programme, Dungeness '*B*'. Mr. Cousins commented that, at present, the difference between British and American prices for fuel was estimated at 10 per cent, but the electricity costs in diffusion were likely to fall, because electricity in Britain was becoming cheaper. The difference to the ultimate consumer was likely to be about 0·25*d*. in the pound. If Britain were to manufacture low-enriched uranium, and if various types of station were to be built around the world, it was possible that Britain would be in the market to sell to them. The Atomic Energy Authority also had in mind the possibility of supplying tailor-made fuel to tailor-made stations. Centrifuge plant might possibly make present methods obsolete, but it would probably be by the late 1970s before centrifuge plant became a possibility. All the information available at the moment led the Authority to believe that it need not worry about the position for the next 10 years. The method under discussion provided opportunity for a changeover at a later stage, if necessary. Non-industrial staff at Capenhurst at present numbered 455, and industrial staff 860. When the plant was in full production a further 200 industrial workers might be required.

A similar statement was made in the House of Lords by Lord Snow, also on December 9, who, in reply to questions, affirmed that the Government was confident that the supply of enriched uranium could be increased to meet any foreseeable demand.

Social Sciences Research Council

In moving approval of the draft Social Science Research Council Order, 1965, in the House of Lords on November 18, the Parliamentary Secretary to the Ministry of Technology, Lord Snow, said that the Government accepted, in general, the functions and powers proposed by the Heyworth Committee. These were embodied essentially in article 2 (1) of the Charter and in the schedule to the Draft Order, which was drawn up in terms

similar to those appearing in the Charters of other research councils. The Social Science Research Council would be breaking more new ground than the other Research Councils, and its first task would be to decide in detail its own programme of activities and methods of working. It would not, for the time being, set up research units of its own and would probably concentrate initially on supporting research at present being carried out in the universities and elsewhere and on providing a focal point for co-ordinating and disseminating information. One of the problems facing the Council would be to define its own sphere of operations; however, economics, sociology, social psychology, social anthropology and political science would be its immediate concern. The Council would assume some of the functions hitherto performed by the Human Sciences Committee of the Science Research Council and the Department of Education and Science in regard to awards to postgraduate students. Arrangements for discussion with the other bodies concerned would include the appointment of assessors from Government departments, as well as the representation of the Council on inter-departmental committees concerned with research in the social sciences.

While regretting that the Council was not immediately to conduct research of its own, Lord Taylor welcomed the Order and emphasized the importance of the provision and operation of services for common use in social science, urging that the proper place for the Government's Social Survey was under the Social Science Research Council, where it would be available, not only for departmental investigations, but also for non-departmental studies.

In introducing a similar motion in the House of Commons on November 22, the Secretary of State for Education and Science, Mr. A. Crosland, said that the Department now had research projects in this field to the value of about £1 million. Neither the Order nor the Charter mentioned the Heyworth Committee's recommendation for a special board for educational research, but this did not mean that the recommendation had been rejected by the Government. They regarded it as a matter for decision by the Council itself, and, so far as special arrangements for the field of the 'built environment' were concerned, the Minister of Housing and Local Government was enthusiastic about the objective; however, he did not think that the particular form of organization recommended by the Heyworth Committee was the most likely to achieve this objective. Mr. Crosland also said that the Government thought it was too early to decide on the exact relation between the new Council and the Council on Scientific Policy and also for the Government to reach a definite decision about the amount of finance and the number of postgraduate awards. The Council would have the opportunity of making its own recommendations, and it was not proposed to decide on long-term finance until the Government had had the advice of the Council. Mr. Crosland also emphasized the importance of there being more than one source of finance for Social Science Research, and hoped that the establishment of the Council would not mean that there would be less money available for, or less interest in, other sources of funds for such research. It was not the intention of the Government that the work at universities, financed from the University Grants Committee or from other Government departments, should be limited in any way in consequence of the establishment of the Council.

In the debate, Mr. D. Price commented on the greater difficulty of isolating a situation for detailed study in social sciences than in physical sciences. This difficulty of providing or creating controlled conditions in social research meant that the quality of the people conducting such research might be even more important than in the physical and natural sciences. This was one reason why he hoped that the Research Council would give particular attention to the supply of trained research workers. He also emphasized the great importance of inter-disciplinary work, and suggested that the immediate problem was for more research in the nature of 'applied' rather than 'pure' research in this field. Mr. Price considered that this particularly applied in regional development and that industry would get a fairly rapid return from further expenditure in sponsoring applied social science research. Finally, he also urged the appointment of a Select Committee on Science and Technology to examine the reports of other Research Councils, including those of the new ones.

Mr. A. Blenkinsop, agreeing that the quality of research workers in this field was of considerable importance, directed attention to the desirability of such workers having had experience in various fields of the social sciences; these should not be exclusively academic. Mr. R. Hornby hoped that the new Council would be able to stimulate co-operation within the universities, as well as the flow of ideas for projects, and that it would have regard to the needs of other specialized institutions for finance. Mr. G. Howe emphasized the importance of wide and effective dissemination of information on social science generally. Mr. E. S. Heffer referred to the need for more social scientists in local government and in industry and also thought that all colleges of technology should have a department which encouraged the development of the social sciences. Sir Edward Boyle agreed as to the assistance which the Social Science Research Council might give to future Royal Commissions and the wider use which might be made of social science; he emphasized the danger of taking important decisions on inadequate knowledge.

Mr. R. E. Prentice, in replying on the debate for the Government, agreed that effective liaison and communication were basic essentials. The co-ordination of relations between the Council and the work being done in this field in the universities and smaller research institutions was a primary function of the Research Council. The Council had been incorporated by Royal Charter on October 29. The Heyworth Committee's recommendation regarding the Social Survey was being studied by the departments concerned. He also agreed that it was important that Parliament should discuss the work of all the Research Councils and thus help to create an informed public opinion which would facilitate the application of the results of their work.

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THE NUFFIELD FOUNDATION

THE report of the Nuffield Foundation for the year ended March 31, 1965*, which includes a tribute to Sir Hector Hetherington, opens by emphasizing once again that the two recurrent themes of the Foundation's work are need and opportunity. The first does not decrease while men remain sensitive to possibilities of knowledge and human benefits, and the second will also be present so long as those who have good ideas and the determination to pursue them will share their thoughts with the Foundation.

Allocations during the year totalled £2,045,741. Of this £205,730 was for biological research and £26,000 for other scientific research; £317,100 was allocated for medical research, £201,065 for social research, and £655,154 for education. Grants for the Commonwealth overseas totalled £182,276 and for fellowship and scholarship schemes, £188,588.

* *The Nuffield Foundation: Report for the year ended 31 March, 1965.* Pp. xlv+161. (London: The Nuffield Foundation, 1965.)

The report expresses concern that the interest of the Foundation in 'food safety' has to some extent failed to communicate the importance and urgent interest of the subject. The Foundation believes that, with the increasing use of additives in food and of chemical and physical procedures in its preparation and storage, there is a corresponding need for more understanding of their biological consequences to ensure that they are not damaging to human health. Too little is known of the long-term biological effects, whether for good or for ill, and it is this programme that is described and delimited by the term 'food safety'.

During November 1964, the Foundation held a one-day conference to examine training needs and possible remedies in the pathology of laboratory animals, and the sum of £2,500 was allocated to meet the cost of a symposium of experts in this subject to be held in April 1966. £250 annually is to be devoted towards the cost of specially arranged lectures in universities on subjects relevant to the Foundation's interest in 'food safety'. A grant of £43,000 over 3 years was made to the Department of Physiology, University of Bristol, mostly towards providing the equipment for recording and analysis of significant features of the apparently random wave-forms of the synaptic activation noise in the control of motoneurones by the brain.

A grant of £9,200 over 3 years was made to the Department of Physiology and Biochemistry, University of Southampton, for research into the trophic factors controlling nerve-muscle functions, and one of up to £8,000 for a pre-fabricated building for ecological research, in the Department of Natural History, University of Aberdeen. A further grant of £10,250, over 2 years, was made to the Department of Anatomy, University College, London, for continued research into the lipid composition of neurones. Other grants for scientific research include a capital grant of £20,000 to strengthen the working scientific fund of the International Council of Scientific Unions and a tapering grant of £6,000 over 3 years, to the Institute of Science Technology, to further its work in the education and training of science laboratory technicians.

The report attributes a decrease in applications and grants in the field of social research to a reluctance to embark on major developments before the Hayworth Committee Report was received. It is anticipated that, in consequence, a more directed approach will now be forthcoming, and emphasis is laid on the importance of drawing more people of ability into the social sciences. Although the Foundation's main concern in this field is a practical contribution to human well-being, it will also support fundamental projects which promise to give form and clarity to the social sciences and to indicate their scope and possible achievements. The Centre for Urban Studies, University College, London, has so far published six reports but it is difficult for the Centre to provide the necessary security for its research staff and carry out the continuing basic work of collecting and analysing data. The Foundation is accordingly making two grants of £20,000 over 2 years in the expectation that, by the start of the new university quinqennium in 1967, funds will be available from public sources for the work of the Centre. A grant of £40,000 was made to the Bartlett School of Architecture, University College, London, for a study of the development of Central London since 1947, and a further grant of £13,000 over 3 years was made to the Department of Architecture, University of Edinburgh, for the continuance of investigations of quantity surveying in the planning of domestic housing. A grant of £14,300 over 3 years was awarded to the Department of Sociology, University of Essex, for measurements of incapacity and disability, in a programme of research into the sociology of handicap, and a grant of £7,500 over the next 3 years was made to the Institute of Commonwealth Studies, University of Oxford, for collecting and sorting records of all kinds from those who have served overseas in former British Colonies. A grant of £23,500 was made to the Institute of Race Relations for its survey of race relations in Britain and a further grant of £30,000 to the National Institute of Economic and Social Research, for the second half of its investigation into the cost of urban redevelopment, which is to be completed by October 1966.

In education, the development of the school curriculum remained a main interest, but the Foundation is now likely to play a diminishing part in sponsoring such work. The School Mathematics Project, which has been assisted by the Foundation over a number of years, has from its start been based on the University of Southampton, and two new projects announced in the report are being sponsored and organized by appropriate university centres, rather than directly by the Foundation; the Trustees are anxious to encourage this trend. Now that the new Schools' Council for the Curriculum and Examinations has taken over the functions of the Secondary Schools' Examination Council and also assumed a national responsibility for stimulating curriculum developments in all subjects at all school levels, the Foundation is likely to turn its attention to other growing-points in education.

Apart from its continuing support for the Foreign Language Teaching Materials Project, the Foundation (in partnership with other organizations) is now sponsoring the work of a national Committee on Research and Development in Modern Languages which will act as a central co-ordinating agency, and a source of stimulus for further advance in this field. Meanwhile a further £528,000 has been set aside for the Science Teaching Project. Three 'O'-Level programmes for the General Certificate of Education in biology, chemistry, and physics were tested in 170 schools in Britain during the academic year 1964–65. Arrangements were made for public examinations to be set at 'O'-Level for pupils from the trial schools in the years up to and including 1967. A new programme was authorized during the year to develop a course for teaching science to non 'O'-Level secondary school children and, to cater for schools which wished to avoid the distinction between physics, chemistry and biology at the beginning of the 5-year 'O'-Level course, a short programme was started to develop a combined science course for the 11–13 age-group. Detailed work on science courses for the sixth form began during the year. In the Mathematics Teaching Project (5–13) a large-scale testing programme commenced in September 1965, with the feeding into the pilot schools of the draft materials for infants and first-year juniors.

A further grant of £13,000 over 3 years was made to the Communications Research Centre, University College, London, for the appointment of four teachers of English with experience at various levels for the English Language Project. A grant of £34,500 over 3 years was made to the Cambridge School Classics Project which is concerned with teaching classics in the initial stages of the school course. A grant of £8,000 was made for a detailed feasibility study of the curriculum needs of the early school-leaver, including a survey of the work at present being carried out in social studies courses for pupils of average and less than average ability. A further allocation of £125,000 until September 1966 was made for the work of the Language Teaching Materials Project in which the Spanish, Russian and German sections now have their own specialist consultative committees.

£100,000 was set aside for programmes recommended by the Committee on Research and Development in Modern Languages formed in September 1964, and sponsored jointly by the Department of Education and Science, the Scottish Education Department, the University Grants Committee, the British Council, the Federation of British Industries and the Foundation. A grant of up to £40,700 over 4 years has been made to the Language Centre, University of Essex, for linguistic analysis of contemporary

Russian, and one of £20,000 over 3 years to the Language Research Centre, Birkbeck College, London, for a scheme of research into applied linguistics and the psychology of language learning. A grant of up to £20,700 over 3 years was made to the Department of Child Life and Health, University of Edinburgh, for investigations of the development of speech in young children. Grants to the new universities include one of £63,000 over 5 years to the University of York toward the development of a Language Teaching Overseas Centre.

Grants for the Commonwealth overseas include a final grant of £15,000 over 6 years to the Plunkett Foundation for Co-operative Studies, for the development of residential training courses for staffs of agricultural co-operative departments and societies in East and West Africa. Further and final support is being given to the Economic Fellowships of the Overseas Development Institutes by a grant of £15,400 over 2 years, and one of £24,000 was made to the Institute of Education, University College of Dar es Salaam, for the building of a Science Centre. A capital grant of £27,600 was made to the University College of East Africa, University College of Makerere Medical School, to build an extension to the medical library, while a grant of £8,250 was made to support, within the Serengeti Research Project, a 3-year study of animal ecology in the Tanganyika National Parks. The Starehe Boys' Centre in Nairobi received a grant of £18,650 to provide a secondary school. A grant of £2,160 over 3 years was made to the University of Melbourne for continuation of investigations of the metabolism and water relations of Australian heath plants. A further grant of £30,000 to the University of the West Indies was partly towards providing guest-house accommodation for postgraduate visitors, and partly for the establishment of a centre for extramural studies in the Leeward and Westward Islands. One of £10,700 was made towards the capital cost of developing the Fields Studies Centre of the University of Malaya, including the provision of accommodation, and one of £25,500 for a 3-year investigation of agricultural marketing in Mauritius.

Awards for the United Kingdom in medicine and dentistry continued unchanged, but the conditions for dental research fellowships were slightly altered to facilitate a wider choice of candidates; one medical research fellowship and two ordinary fellowships were awarded and twenty-four scholarships in tropical medicine. Two scholarships and four bursaries were awarded in biology and four scholarships in sociology. Under schemes for the Commonwealth, overseas travelling fellowships for graduates for one year's study were awarded to scholars from Australia, Ceylon, India, Pakistan and New Zealand. Two of these were in medicine, eight in the natural sciences and six in the humanities and social sciences. Shorter travelling fellowships for six months were awarded to three public officials and two Trade Unionists from Canada, and nine short-term travel grants were made to senior Canadian scholars (two being in the natural sciences, three in the humanities and four in medicine). The travelling fellowship for a Dominion practitioner was last year awarded to Canada; Dominion travelling scholarships were again awarded to two farmers each from Canada, Australia and New Zealand, and one each from Rhodesia, Zambia and Kenya. The Foundation's original grant of £50,000 over 10 years to the Royal Society and Nuffield Foundation Commonwealth Bursary Scheme was renewed for a further and final period of 5 years at the same rate of £5,000 a year and, in 1964, twenty-two awards were made. So far, 330 grants have been made from the Elizabeth Nuffield Educational Fund, fifty-two being made during the year.

PUBLICATIONS OF THE WORLD HEALTH ORGANIZATION

THE World Health Organization, in accordance with its Charter, publishes a large number of authoritative books and pamphlets in various fields of public health, and in more specialized fields of medicine. The following recent publications will be of interest to a wide spectrum of workers in these fields.

The Animal Health Yearbook for 1964 is the eighth issue of the series of yearbooks bearing this title published jointly by the Food and Agriculture Organization of the United Nations, the World Health Organization and the Office Internationale des Épizooties (FAO–WHO–OIE. *Animal Health Yearbook, 1964*. Pp. vi+316. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1965. 17*s*. 6*d*.; 3.50 dollars). The present volume, like its predecessors, contains 316 large pages of detailed information about the incidence and distribution of diseases of livestock all over the world. Printed in English, French and Spanish, it also gives the Latin names of the causative agents of all the diseases listed. A triumph of clear printing and classification of the astonishing range of information collected from replies to questionnnaires sent to the veterinary services in all the countries represented, it constitutes an invaluable summary of the state of animal health all over the world. This year it includes information about the diseases of fish and bees and also an article on the livestock disease position in 1964, and this issue contains for the first time a Russian glossary compiled at the headquarters of the Food and Agriculture Organization from the veterinary literature consulted. An additional valuable feature is the reference index at the end of the volume containing all the terms applied to the various diseases listed in the tables, together with secondary titles, synonyms and causal agents in English, French and Spanish and also other accepted international nomenclatures.

Another valuable publication of the Food and Agriculture Organization is the paperback volume entitled *Fascioliasis and the Liver Fluke* (Food and Agriculture Organization of the United Nations. FAO Agricultural Studies No. 64. Pp. xxi+234. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1964. 15*s*.; 3 dollars), the publication of which has been made possible by a generous grant from Ciba, Ltd., of Basle, as a contribution to the Freedom from Hunger campaign. Written by Dr. R. L. Taylor, who, until recently, was deputy director of the Central Veterinary Laboratory of the Ministry of Agriculture at Weybridge, Surrey, this volume carries all the authority of an author whose knowledge of the subject is derived from many years of practical investigation of fascioliasis, in collaboration with his colleagues at the Weybridge laboratory. In this book, Taylor emphasizes the ecological aspects of the disease, which he regards as being a derangement of a naturally healthy relationship between grazing animals and the flukes. He presents the methods of control on farms as artificial adjustments which endeavour to re-establish a normal situation rather than to eradicate a harmful parasite. Taylor does not discuss the serious disease that this fluke may cause in man and, no doubt, he would agree that human fascioliasis is anything but a normal healthy relationship between parasite and host.

Discussing the grave losses caused among livestock by *Fasciola hepatica*, Taylor points out the great difficulty of assessing these accurately, but there is little doubt that, when severe outbreaks of fascioliasis occur, in any country, millions of sheep may be killed by it. He then describes

the anatomy of *F. hepatica*, and its biology, and in an interesting chapter he traces the development, step by step over the years, of our knowledge of the life-history of this fluke, from the first recorded reference to it by Jean de Brie in the time of Charles V of France. He then discusses in considerable detail the ecology, life-history and habitats of the intermediate host, the snail, *Lymnaea truncatula*, which is the chief intermediate host in Britain, although other species of snail are intermediate hosts in other countries. Succeeding chapters discuss the pathogenicity of *F. hepatica*, the pathology of fascioliasis, the clinical features of the acute and chronic forms of the disease, and its treatment. Next is discussed the rapidly fatal black disease of sheep, caused by *Clostridium oedematiens* infecting the liver when this has been damaged by *F. hepatica*. Another chapter discusses the disease caused by *Fasciola gigantica*, the differentiation of this species from *F. hepatica* and the various species of *Lymnaea* that are its intermediate hosts, together with their biology, the losses caused by this species in Pakistan and Uganda and methods used for control of the disease.

In a final chapter, Taylor discusses prospects for the future control of this very grave disease. A valuable bibliography concludes a volume which cannot fail to be of great value and interest to all agriculturists, especially those who are concerned with the rearing of sheep and cattle. Many readers will, no doubt, wish that it could have been issued in a more durable binding.

Aspects of Family Mental Health in Europe (World Health Organization. Public Health Papers, No. 28. Contributed by D. Buckle *et al.* Pp. 123. Geneva: World Health Organization; London: H.M.S.O., 1965. 5 Sw. francs; 8*s.* 6*d.*; 1.75 dollars), by ten European authors, has as its theme that mental illness cannot adequately be treated by confining treatment to the individual who is mentally ill, but must be dealt with in the light of the individual's social environment and of disturbances which affect other members of his family. The papers deal with the work of the School for Parents which originated in Paris and that of the Hampstead Child Therapy Clinic and with family psychotherapy as it is practised in Czechoslovakia. They demonstrate the complexity of the subject and of the various approaches being made to it in different parts of Europe.

Mass Campaigns and General Health Services, by C. L. Gonzalez (World Health Organization. Public Health Papers, No. 29. Pp. 87. Geneva: World Health Organization; London: H.M.S.O., 1965. 4 Sw. francs; 6*s.* 8*d.*; 1.25 dollars), gives the preliminary results of an investigation of this complex subject. It is so complex that it is impossible to summarize briefly the contents of this volume, but it should interest everyone who has responsibility for health services in areas in which mass campaigns against particular diseases may have to be integrated into general health services.

Nutrition and Infection—Report of a WHO Expert Committee (World Health Organization. No. 314. Pp. 30. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. francs; 3*s.* 6*d.*; 0.60 dollar) discusses the effects of malnutrition on morbidity and mortality caused by infectious disease and experimental investigations of this. Other sections of the report discuss the effect of infection on nutritional investigations, mechanisms of synergism and antagonism and the education of physicians and public health workers. Other sections offer recommendations and suggestions for research.

Special Courses for National Staff with Higher Administrative Responsibilities in the Health Services (World Health Organization. No. 311. Pp. 31. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. francs; 3*s.* 6*d.*; 0 60 dollar) gives the views of a World Health Organization Study Group on the training required by those who adopt health services as a career. Annexes give details of existing training programmes in the University of Zagreb, the School of Hygiene in the University of Toronto, the Johns Hopkins University, the National Institute of Health Administration and Education, New Delhi, and in the Staff Training Programme of WHO.

Diabetes mellitus—Report of a WHO Expert Committee (World Health Organization. No. 310. Pp. 44. Geneva: World Health Organization; London: H.M.S.O., 1965. 3 Sw. francs; 5*s.*; 1 dollar) discusses the natural history, detection, prevention and control of diabetes and the employment of diabetics, with suggestions for research on the disease.

Vol. 19, No. 11, of *WHO Chronicle* (price 1*s.* 2*d.* from H.M.S.O., P.O. Box 569, London, S.E.1) discusses hospital organization in developing countries, the health problems of adolescence, the World Health Organization's programme on nutrition and international non-proprietary names given to pharmaceutical preparations. In the "Notes and News" section there are notes on cholera outbreaks in Iran, Afghanistan and the U.S.S.R., on mycoses in Africa and on the collaboration between the World Health Organization and the London Zoo in comparative medicine.

Dependence-producing Drugs (World Health Organization. No. 312: WHO Expert Committee on Dependence-producing Drugs—Fourteenth Report. Pp. 16. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. francs; 3*s.* 6*d.*; 0.60 dollar) contains an annexe which gives a list of drugs that are under international narcotics control.

The August issue of the *WHO Chronicle* (price 1*s.* 9*d.*, from H.M.S.O., P.O. Box 569, London, S.E.1) contains a report on the Proceedings of the eighteenth World Health Assembly held in Geneva in May 1965, an article on the work of the World Health Organization in relation to population problems, and another on activities assisted by the World Health Organization in the campaign against tuberculosis, an account of the report of the World Health Organization Expert Committee on nutrition in pregnancy and lactation, together with the customary feature entitled "Notes and News" and short reviews of three World Health Organization publications. The September issue of this *Chronicle* contains articles on malaria eradication in 1964, research on cardio-vascular diseases, work on tuberculosis assisted by the World Health Organization, the chemoprophylaxis of primary tuberculosis, and a useful article on changes in health legislation in various countries in Europe, South America, India and Australia.

World Health Organization Technical Report Series No. 309 is a joint publication with the Food and Agriculture Organization entitled *Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation, Food Colours and some Antimicrobials and Antitoxidants* (World Health Organization. Eighth Report of the Joint FAO/WHO Expert Committee on Food Additives. Pp. 25. Geneva: World Health Organization; London: H.M.S.O., 1965. 2 Sw. francs; 3*s.* 6*d.*; 0.60 dollar). It emphasizes the need for international establishment of specifications for the identity and purity of food-additives, which should not only protect the consumer, but should also help the manufacturers and those who process foods.

No. 26 of the invaluable series of World Health Organization Public Health Papers, written by Prof. E. M. Blackett, of the University of Aberdeen, deals with the grave subject of domestic accidents, which, unlike the road accidents which are so often in the news, receive little publicity (World Health Organization. *Domestic Accidents*. Pp. 137. Geneva: World Health Organization; London: H.M.S.O., 1965. 6 Sw. francs; 10*s.*; 2 dollars). Yet in many countries deaths due to domestic accidents are comparable in number to those due to tuberculosis. The study of them on an international basis is complex and much of the literature about them is obscure, yet these accidents are, this publication suggests, likely to

become more and more a public health problem in the future. Control of disease and the resulting increase of the population are leading to increasing numbers of old people and children, who are particularly liable to domestic accidents. Mechanization in the home also tends to increase their number and variety.

No. 27 of the same series is entitled *Trends in the Study of Morbidity and Mortality*, and it contains articles on this subject by fourteen contributors (World Health Organization. Public Health Papers, No. 27: *Trends in the Study of Morbidity and Mortality*. Dario Curiel and thirteen other authors. Pp. 196. Geneva: World Health Organization; London: H.M.S.O., 1965. 8 Sw. francs; 13*s*. 4*d*.; 2.27 dollars). In general, the articles survey the methods now in use for obtaining statistics on morbidity and mortality in the United Kingdom, Europe, Romania, Japan and India, and the problems of making the data obtained internationally comparable. There is need for further research into methods of reporting and for the establishment of some degree of uniformity.

RESEARCH FACILITIES AVAILABLE TO INDUSTRY AND THEIR INTER-RELATION

CLOSER collaboration between industrial, governmental, academic and independent research establishments was the principal theme and objective of the symposium arranged jointly on September 14, 1965, by the Scottish Office of the Ministry of Technology and the Arthur D. Little Research Institute, at the Royal Society of Edinburgh. The seven speakers at the symposium were followed by Sir Harry Melville, who described the varied work of the newly formed Science Research Council, of which he is chairman, this lecture being the first of the Arthur D. Little 1965–66 series of Acorn Lectures on various scientific topics.

Mr. P. M. Thomas (managing director, William Beardmore and Co., Ltd.), in the chair for the morning session, directed attention to the necessity for industry to make proper use of research facilities and so be aware of the present status of any topic relevant to the particular industry and the appropriate place to which to direct problems.

Prof. Manfred Gordon (Department of Physical Chemistry, University of Strathclyde) said that his University, formerly the Royal College of Science and Technology, always had a technical bias and strong links with industry. For example, the idea of industrial liaison officers, whose purpose is to establish co-operation between an academic establishment and industry, originated in 1956 at the then Royal College of Science and Technology. The University and the National Engineering Laboratory have special relationships and that some of the National Engineering Laboratory staff are registered as Ph.D. students. Many of the University staff act as consultants, but Prof. Gordon expressed the desirability of younger members spending a few weeks of the summer vacation in industry. Strathclyde now uses closed-circuit television for teaching, which could be a valuable technique to assist firms with their training problems. The computer service at the University was also mentioned.

Dr. Magnus Pyke (manager, Glenochil Research Station, Distillers Co., Ltd.) recalled that a few years ago some firms believed in leaving the research staff alone, in the hope that profitable ideas and processes would emerge. This procedure was found very expensive and not very profitable. To-day, practical problems are chosen for the research staff. However, Dr. Pyke stressed that applied research should be tackled at an intellectual depth in order for the results to be financially worth-while and stimulating to the staff. Non-secret technical know-how should be more widely disseminated between firms by personal contacts and a new journal.

The problem of assimilating the scientific literature could, he suggested, be solved by using computers to store information which would be released by 'telephoning'. Dr. Pyke, like other speakers, deplored the gap between industry and academic establishments in Britain, a situation which does not occur to the same extent on the Continent or in the United States, and called for more industry-orientated projects for research students in order to counteract an 'ivory tower' attitude.

Mr. F. D. Penny (deputy director, National Engineering Laboratory) gave a short historical survey of Government laboratories. Initially, during the seventeenth and eighteenth centuries, these were for defence purposes; later, civil laboratories were established, the Geological Survey being founded in 1835 and the Inland Revenue Laboratory in 1842. Others, of which the National Physical Laboratory was the first in 1900, were intended to determine fundamental and applied standards. In 1915 the important White Paper setting up the Department of Scientific and Industrial Research, which represented a conscious effort by the Government, appeared to take responsibility for improving industrial progress. Research programmes were not evolved in isolation but were controlled by committees of leading academics and industrialists.

The National Engineering Laboratory was opened in 1947, and, as with other Government laboratories, the work can be classified as follows: (1) particular problems —testing and longer-term research (the latter represents 10 per cent of the total work at the National Engineering Laboratory); (2) safety, for example, tests on gas cylinders; (3) problems applicable to a class of industry but not sponsored by any firm, for example, investigations of metal fatigue; (4) special facilities—testing of pumps and turbines; (5) research in a field of industrial importance in which the Government believes there should be a greater rate of innovation; (6) standards of goods for export; (7) advisory services.

The National Engineering Laboratory issues reports (8,000 total so far) and papers in scientific journals, welcomes visitors and holds open days. The total staff is 800, and they spend about half their time on research and half on application.

Dr. H. P. Stout (director of research, British Jute Trade Research Association) pointed out how a research association benefits especially the smaller firms of an industry, since such firms usually cannot afford adequate scientific staff and, where facilities are available, may have difficulty in the recruitment of staff because there is no suitable environment for them. The research association can provide a scientific atmosphere, but there is still the problem of getting *en rapport* with the firms. Commenting on the statement sometimes heard that arts graduates were more use as management material than science graduates, he thought this was usually the result of the scientist being given a specialist function and not acquiring the broad training and experience which was given to the arts man. Dr. Stout noted that innovation costs money and manufacturers are unwilling to develop ideas where the market is small, even after the research association has done the 'spade work'. He instanced new weaving machinery developed

for the woollen and cotton industry which had not been adapted for the jute industry because of its comparatively small size.

Opening the afternoon session, the chairman, Dr. E. A. C. Chamberlain (divisional chief scientist, National Coal Board), questioned whether Britain was really research-oriented and said that Britain tends to ignore the real achievements which have been made. Personal contact is essential to break down the barriers of ignorance, laziness and snobbery between scientists, technologists and industrialists, and he therefore welcomed this symposium.

Dr. F. N. Woodward (director, Arthur D. Little Research Institute) recalled the Jubilee Memorial Lecture given to the Society of Chemical Industry by Prof. J. W. Mitchell, in which he depicted the four sources of scientific information, that is, industry, academic institutions, Government laboratories and sponsored research institutes, as standing at the corners of a tetrahedron. In the United States, industry uses the four sources equally and there is free movement of people and ideas between them—to which is ascribed the fact that industry in the United States has a growth-rate twice that in Britain. He went on to describe the growth of sponsored research institutes, particularly those of Arthur D. Little, first in the United States and later in Europe. Enumerating the reasons for the rapid growth of sponsored research institutes, Dr. Woodward cited their independence; the fact that they are professional business organizations without other ties such as teaching; and their versatility, speed of action and flexibility through a multi-disciplinary staff (which includes management skills). The sponsored research institute obviates the need for a firm to augment its own research facilities to deal with special problems. They have close ties with academic establishments and use academic consultants regularly. While working for a firm they make a point of trying to become a temporary but integral part of the management.

Prof. W. E. J. Farvis (Department of Electrical Engineering, University of Edinburgh), while speaking of the relationships between industry and university engineering departments, complained about the difficulty of deciding on suitable student curricula in view of the changing face of the engineering industry. He supported the concept of colleges of advanced technology and the Diploma in Technology, and deplored the present desire, partly based on snobbery, for such colleges to become universities. Prof. Farvis wished to see a better liaison and understanding between industry and academies. Thus, industry could help engineering departments and themselves by promoting research projects and releasing staff to help teach design. Next year, in the Department of Electrical Engineering at Edinburgh, design experiments will be included in the curriculum to help the student to feel that he is part of industry as well as of a university. Prof. Farvis would like suggestions from firms as to small problems, possibly of dubious solutions. Little would be lost financially if there were no satisfactory outcome, but meanwhile students were being trained while worrying at a practical problem in close contact with industry.

W. S. Robertson (chief executive, Scottish Council for Development and Industry) spoke fluently about the past, present and future industrial situations in Scotland. Industrial development in Scotland first 'sheared off' from the rest of the world during 1880–90 although the effects were scarcely noticeable until the 1920's. Until 25 years ago, industry in Scotland suffered from two weaknesses: (1) there was no production of engineering components in quantity; (2) there was little applied research. Scotland had only a weak technological push, there were many gaps in the industrial scene, and few opportunities for graduates. Since 1925 much progresss has been made in getting quantity production and fairly good progress in applied research. Mr. Robertson said that distribution as well as number should be considered with regard to graduates. He defined:

Expansive force = No. of scientists and engineers multiplied by communications

The pattern of industry is closely associated with the distribution of Government defence spending. Thus, in south-east England there are many Government research laboratories; once these had been established, industry rose up to feed them. The benefits from the National Engineering Laboratory, founded in 1947 at East Kilbride, have begun to mature and Mr. Robertson predicted that this area of Scotland will become one of the best engineering centres in Europe. Such experience suggests the need for similar Government spending to stimulate industry in other parts of Scotland and the United Kingdom. For a thriving research community, communications, both physical and intellectual, are vital, and he instanced the growth of the electronics industry in the area around Edinburgh, well served by air and rail, which is expanding at a higher rate than anywhere else in Britain. The air service north of the industrial belt, however, is poor, and no direct communication exists between Scotland and the European Continent.

Sir Harry Melville (chairman, Science Research Council), speaking on "Collaboration between Industry and Government in Fostering Scientific Research", began by asking for better communications and exchange of personnel between centres of learning, industry and Government laboratories. The Science Research Council, inaugurated in April 1965 following the replacement of the Department of Scientific and Industrial Research, has promoted, or will promote, research studentships, fellowships and industrial fellowships at academic institutions, and make grants towards research work undertaken in a research association, sponsored research institute or industrial laboratory, provided the work is of outstanding public and scientific interest and the recipient of the grant uniquely qualified to undertake it.

The Science Research Council is paying about £4 million a year in grants to universities for expensive equipment. Usually the Council stipulates that the equipment be shared with other departments and available to outside users. An outstanding example is the Scottish Universities Reactor, which cost £500,000. However, some equipment, such as particle accelerators, costing up to £10 million, is of little direct use to industry.

In the United States the same proportion of the national income is spent on research as in Britain but the total is ten times as great. Hence, while in the United States some of the national income can reasonably be spent on pure research from which no practical results are expected, Britain should not, he believes, do so as a rule. The Science Research Council, however, does make exceptions, one of which is support for a very expensive space science programme.

During discussions several deprecated the 'gap' between the industrial and academic spheres. It was generally agreed to be mutually advantageous for institutions of higher learning to undertake problems suggested and partly supported by industry and Government departments; however, Prof. Farvis quoted Sir Edward Appleton's remark that research, even if apparently trivial, serves to train the student into thinking in an original manner—which is what is required of the graduate in industry. There is need to attract creative minds into industry. The need for personal communication was emphasized: research workers should go out to non-scientists to persuade them of the desirability and application of particular research ideas.

We look forward to translation of the sentiments of the symposium into action.

A. C. Low
A. J. Rostron

FUNDAMENTAL CANCER RESEARCH

THE nineteenth annual symposium on "Fundamental Cancer Research", sponsored by the M.D. Anderson Hospital and Tumour Institute of the University of Texas, was held at Houston, Texas, during March 4–6, 1965, the subject being "Development and Metabolic Control Mechanisms and Neoplasia". The sessions at the Symposium dealt with the following topics: "Biosynthesis and Control Mechanisms", "Molecular Basis of Early Development", "Molecular Basis of Later Development and Control" and "Comparative Studies of Control Mechanisms in Normal and Neoplastic Tissues". Several papers dealt with various aspects of the currently accepted sequence of events in biosynthesis, namely, DNA → RNA → protein, and especially aspects relating to development and differentiation.

R. M. S. Smellie (Glasgow) gave a review of the enzymatic synthesis of nucleic acids in bacteria and animal tissues, describing the various RNA and DNA polymerases involved. He pointed out that DNA is present in animal cells as a complex with protein, and that the mechanism by which it directs synthesis of new DNA remains to be determined. One function now generally assigned to histones is that of acting as gene repressors and they would accordingly play an important part in differentiation and development. Since different RNA fractions of the cell differ in their base composition, it is possible that the action of RNA polymerase is restricted to transcribing certain DNA regions in the nucleus. One approach to the study of these problems is by investigations on the synthesis of RNA in isolated nuclei and nucleoli the structural organization of which is maintained as intact as possible. R. B. Hurlbert (Houston), working with such systems, has presented evidence that the base composition of the newly formed labelled RNA can be altered by pretreatment of nuclei with different fractions of histone, or with trypsin to remove part of the histone originally present. The synthesis of the labelled RNA is inhibited by actinomycin *D*, indicating that the RNA is formed on a DNA template.

In the past few years a new approach has been developed in the study of the functions of hormones involved in development and growth. It is thought that their action is mediated through cellular mechanisms regulating nucleic acid and protein synthesis in their target tissues. It is possible that, in response to hormones, particular types of *m*RNA are formed; these then direct the coding of particular proteins, including enzymes which bring about specific developmental changes. This was discussed by J. R. Tata (London), who has shown that RNA polymerase of liver nuclei is increased by administration of thyroid and growth hormones. The effect is additive, indicating that each hormone enhances the formation of different fractions of RNA, including *r*RNA and *m*RNA. Also, incorporation of amino-acids into protein by mitochondria and microsomes is stimulated by thyroid hormone. Another example of the effect of thyroid hormone which was mentioned is the induced metamorphosis in the frog. The effects of other hormones involved in development were also discussed. In insect metamorphosis, the hormone ecdysone controls new differentiation processes by regulating the activity of specific genes involved in these processes (U. Clever, Lafayette, Ind.). A similar conclusion is arrived at also from the work of J. D. Wilson (Dallas, Texas) on the binding of [^{3}H]testosterone to chromatin fragments of nuclei from duck preen glands; the hormone binds to the fragments which are the major sites of synthesis of RNA in the nucleus. S. Cohen (Nashville, Tennessee) presented work on the isolation from mouse salivary gland of a protein of low molecular weight which stimulates epidermal growth. The stimulation of RNA and DNA synthesis in organ culture by this factor was also demonstrable.

Comparison of *m*RNA templates in rat liver and hepatomata were discussed by H. C. Pitot (Madison, Wisconsin). Differences in the lifetime of templates for synthesis of enzymes concerned in amino-acid degradation were stated to occur in liver and hepatomata. H. G. Wittmann (Tübingen) reported work that is being carried out on the correlation between nucleotide changes in an *m*RNA and the resulting amino-acid replacements in the protein synthesized. This is done in tobacco mosaic virus RNA and its coat protein. An investigation of mutants of the virus obtained by treatment with mutagens has been carried out.

Investigations of the RNA and ribosome content of the unfertilized amphibian egg and embryo by D. D. Brown (Baltimore, Maryland) have shown that, while ribosomes are present mainly as monosomes in the unfertilized egg, polysomes are predominant in the early stages of the developing embryo, indicating that either more *m*RNA is formed or that its attachment to ribosomes is then initiated. Also the *s*RNA content of the egg is extremely small —possibly a further factor controlling protein synthesis. Modification of *s*RNA, which acts as an adaptor in protein synthesis on polysomes, may occur prior to differentiation, as shown by the work of N. Sueoka (Princeton, N.J.), who found that, as a result of infection of *Escherichia coli* with phage, a modified type of leucyl-*s*RNA is formed. S. Penman (New York) reported work on the relationship between protein synthesis and lipid metabolism in HeLa cells infected with polio virus. The polysomes of infected cells are found attached to lipoprotein membranes in contrast to those of normal cells. The infection is also accompanied by a stimulation of the lipid metabolism of the cell.

In a cell-free system *in vitro*, protein synthesis can be investigated by the rate of incorporation of labelled amino-acids; the presence of the necessary factors can thus be verified. The attachment of ribosomes to *m*RNA to form polysomes and the binding of aminoacyl-*s*RNA are prerequisites for polypeptide synthesis. The role of several factors involved in these processes, including enzymatic ones, has been discussed by R. S. Schweet (Lexington, Kentucky) and by H. Noll (Evanston, Illinois). The interchangeability of the various fractions from Novikoff tumour, rat liver and *E. coli* in such *in vitro* systems has been studied by A. C. Griffin (Houston). The components of the systems included amino-acid-activating synthetases, ribosomes, transfer enzyme fractions, *s*RNA and [^{14}C]aminoacyl-*s*RNA. It is interesting that, in general, a much greater interchangeability was observed between the components of tumour and liver than between those of *E. coli* and the mammalian tissues.

Aspects of metabolic regulations at the enzymatic and metabolic levels were discussed at the Symposium. Since the discovery, by Monod and his co-workers, of allosteric effects, many enzymes which show these properties have been described. Some enzymes exist in two configurations while others occur in sub-units which show reversible association and dissociation. A discussion of the characteristics, kinetics and behaviour of such enzymes towards substrates and inhibitors as compared with those of the more general type of enzyme was given

by C. Frieden (St. Louis, Missouri). Regulation of substrate concentration in the cell is another factor in control of biochemical reactions. Injection of creatine into chick embryo inhibits the endogenous formation of creatine by repressing the first enzyme involved in its biosynthesis. This inhibition is specific to creatine since its structural analogues are not effective (J. B. Walker, Houston). A similar negative feed-back control is found in the case of cholesterol synthesis by normal and regenerating liver. However, it is interesting that such a feed-back system is absent in all spontaneous and induced hepatomas tested (M. D. Siperstein, Dallas). Changes in the levels of substrates used as building-blocks in the synthesis of polysaccharides and in the activities of the enzymes concerned, and their correlation with different stages of differentiation of the slime mould, were discussed by B. E. Wright (Boston, Massachusetts). In another contribution, W. J. Rutter (Urbana, Illinois) discussed the two variants of fructose-diphosphate aldolase that have been found in animal tissues. During the development of the embryo there is a change-over from the predominance of one to that of the other. Two different metabolic roles are suggested for the two enzymes. Since the enzymes have distinct differences immunologically and in their fingerprint patterns, it is possible that they are coded by separate regions on the genome at different stages of cellular differentiation.

The Bertner Foundation Award for Outstanding Achievement in the Field of Cancer Research was presented to Prof. E. Chargaff (New York), who gave the Bertner Foundation Lecture at the Symposium on the topic: "On the Biological Consequences of Base-Pairing in Nucleic Acids". He discussed problems relating to the study of the primary structure of DNA. Although the regularities in the base composition of DNA apply to the whole DNA molecule, it is not known how far this is true of any given segment of the molecule. Several chemical methods for partial degradation of DNA have been developed. Clusters of nucleotides from the DNA chain can thus be obtained and identified and this may constitute a valuable approach to base sequence studies.

S. Itzhaki*

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POSSIBLE STRUCTURES FOR TRANSFER RIBONUCLEIC ACID: A TRIPLE-STRANDED MODEL

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ALTHOUGH considerable interest has been shown in the ribonucleic acid species called soluble, acceptor or transfer RNA (*t*RNA)[1] ever since its discovery[2,3], few concrete suggestions have appeared as to its structure. It is now believed from hydrodynamic measurements, X-ray investigations and electron micrographs[4] that *t*RNA is a rather rigid rod, although the evidence is not too convincing since these techniques are presently not refined enough to settle this point. Fresco *et al.*[5] first summarized the experimental evidence existing and brought forward two structures which are the essential basis of all models suggested to date. A chain bent back on itself in the form of a hairpin to form a helix, and a model with two hairpins joined together (V-model) were proposed. These authors believed that the helices in either model were interrupted to a varying degree by loops. A single completely helical (DNA-like) hairpin was also proposed[6-8]. Brown *et al.*[9] also envisaged the possibility that the hairpin and V model could be interchangeable. Sequence studies on unfractionated *t*RNA[10] led to a model only about 70 per cent helical where the bend was expanded to a large loop of about 15–20 nucleotides accommodating the anticodon and the rare bases. Since by definition *t*RNA has two distinct properties, that is accepting and transferring amino-acids, and since the phosphate backbone will provide little specificity for recognition, two non-helical regions should be necessary which might or might not be separated by helical regions. Novelli and others[11] suggested distinct acceptor and transfer sites, and recent work by Weill *et al.*[12] further supports the concept of two different operational sites, difficult to reconcile with the hairpin model, although evidence to the contrary has been presented too[13,14]. Spectral studies by Felsenfeld and Cantoni[15] point to a clustering of *A–U* and *G–C* pairs in serine specific *t*RNA, similar to previous findings of Fresco[16], who first reported two-step melting profiles for amino-acid specific *t*RNAs. From difference spectra studies on these purified *t*RNAs it was inferred[17] that each molecule contained two helical regions which melted separately and co-operatively. These data suggested that the ratio of *A–U* to *G–C* pairs was always considerably lower in the higher melting region.

All these reports have centred on a highly helical double-stranded structure of the Watson–Crick type, although no rigorous evidence has been presented so far for or against double strandedness in *t*RNA. In this article a triple-stranded model for *t*RNA will be offered to emphasize that other than double-stranded structures cannot be ruled out *a priori* at present. Evidence will be brought forward which will demonstrate the feasibility, advantages and disadvantages of such a structure as compared with various double helical models.

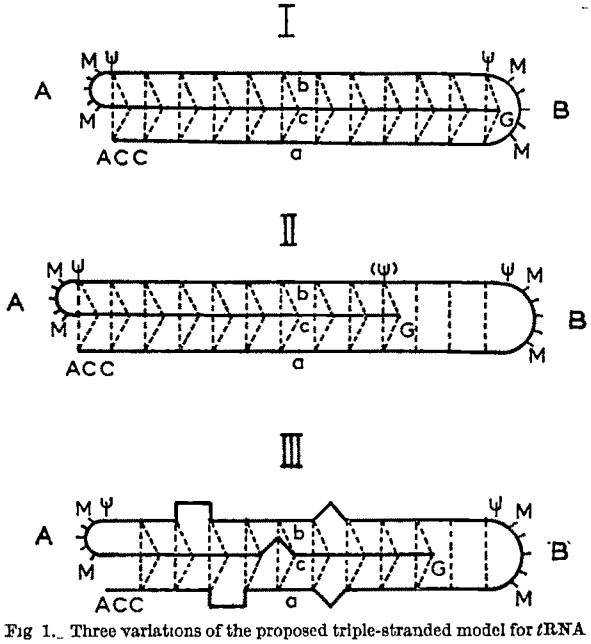


Fig 1. Three variations of the proposed triple-stranded model for *t*RNA

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The possibilities for a triple-stranded model for *t*RNA are shown schematically in Fig. 1. Model I involves three equally long strands, hydrogen bonded to each other and connected with each other by two loops of at least three but probably not more than five or six nucleotides. The *Gp* end of the molecule could be hydrogen bonded too, as well as the *pCpCpA* amino-acid acceptor terminal. Neither, however, needs to be bonded. This would place the amino-acid acceptor end in close vicinity of the loop *A*, which could serve as the attachment and recognition site for the amino-acid RNA synthetase.

The second loop *B* would contain the anticodon (either a triplet[18,19] or a doublet[20]) to attach to the messenger RNA. In the present model the methylated purines are placed on the first and last position of each loop (*M*, Fig. 1), where they could serve as punctuation bases because of their incapacity to form hydrogen bonds[21]. Pseudouridine residues, because of their strong hydrogen-bonding capacities[22], are pictured to be on the ends of the strands in the helix (ψ, Fig. 1), acting like clamps and holding together the rather short helices of 20–30 triples. ψ–*U* and the methylated purines would thus be immediate neighbours in the sequence. It is interesting to note that several investigators[23] have found oligonucleotides containing one ψ–*U* residue and one methylated purine. In either model the rare bases would occur in the centre third of the molecule in agreement with the results of Nihei and Cantoni[24]. The completely triple-stranded model I should be considered as a special case of the more general model II.

In model II one strand is shorter than the two others, so that only part of the helix is triple stranded, while the rest is double stranded. The two loops as well as other features are essentially similar to model I. Another ψ–*U* residue might be pictured on strand *B* close to the area where the *Gp* terminal could be bonded. It is clear that looping out in either one of the three strands could be envisaged to accommodate non-fitting bases[25], as depicted on model III.

Either of these models could serve as the 'adaptor'[2], combining the properties suggested for the double-stranded models for the attachment to the messenger RNA with the additional feature that the acceptor site of the model will also be specified to accommodate the enzyme for the attachment of the amino-acid to the *t*RNA. This would also be the cause with the double-stranded V-shaped model. However, the latter would not be a single rigid rod, since its centre loop will permit free rotation of the two arms. The second loop would also be rather far away from the amino-acid accepting end, except if the centre loop would serve as the attachment or coding site[6]. One of the main advantages of the present model is its compactness, which would render it less susceptible to attack and degradation.

Several years ago Stent[26] and Zubay[27] had already shown in their investigation of the possible arrangement of messenger RNA during its synthesis on DNA that triple-stranded nucleic acid helices are feasible on spatial grounds. The fact that a single strand of RNA can be accommodated in the large groove of the Watson–Crick helix with ample possibility for hydrogen bonding to the already existing base pairs without distortion of their arrangement to form triples seems unlikely to be fortuitous in Nature. Such a scheme can essentially be applied to the present triple-stranded model (substituting *U* for *T*). Zubay[27] suggested two models, each involving four different triples. Each triple consists of a Watson–Crick base pair plus an additional base in the 'third strand'. They are summarized in Table 1.

Table 1. BASE TRIPLES
(After Zubay (ref. 27))

Model *A*		Model *B*	
Watson–Crick pair	Third strand	Watson–Crick pair	Third strand
U+A	*C*	*U+A*	*A*
A+U	*G*	*A+U*	*U*
C+G	*U*	*C+G*	*G*
G+C	*A*	*G+C*	*C*

Although there is experimental evidence from polynucleotide studies for only two of the complexes of model *B*[28,29] so far, model *A* cannot be excluded *a priori*.

After melting of the third strand a double helix of about 20 base pairs will remain[30]. Either strand *A* or strand *C* in Fig. 1 could be topologically the third strand. *A priori*, there seems to be no reason to assume that, if, for example, the acceptor strand *A* were the third strand in one amino-acid specific *t*RNA, it should not be so in all types of *t*RNA. The experiments of Monier and Grunberg-Manago[31] point indeed to a structural differentiation among different *t*RNAs.

The difference spectra studies of Fresco *et al.*[17] make model I less probable. Three results of this work must be taken into account: (*a*) the co-operativeness of the melting of each region (which seems to be unequivocally proven by these experiments); (*b*) the length of each region is not equal; (*c*) there are less *A–U* containing complexes (pairs or triples) in the higher melting region. This latter region seems to be always shorter in the three types of *t*RNA studied (between 30 and 45 of the total helix) than the lower melting one. This, however, need not necessarily be true for all other *t*RNAs. The absolute molar extinctions of the difference spectra of the various transitions of the *G–C* series are at present not sufficiently exactly known to allow any definite conclusions so far as strandedness is concerned. In addition the shapes of the difference spectra between the transitions 2 to 1 and 3 to 2 strands are quite similar[28,29], which could reconcile these results even with model I. In addition, the additivity of component difference spectra which seem well documented for long chains of DNA[17] and RNA[32] might not necessarily hold exactly for short RNA helices. Model II would be in agreement with these findings.

There might also be another, at first sight less attractive, possibility for the melting of the third strand which will not always lead to a Watson–Crick type double helix. Donohue[33] showed that 29 different base pairs are possible, several of which could appear in a helix with Watson–Crick dimensions. Some distortions of the bases would, however, be necessary to form a double helix after removal of the third strand. Such unconventional base pairs would probably be present only in small amounts. The difference spectra studies of Fresco[16,17] exclude extensive bindings of this kind. Fresco and Alberts[25] also showed that looping out would be the preferential arrangement for non-complementary bases. Recent work by Bautz and Bautz[34], however, suggests that non-complementary bases could as well be accommodated simply by stacking in the helix, without hydrogen bonding. Model-building studies[35] make several other triples quite possible which do not have a Watson–Crick base pair as basis, and might even involve other bases like I. It is clearly not to be expected that difference spectra studies will distinguish single pair or triple arrangements of this type.

All macromolecular and biological features of *t*RNA known so far can equally well be applied to the suggested triple-stranded model as to the double-stranded forms. There are, however, several findings which would be difficult to reconcile with a double-stranded model, but would be expected in a triple helical structure.

(1) The reluctance of polyribonucleotides to form double-stranded structures makes heavy demands on a double helical model of *t*RNA. It has been shown that mixtures of poly *A* and poly *U* can form both double- and triple-stranded helices, depending on the conditions[16,28]. In the *G–C* series only recently attempts to form a double-stranded structure were successful[29]. A triple-stranded complex poly (*G+C+C*) will be formed exclusively, however, if short chains are involved in the helix formation. Helices of poly *X* with poly *A* or poly *U* are only triple-stranded, as well as all complexes involving 5-substituted poly *U* with poly *A*[36]. Poly ψ*U* seems

to form an unusually strong triple helix with poly *A* [22] and probably also with other homopolymers. Recently, however, the RNA of Rheo virus has been found to be double-stranded and several reports on double-stranded RNA have appeared since[37–39].

(2) Two-step heating curves[16] would be expected for the triple-stranded models of *t*RNA. The implications of the difference spectra studies[17] have been discussed here.

(3) Holley *et al.*[40] and Cantoni *et al.*[41] have shown that the base composition and sequence of purified amino-acid specific *t*RNAs does not always reflect a complete Watson–Crick scheme. The recently published complete sequence of alanyl-*t*RNA shows clearly that only a loop-containing model[40] could reconcile the sequence with a Watson–Crick double helix. It can be accounted for in terms of a triple-stranded structure with a minimum of non-helical bases[35].

(4) Marciello and Zubay[42] examined the reaction of formaldehyde with the amino groups of *A*, *G* and *C* of *t*RNA which do not react, if they are bonded in a helix, as a function of temperature. Additional attacking sites were freed at lower temperatures because of the denaturing effect of formaldehyde. The extent of the reaction increased stepwise and it was concluded that there are three groups of bases according to their reactivity. The authors estimated that the last group (the least reactive and strongest bound bases) comprised about two-thirds of the total bases or about 24 base pairs, which is in excellent agreement with the number of base pairs and the relation to the total molecule for the second double-stranded region in the present model[30]. A V-shaped model with one longer, *G–C* rich branch would also account for this result.

(5) The fact that only recently have satisfactory X-ray diffraction patterns[7] of what supposedly was *t*RNA [38] been obtained, and this only after special pre-treatment[8] which involved chain breakage, clearly shows that *t*RNA can be obtained in a DNA-like structure only under very special conditions, if at all, while several other, double-stranded RNAs [37] have given very satisfactory X-ray pictures. Unfortunately the only existing X-ray data on triple-stranded polynucleotides (poly I) are rather inconclusive[43]. It is clearly evident, however, that all other physico-chemical techniques, except very refined X-ray work, will not solve the problem.

(6) Monier and Grunberg-Manago[31] showed that polynucleotide phosphorylase (PNPase) attacked unfractionated *t*RNA to only about 30 per cent at 37 degrees. The acceptor activity for several amino-acids was unchanged, indicating that these *t*RNAs were not attacked at all. This finding is apparently incompatible with a non-hydrogen-bonded *pCpCpA* amino-acid acceptor end, since PNPase should remove these three nucleotides first on all molecules, if they are not hydrogen bonded. Only the V-shaped model[5,8] could account for this finding among the double-stranded models. In a triple-stranded model attack by PNPase will be possible only if the *pCpCpA* carrying branch *A* is the third (lower melting) strand. If strand *C* (with the *Gp* terminal) will melt lower, no attack by PNPase would be possible, since this enzyme does not act on the 5′-phosphate end of the polynucleotide chain. Such *t*RNAs should be resistant to the action of this enzyme up to about 60°, where in the cases tested so far[16] the second helical region will start to melt, thus freeing the acceptor terminal too, which now can be attacked by the enzyme. When phosphorolysis is carried out at 60°, complete digestion is indeed obtained[44].

From the foregoing it is clear that a triple-stranded model for *t*RNA is feasible and cannot be excluded *a priori*. It should, however, be pointed out that, although less attractive, even a double-helical structure could form a triple-stranded arrangement under suitable conditions, and vice versa, if both schemes of Zubay[27] (and possibly the base pairs of Donohue[33]) are considered. Such changes in conformation could possibly happen during the transition *in vivo–in vitro*, particularly since the formation of an artefact is known to occur in the case of TMV–RNA, as pointed out by Timasheff *et al.*[45]. A change in conformation of this kind might also distinguish charged from uncharged *t*RNA molecules. Although no differences in molecular weight (determined hydrodynamically) have been found[46], there is some, admittedly sketchy, evidence that *t*RNA might have different shapes under different conditions[47]. Here again, only X-ray techniques can be invoked to settle the question. Therefore, even a complete nucleotide sequence[40] of an amino-acid specific *t*RNA will not clear the problem of *in vivo* secondary structure. In addition to the several double-stranded models proposed by Holley *et al.*[40] (which incidentally do involve some base pairs other than *A–U* and *G–C*), at least two rather satisfactory three-stranded structures can be constructed with a few loops and little complexing other than in the schemes of Zubay[35].

Of several double-stranded models suggested the V-model or some variation of it[5,8] has the most experimental support. Particularly an arrangement where the anticodon triplet is envisaged between the two branches[7] would also account for most of the results in the literature.

Considering the aforementioned results, triple-stranded arrangements could also be involved in other conformational problems of RNAs. Although double-strandedness has now been well documented for Rheo virus[39] and several other RNAs [37], triple-strandedness might be invoked for the structure of the RNA of spherical viruses and of ribosomes where close packing would be favoured by stabilized hydrogen-bonded structures. Another case which might involve triple-stranded hydrogen bonding is the 'three body problem' ribosomes–messenger–*t*RNA. Since, as shown by Zubay[27], for any base pair more than one triple could be possible, the third base could be any one of the four bases (Table 1). This might be a clue to the degeneracy of the genetic code.

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[1] The following abbreviations are used: *t*RNA—transfer (soluble) ribonucleic acid; *A*, *G*, *U*, *C*, *T*—the five common bases; I—hypoxanthine; *ψU*—pseudouridine; 5-met-*C*—5-methyl-cytosine, *A–U* and *G–C*—the Watson–Crick base pairs; PNPase—polynucleotide phosphorylase; TMV—tobacco mosaic virus.

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[18] In contrast to doublet and triplet which define an arrangement of two or three nucleotides, respectively, along the helix axis (or the ribose-phosphate backbone), the terms 'pairs' and 'triples' are used to designate (hydrogen-bonded) arrangements in a plane perpendicular to the helix axis.

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[30] Assuming an average molecular weight of 350 per nucleotide residue, model I would give three branches of about 24 nucleotides (mol wt 8,500) each, plus two loops of five nucleotides (mol. wt. 1,800) each, totalling a molecular weight of about 29,000. In Model II two branches with 27 nucleotides (mol wt. 9,500), one branch with 15 nucleotides (mol. wt. 5,000) and two loops with five nucleotides (mol. wt 1,800) each would add up to about 28,000 mol. wt Similar considerations hold for the loop containing model III. It should be pointed out that models II and III could be different in different *t*RNAs, for example, two branches with about 30 residues (mol. wt. 11,500), one strand with about 10 residues and the two loops (mol wt 3,500), totalling about 29,000 mol. wt
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GENETICALLY DETERMINED VARIATION OF ADENYLATE KINASE IN MAN

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ADENYLATE kinase (AK), also known as myokinase, is a phosphotransferase (EC 2.7.4.3) which catalyses the reversible reaction:

$$2\ ADP \rightleftharpoons ATP + AMP$$

The enzyme has mainly been studied in muscle[1]. However, it is also known to occur in other tissues[2], and red cells in particular have been found to show considerable activity[3–5]. We describe in the present article a procedure for the detection of the enzyme after starch-gel electrophoresis, and report the results obtained with human red cell lysates and muscle extracts. It has been found that several isoenzyme components with adenylate kinase activity occur, and that there are also person to person differences in the pattern of components present. These differences are genetically determined.

Horizontal starch-gel electrophoresis was carried out at 10 V/cm for 4 h in the laboratory, using metal cooling plates with circulating tap water. Samples were examined at *p*H 7·0 using a discontinuous buffer system (gel buffer 0·005 M in histidine, bridge buffer 0·41 M in citric acid: both adjusted to *p*H 7·0 with sodium hydroxide). The inserts were made from Whatman No. 3 filter paper. Following electrophoresis the gels were sliced and then covered with a 0·75 per cent agar solution at 45° C made in 0·1 M *tris* buffer *p*H 8·0 and containing glucose 10 mM, magnesium chloride 20 mM, adenosine diphosphate (ADP) 1·0 mM, nicotinamide adenine dinucleotide phosphate (NADP) 0·4 mM, phenazine methosulphate (PMS) 0·012 per cent, tetrazolium salt MTT (ref. 6) 0·012 per cent, glucose-6-phosphate dehydrogenase (G6PD) 0·04 units/ml., and hexokinase 0·08 units/ml. The agar 'overlay' was allowed to set and then the gel was incubated at 37° C for up to 2 h. The reaction mixture was designed to locate AK according to the reaction sequence shown in Fig. 1. At the sites of AK activity ADP is converted into AMP and ATP. The ATP so formed reacts with glucose in the presence of hexokinase to produce ADP and glucose-6-phosphate (G6P). The latter is oxidized by G6PD to 6-phosphogluconate (6PG) with the concomitant reduction of NADP. The reduced NADP in the presence of PMS, which is thought to act as an electron carrier, causes the reduction of MTT to give a blue-coloured insoluble formazan which is thus deposited at the sites of AK activity.

Red cell lysates (prepared by mixing one volume of packed cells with two volumes of water) from a large number of different individuals have been examined by this procedure. Three distinct types of electrophoretic pattern have been recognized (Fig. 2), and they will be referred to as AK 1, AK 2–1, and AK 2. About 90 per cent of individuals in the English population have the AK 1 phenotype and about 10 per cent the AK 2–1 phenotype. AK 2 is very uncommon, and so far only a few individuals with this phenotype have been identified. In AK 1 two major components are seen. The more active of these does not migrate appreciably at *p*H 7·0, while the other is negatively charged and migrates towards the anode. Besides these, at least two, relatively much less active, components also occur and they migrate

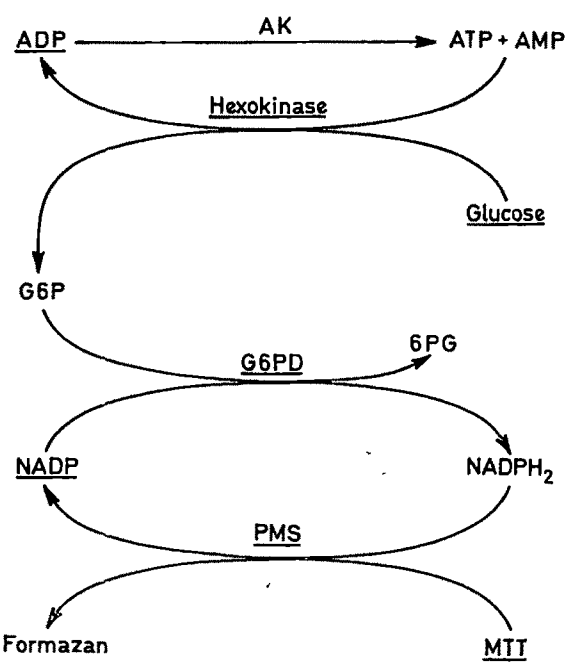


Fig. 1. Sequence of reactions for detection of adenylate kinase following starch gel electrophoresis. In this system ADP is used as substrate, and the other components underlined are contained in the reaction mixture

even more rapidly towards the anode. In AK 2–1 components with mobilities of each of those present in AK 1 are found. However, there is in addition a further major component which is positively charged and migrates towards the cathode. This component is somewhat less active than the component present on the origin, but more active than any of the anodically migrating components. In AK 2 most of the activity is concentrated in a single positively charged component which has the same mobility as the cathodically migrating component of AK 2–1. Other components with mobilities similar to those seen in AK 2–1 are also present, but they are relatively very much less active. Artificial mixtures of equal volumes of haemolysate of type AK 1 and AK 2 give an electrophoretic pattern indistinguishable from AK 2–1. It has also been shown using serially diluted haemolysates that the component on the origin and the main anodically moving component present in AK 2–1 are on the average appreciably less active than the components with similar mobility in AK 1.

Adenylate kinase in skeletal and heart muscle has also been examined by this procedure. The muscle samples were obtained *post mortem*. Extracts were prepared by homogenizing in an equal volume of water and, after centrifuging, diluting the supernatant 1 in 10 with buffer. All the zones of activity seen in the haemolysates were observed in the muscle extracts. Furthermore, the distinctive AK 1 and AK 2–1 patterns were found in muscle samples obtained from different individuals. In every case the AK phenotype observed in the muscle extract was the same as the AK phenotype found in red cells from the same individual. The muscle extracts utilized for electrophoresis were generally more active than the haemolysates, and they usually showed several additional weak zones of activity migrating relatively rapidly towards the anode. Similar zones have occasionally been observed with haemolysates.

Evidence that the zones of activity seen under these conditions are indeed attributable to adenylate kinase activity may be summarized as follows:

(1) If ADP is omitted from the reaction mixture none of the zones develops. Increasing the concentration of ADP in the reaction mixture up to at least 10 mM results in an increasing rate of development of the zones.

(2) The reaction catalysed by adenylate kinase is freely reversible and it has proved possible to detect the zones of activity using ATP and AMP as substrates instead of ADP. For this purpose a 0·75 per cent agar solution at 45° C made in 0·1 M *tris* buffer and containing ATP 1·0 mM, AMP 1·0 mM, reduced nicotinamide adenine dinucleotide ($NADH_2$) 0·3 mM, phosphoenolpyruvate (PEP) 1·0 mM, potassium chloride 0·1 M, magnesium chloride 0·04 M, lactic dehydrogenase (LDH) *c.* 1·0 units/ml., pyruvate kinase *c.* 0·1 units/ml., is poured over the starch gel, allowed to set and then incubated for up

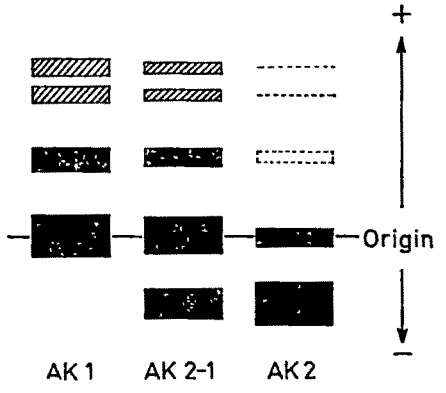


Fig. 2. Diagram showing electrophoretic patterns of three different human adenylate kinase phenotypes

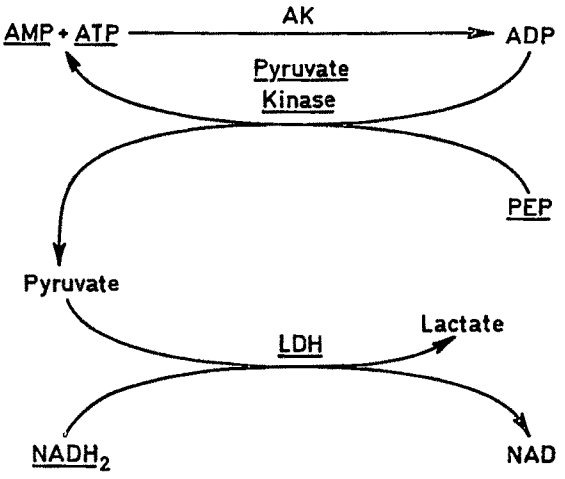


Fig. 3. Sequence of reactions for detection of adenylate kinase following starch-gel electrophoresis. In this system ATP and AMP are substrates, and the other components underlined are contained in the reaction mixture

to 2 h at 37° C. This reaction mixture was designed to locate AK according to the reaction sequence shown in Fig. 3. The ATP and AMP react at the sites of AK activity to give ADP, which is then phosphorylated by PEP under the action of PK to yield ATP and pyruvate. The pyruvate is reduced by LDH with the concomitant oxidation of $NADH_2$ to form lactate and NAD. The sites of AK activity appear as zones of absorption (NAD) on a fluorescing background ($NADH_2$) when the gels are examined in ultra-violet light (Hanovia lamp with peak transmission 3660 Å units). Despite the quite different reagents and reaction system utilized in this procedure, the patterns of activity observed were the same as those found with the procedure using ADP as substrate.

(3) It has been reported[7] that AK is noticeably stable to acid conditions. We have found that red cell lysates adjusted to *p*H 2·0 with 2 N hydrochloric acid and then after 10 min at room temperature neutralized with 2 N sodium hydroxide still gave the same AK electrophoretic pattern. Other enzyme electrophoretic patterns such as lactic dehydrogenase and 6-phosphogluconate dehydrogenase were destroyed by this treatment, which also resulted in a heavy precipitate of denatured haemoglobin.

(4) A comparison has been made between a purified crystalline adenylate kinase preparation made from rabbit muscle obtained commercially, and a crude rabbit muscle extract made by homogenizing the muscle in an equal volume of water. The crude extract diluted 1 in 10 with gel buffer was subjected to starch-gel electrophoresis under the standard conditions side by side with a suitable dilution of a crystalline AK preparation. Identical patterns of activity were obtained. There were at least three zones and they had the same mobilities in the two preparations. The mobilities were different from those observed using human material.

Haemolysates from 960 unrelated English people have been examined. Among 540 males there were 60 (11·1 per cent) who were AK 2–1, and among 420 females there were 35 (8·3 per cent) who were AK 2–1. The difference between the sexes is not statistically significant and one may conclude that AK 2–1 occurs with a frequency close to 10 per cent in the general population. Only one

Table 1. AK Phenotypes among 136 Children of 54 Matings where one Parent was AK 1 and the Other AK 2–1

Parents		No. of matings	Children				Total
			AK 1		AK 2–1		
Father	Mother		Males	Females	Males	Females	
1 × 2–1		28	23	17	14	16	70
2–1 × 1		26	17	15	17	17	66
		54	40	32	31	33	136

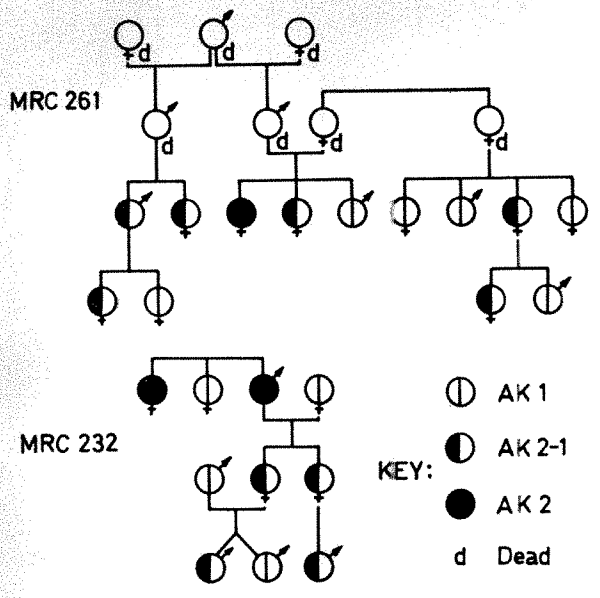


Fig. 4. Pedigrees of two families in which the uncommon phenotype AK 2 was found to occur

instance of AK 2 (a female) was observed in this population sample. Family investigations make it clear that these phenotypic differences are genetically determined. Table 1 summarizes the findings among the offspring of 54 matings in which one parent was AK 1 and the other AK 2–1. Among the 136 children tested, 72 were AK 1, 64 AK 2–1 and there were no examples of AK 2. This is in striking contrast to the findings in 62 families where both parents were AK 1. Here all the 141 children tested were also AK 1. These results and also the segregation patterns in several quite extensive pedigrees suggest that the differences may be attributed to a pair of autosomal allelic genes (AK^1 and AK^2), AK 1 individuals being homozygous (AK^1AK^1) and AK 2–1 individuals heterozygous (AK^1AK^2). If this were so the gene frequency of AK^2 in the English population should be about 0·05, and the homozygote AK^2AK^2 might be expected to occur in about 1 in 400 of the population. It seems probable that the uncommon phenotype which we have designated AK 2 represents the homozygous genotype AK^2AK^2. Although only one individual with this phenotype was encountered in the series of 900 unrelated individuals mentioned here, three other examples of the phenotype have been encountered in the course of our family investigations. The relevant parts of the two pedigrees in which they occurred are shown in Fig. 4. In both these families the difference between the AK 2 and AK 2–1 electrophoretic patterns was quite clear cut and there was no difficulty in differentiating the two phenotypes. The segregation of the phenotypes in these families is clearly consistent with the hypothesis that AK 2 individuals are homozygotes AK^2AK^2. However, further family data are obviously needed on this point.

The preparations of adenylate kinase, glucose-6-phosphate dehydrogenase, hexokinase, pyruvate kinase and lactate dehydrogenase were obtained from Boehringer Corporation (London), Ltd.

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PROBABLE *in vivo* ORIGIN OF MULTI-NUCLEATED GIANT CELLS FROM CIRCULATING MONONUCLEARS

By Dr. T. GILLMAN and L. J. WRIGHT

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LEWIS[1] demonstrated that, in culture, multi-nucleated giant cells could be formed by fusion of blood monocytes—a view supported by many other workers since (see reviews in refs. 2 and 3). Willis[4] states crisply the opinions at present most widely held regarding some of the possible sources of giant cells *in vivo*, namely: "Some workers believe that foreign-body giant cells may develop, not only by fusion, but also by amitotic nuclear division of macrophages, but from the appearances observed it is clear that fusion is their main, if not their only, mode of formation". Objective proof of these views is, however, still lacking.

During the course of recent radioisotope investigations of the histo- and cyto-genesis of fibrous tissue in wounds and around implanted foreign bodies, evidence emerged that at least one type of multi-nucleated giant cells, related to fibrosis around foreign bodies, is formed by fusion of circulating blood mononuclears (that is, lymphocytes and/or monocytes) after they have emigrated into injured tissues.

The experiments were of two types, namely: (1) tritiated thymidine was injected intravenously into adult rats either at 18 h or at both 18 and 5 h before wounding and implanting foreign bodies (Millipore filters or polyvinyl sponges) subcutaneously and/or intraperitoneally; the foreign bodies were removed at various times from 1 to 14 days after implantation; (2) previously unlabelled rats, carrying identical types of excised wounds and implanted foreign bodies (intraperitoneally or subcutaneously), received, only post-operatively, a single intravenous injection of tritiated thymidine just 2 h before killing and removal of the wounds and foreign bodies. In all the experiments, touch or imprint preparations were made of portions of each of the excised wounds and foreign bodies[5]. To avoid chemiograms, it was found necessary to fix the imprints in C.P. methanol for 24 h. Then, after coating with liquid emulsion and exposure for 14–92 days, the imprint preparations were developed and stained by modifications of the Giemsa method originally described by Speirs *et al.*[6]. Autoradiographs were also made of serial paraffin sections of these same wounds and foreign bodies. The imprint preparations provided the most striking and reliable cytological pictures because of minimal cell shrinkage; Giemsa staining also permitted direct morphological comparison of the cytology of cells in the imprints with those seen in smears of the bone marrow and circulating blood prepared by accepted standard haematological methods and treated in the same way as tissue imprints. On the other hand, autoradiographs of the sections permitted both precise localization of all labelled cells and determination of their relations to pre-existing and newly formed vessels and fibrous tissue.

The results of these two sets of experiments were different although complementary. Multinucleated giant cells, containing 5–20 nuclei, were commonly seen in imprints and sections, and appeared as early as the third

post-operative day. Those multi-nucleated giant cells, found in the rats which had received isotope only prior to the operation, had many of their nuclei labelled (Figs. 1, 3 and 4). The same cells from animals flash-labelled only post-operatively, that is, just 2 h before killing, had completely unlabelled nuclei (Figs. 2 and 4).

Volkman and Gowans[7,8] showed clearly, in Rebuck-type skin windows, that labelled cells, which they named macrophages (whether or not they contained phagocytosed material), appeared in large numbers 24 h after operation only in rats that had received isotope 18–24 h pre-operatively. We have confirmed and extended these observations by showing that, within the first 1–4 days post-operatively, the greatest number of labelled mononuclear cells is obtained in wounds and implants by injecting rats with tracer 18–24 h before operation. By giving two pre-operative isotope injections, that is, at 18 and 5 h before operations, we could label between 60 and 80 per cent of the mononuclears found 24–48 h post-operatively in excised wounds and around implants.

There seems no reason to doubt that these lymphocytes and monocytes, in the inflamed and fibrosing tissues, were derived from the blood, especially since, at the time of operation and of killing, relatively few cells were labelled in the untraumatized connective tissues in comparable sites in the same rats. Many labelled lymphocytes and monocytes (5–15 per cent), as well as granulocytes, were consistently seen in the blood smears taken from these rats.

'Round cell' (mononuclear) emigration is a recognized sequel to the initial neutrophil infiltration seen in the early stages of any inflammatory response, including that preceding fibrosis in wounds and around foreign bodies. The fact that not all nuclei in the giant cells were labelled in rats injected pre-operatively with tritiated thymidine is to be expected, since 20–40 per cent of mononuclears in skin windows[7,8] and infiltrating injured tissues, in our experiments, were unlabelled.

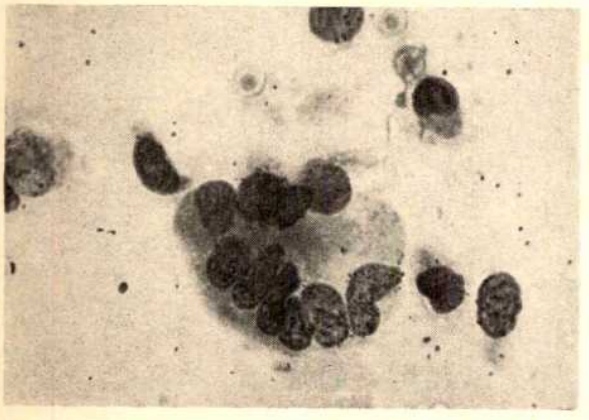


Fig. 1. Multi-nucleated foreign body giant cell and several round cells in an imprint of a 4-day-old subcutaneous sponge from a rat that had received 1 μc. per g body-weight of ^{3}H-thymidine intravenously at 18 and 5 h before operation only. Note clear labelling of at least two giant cell nuclei and of several of the round cells. Autoradiograph, Giemsa counterstain (× *c.* 703)

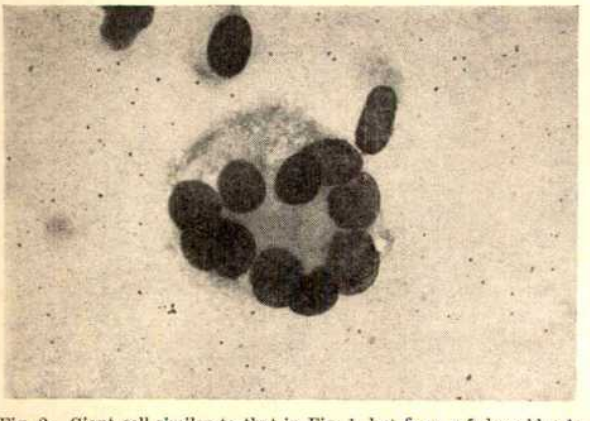
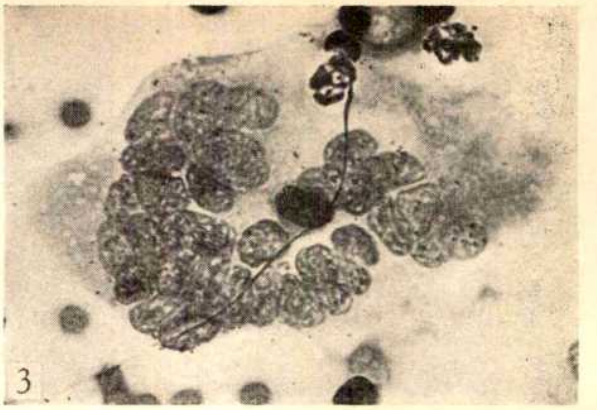
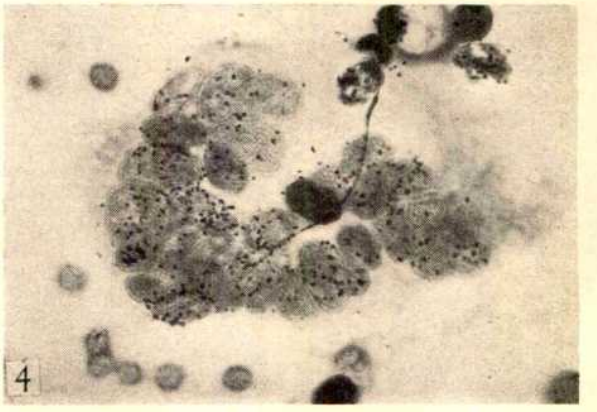


Fig. 2. Giant cell similar to that in Fig. 1, but from a 5-day-old subcutaneous sponge from a rat that received one injection of the same dose of label but only 2 h before death. No nuclei labelled. Autoradiograph, Giemsa counterstain (× *c.* 703)





Figs. 3 and 4. A very large giant cell from another rat injected once only, 18 h before operation with the same dose of isotope. These two figures, of the same cell, at two different levels of focus, show how many nuclei were labelled. Note also label in nuclei of adjacent neutrophils. The findings indicate formation of giant cells by fusion of labelled round cells. Autoradiograph, Giemsa counterstain (× *c.* 703)

The total absence of nuclear label in *any* of the nuclei of giant cells, in rats injected only post-operatively, that is, 2 h before being killed (Figs. 2 and 4), suggests that these nuclei were not synthesizing DNA. This fact, taken in conjunction with the labelling of some, but not all, giant cell nuclei in rats injected pre-operatively (Figs. 1 and 3), indicates that multi-nuclear cells were probably derived by fusion of both labelled and unlabelled local or, more likely, emigrated blood mononuclears.

Such giant cells, with a proportion of their nuclei labelled, were frequently found even 14 days after the operation, and hence 14 days and 18 h (or 14 days and 5 h) after the last pre-operative injection of isotope.

Shelton and Rice[9] described the presence, within histiocytes, of many lymphoma cells cultured *in vivo* in 'Millipore' chambers. In fixed material, such histiocytes could, in all probability, have been taken to be one type of multi-nucleated giant cells. It is also possible that, in our experiments, the presence of many labelled nuclei within a single giant cell could also have arisen by 'ingestion' of labelled blood lympho- or mono-cytes by tissue macrophages around the foreign bodies. It is interesting to note that, as shown in Figs. 1, 3 and 5, the size and other characteristics of the nuclei of the giant cells indicated that they were primarily lymphocytic rather than monocytic in origin. This was especially so in the subcutaneous giant cells. In the peritoneum, however, the giant cells were either attached to the peritoneal

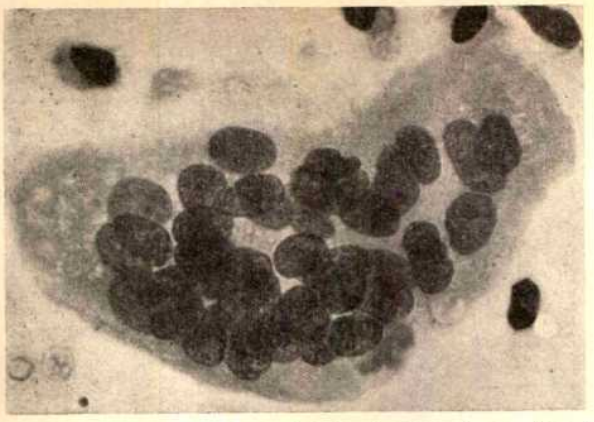


Fig. 5. Another very large giant cell, similar to that in Figs. 3 and 4 but in an imprint from a 7-day-old sponge from a rat injected with isotope only 2 h before death. Note, again, absence of any nuclear label indicating probable absence of active DNA synthesis during genesis of the many nuclei in this cell which was therefore probably formed by fusion of unlabelled round cells. Autoradiograph, Giemsa counterstain (× *c.* 703)

implants or were floating freely in the peritoneal fluid; they often contained large bi-lobed nuclei reminiscent of monocyte nuclei. Similar monocytoid (or paramonocytic)[5] giant cells, often resembling Reed–Sternberg cells, were a feature of the lymphomata produced in rats treated chronically with trypan blue[10,11]. These cells, also, may well have been derived from the circulating 'polymorphonuclear type' blood monocytes so common in these rats[11,12] and in which lympho- and mono- as well as erythrophagocytosis was shown to be a consistent finding in lymphoid tissues and liver.

These observations from our labelling experiments would seem to leave little doubt that at least the foreign-body-type multi-nucleated giant cells seen in these experiments were derived from previously labelled blood mononuclear cells either by their fusion, once they had emigrated into tissues, or through their being phagocytosed by locally resident macrophages. Our findings that, during the first 3 days post-operatively, a far higher percentage of infiltrating mononuclears were labelled if rats were injected with isotope many hours before operation than when isotope was given post-operatively confirm Volkman and Gowans's reports. However, we have here extended their observations to show that, once they have entered injured tissues, many of these labelled inflammatory cells may remain there for days, or even for 2 weeks post-operatively, where, in appropriate circumstances, they may fuse to form foreign body giant cells. The further implications of these findings for the possible origins of fibroblasts, as well as giant cells, has been discussed elsewhere[13,14].

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VARIABLE AND INVARIABLE COMPONENTS OF THE ANTICOAGULANT FRACTION OF INCUBATED FIBRINOGEN

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TRIANTAPHYLLOPOULOS[1] and Niewiarowski and Kowalski[2] showed independently, and it was later verified by others, that the breakdown products of fibrinogen possess anticoagulant properties. Most of the anticoagulant activity is exhibited by the protein which is precipitated between 25–50 per cent ammonium sulphate saturation. Since this fraction was originally isolated from solutions of incubated (spontaneously lysed) fibrinogen, it was called the anticoagulant fraction of incubated fibrinogen (AFIF)[3]. In 1962 we showed that AFIF can be separated by continuous-flow paper electrophoresis into two main sub-fractions which we found able to prolong the thrombin clotting time of intact fibrinogen to the same extent[4]. We later demonstrated that in human AFIF the larger of these sub-fractions is 70 per cent thermolabile, while the smaller is completely thermostable[5]. Since, however, both the thrombin time prolonging effect[6] and the thermoresistance[5] of AFIF have been shown to vary considerably with the length of the incubation of the parent fibrinogen solution from which a sample is isolated, it became necessary to investigate the effect of incubation on the activity and thermosensitivity of each electrophoretic fraction and estimate the extent to which each of these constituents is responsible for the variations observed with unfractionated AFIF preparations.

Human fibrinogen was isolated from outdated Red Cross plasma by saturation to 25 per cent with ammonium sulphate and reprecipitation of the protein by 50 per cent saturation with sodium chloride, as described earlier[6]. Bovine fibrinogen was prepared by the method of Laki[7]. Only preparations with a clottability of at least 92 per cent (human) or 84 per cent (bovine) were used.

Solutions of human fibrinogen at 10 mg/ml. in physiological saline (8·5 per cent w/v), buffered to *p*H 7·4 with 0·025 M imidazole and containing 0·028 M sodium citrate, were sterilized by passing them through Seitz filters and were incubated at 37° C. Bovine fibrinogen solutions in 0·3 M potassium chloride similarly buffered and citrated were digested at 37° C by a streptokinase-activated human fibrinolysin preparation (Thrombolysin) which was added at 0·12 casein and 60 casein activator units/ml. of final solution. As soon as each of the fibrinogen solutions became completely unclottable by thrombin (human fibrinogen was lysed spontaneously within 3–5 days, that of bovine origin within 30–80 min), aliquots of 60–100 ml. were removed at various intervals and fractionated with ammonium sulphate. Pancreatic trypsin inhibitor ('Iniprol' of the Choay Laboratories, Paris) was added to each fraction at a final concentration of 100 anti-Schwert–Takenaka[8] units per ml. before fractionation. The 25–50 per cent precipitates (AFIF preparations) from the successive samples were collected and an aliquot of each was subjected to either continuous-flow paper[4] or column electrophoresis. In the latter case, the Porath electrophoresis apparatus 3340 of the L.K.B. Co., Stockholm, was used. Munktell's cellulose powder especially developed for zone electrophoresis was pur-

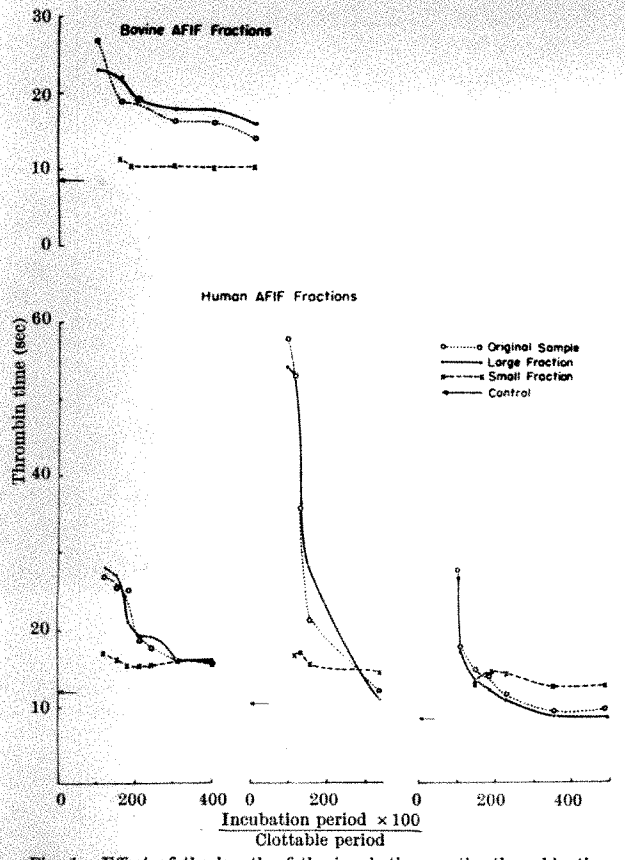


Fig. 1. Effect of the length of the incubation on the thrombin time prolonging ability of the main electrophoretic fractions of AFIF. The concentration of each fraction was adjusted to 4 and that of fibrinogen to 2 mg/ml. Thrombin times were determined at room temperature (25° C)

chased from Grycksbo Paperback A.B., Sweden. The buffer was veronal 0·02 M, *p*H 8·6. The sample (3–5 ml. at 40–70 mg/ml.) was stained with bromphenol blue and dialysed against 3–4 h changes of the veronal buffer under continuous stirring, before electrophoresis. The current was applied at 17 m.amp and ≃ 560 V until the blue-coloured band reached the bottom of the column (15–18 h). The flow rate of the eluting buffer was adjusted to one drop/10 sec and the fractions were collected at 20-min intervals. All operations were carried out at 2°–4° C. The absorption of each fraction at 280 mμ was measured in a Beckman *DB* spectrophotometer and the protein content was calculated from a curve which was prepared with AFIF solutions of known concentration. The protein pattern was the mirror image of that obtained with continuous-flow paper electrophoresis[6] since the most electro-negative components emerged from the column first. The central fractions belonging to each main peak were pooled together and concentrated by lyophilization. The dry powders were dissolved in saline and dialysed against it until free of veronal (no absorption at 240 mμ). Their effect on the thrombin time of intact fibrinogen was tested as described in an earlier publication[6].

Thrombin time prolonging effect of the electrophoretic fractions as a function of the incubation stage. Seven series of experiments were performed, five with human and two with bovine AFIF fractions. Separation of bovine AFIF into fractions under the conditions of these experiments was never so good as that obtained with human preparations. In the majority of cases it was not possible to isolate small-fraction material free of large-fraction contamination in quantities sufficient for these investigations and these experiments were not taken into account. The incubation period of each sample was expressed as a percentage of the clottable period (initial stage of incubation during which the fibrinogen was still clottable by thrombin) in order to facilitate the comparison of the results from experiments with different degrees of fibrinolytic potency. The prolongation of the thrombin time exerted by the large fraction of human AFIF (Fig. 1) was high at the beginning of unclottability (when separation into fractions could first be obtained) but decreased considerably during advanced stages, following the same pattern which the original non-electrophoresed sample exhibited. The inhibition obtained hy the small fraction was much lower and changed little with incubation. Due to the progressive decrease in the activity of the large fraction, there were instances where this fraction was more, equally or in rare cases less active than the small fraction. The results obtained with the two series of bovine fractions (Fig. 1, upper part) indicate that the activity of the large fraction of these preparations was similarly reduced during advanced stages. However, the reduction was not as great as with human specimens and this fraction seemed to remain significantly more inhibitory than the smaller one at all stages. Some of the large peak fractions which were obtained from samples isolated at about the end of the period during which clotting could take place showed a lower antithrombic effect than their parent non-electrophoresed preparations, although the opposite was expected. This indicates that the large peak fractions of this period are labile and can lose part of their activity during electrophoresis, lyophilization or dialysis.

Thermosensitivity and relative concentration of the electrophoretic fractions of AFIF as a function of the incubation period. Aliquots of the two fractions were heated in a water bath at 60° C for 15 min. The precipitates were removed by centrifugation and the protein content of the supernatants was determined together with the concentration of an unheated aliquot of each preparation by Kingsley's biuret method for total protein[9]. The thermostable protein was then calculated as a percentage of the amount present before heating. Small peak fractions (upper half of Fig. 2) were found to remain completely thermoresistant throughout incubation. The resistance of the large peak moiety (middle left of Fig. 2), however, and the relative concentration of the small

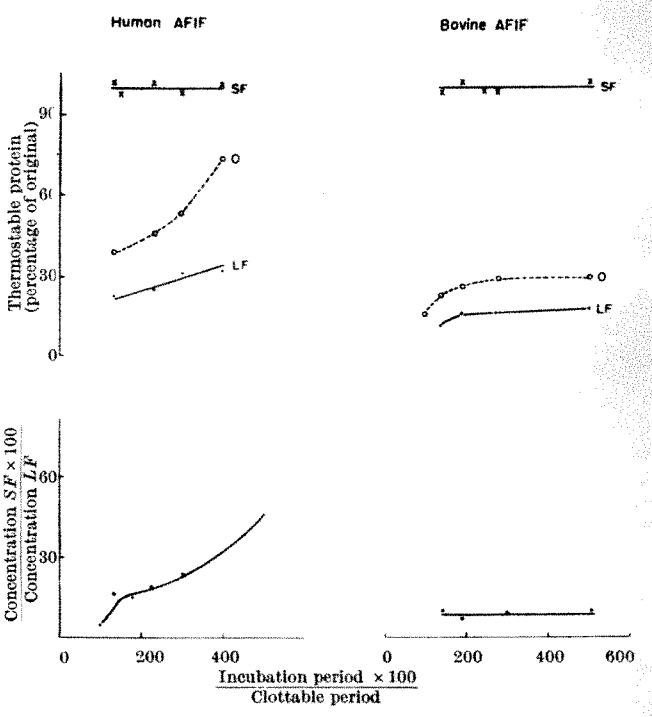


Fig. 2. Effect of the incubation period on the thermoresistance and relative concentration of the electrophoretic fractions of AFIF. The thermostable protein is expressed as a percentage of the protein concentration of the unheated samples. *SF*, Small fraction; *LF*, large fraction; *O*, original sample

fraction in human AFIF (lower left of Fig. 2) seemed to increase appreciably with incubation. One group of experiments with bovine fractions indicated that: (*a*) the stability of the large fraction of these preparations increased very little with incubation; (*b*) the ratio of the two fractions remained constant after 140 per cent of the clottable period. (Small fractions from bovine AFIF were never obtained at earlier periods under the conditions of these experiments.) So far as the ability of the heated fractions to prolong the thrombin time was concerned, the small fraction retained all its activity throughout, while the larger one was completely inactivated, for all practical purposes, at all stages.

It becomes obvious from the results of these experiments that both the increase in thermoresistance[5] and the reduction of the ability of AFIF preparations to prolong the thrombin time during advanced stages of incubation[6] are the result of the same factors: (*a*) appearance and gradual increase in relative concentration of the resistant, less active small fraction; (*b*) change in the quality of the larger fraction with increasing incubation. The latter factor is probably the most significant considering the higher relative concentration of the large fraction, especially between 100 and 200 per cent of the period during which clotting could take place when the sharpest changes in the activity and thermoresistance of AFIF occurred. Both the change in the quality of the large peak and the increase in the concentration of the small peak are minimal with bovine preparations but quite pronounced with human samples: this explains the greater variability of human AFIF[5,6].

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PERCEPTION OF MOVEMENT BY THE CRAB

By Dr. G. A. HORRIDGE and P. R. B. SHEPHEARD
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PERCEPTION of movement describes, in essence, a relation which is derived from two successive intensity changes at nearby points in the visual field. When a contrasting edge moves, a simple means of determining the movement would be to remember the old position and compare it with the new one in a system containing an absolute spatial representation of the retina. The following experiments on the crab *Carcinus* suggest that, in this animal, this may well be an important part of the mechanism for the perception of slow movement.

The eyes of a crab are on stalks and normally they will follow the movement of a striped drum placed around the animal. Over a range of drum speeds the eye moves more slowly than the drum, leaving a residual slip speed which maintains the stimulus for the eye movement[1]. The percentage following, defined as the ratio of eye speed to drum speed multiplied by a hundred, varies with the speed of movement and is low for very slow or very fast shifts of the drum. With the crab out of water, the eye is capable of following drum movements down to about 0·002 degrees/sec, that is, several times slower than the hour hand of a clock, which is 0·0083°/sec.

A crab was placed with one eye at the centre of a striped drum. The movements were recorded by a photocell activated by a narrow infra-red beam and the other eye was kept covered. In our experiments, the drum radius was 8 in., and the repeat pattern of the stripes subtended 28·7° at the eye, but the details of the pattern, so long as it is large, or its exact regularity and illumination are of little importance for the experiment described here. The crab was left undisturbed for 15 min and the light was then turned out. Movements of the drum now produced no response. During an adjustable period in darkness the drum was moved through a controlled angle. When the light was switched on again, the eye swung to a new position by moving in the direction in which the drum had been turned. In all experiments, 3 min was allowed for the eye to reach its new equilibrium position. It was possible to show a directional response to a movement of 0·5° over a dark period of 2 min or of an angle of 1° over 15 min. A response to 1·5° over 40 min was achieved. Lower thresholds and longer times could not easily be reached in single trials because the eye moved and drifted in the dark, and switching on the light suddenly after long periods in the dark may have caused flicking or retraction of the eye or leg movements so that it was necessary to discard the result.

Results for different drum movements are shown in Fig. 1. The ordinate percentage following is the ratio of eye movement to drum movement, expressed as a percentage. When the light was switched on, the drum was stationary, so that during the eye movement the stripes moved across the eye as if the drum had been moving in the opposite direction, but this did not appear to inhibit the actual percentage following so much as the time taken to reach the final position.

Movement of the drum in the dark by one black–white repeat distance caused no response, showing that it was the stripes which were perceived. Movement through half this angle, so that white replaces black, also caused no response. In this instance no clue as to the direction of the movement was given. It is curious that for some degrees on each side of this position the direction of the slope of the curve in Fig. 1 suggests that a black–white edge is confused with a white–black one. Large angles cannot be tested with a striped drum, but when a single black–white edge was moved around the eye in the dark, responses to much larger angles occurred when the illumination was resumed. For angles up to 180° the results are shown in Fig. 2, for a period of 1 min in the dark. The actual angle of the response increased for rotations up to about 160°, showing that the crab took a measure of these large rotations. It seems most unlikely that integration is limited to subjacent ommatidia. The percentage following is low, as it always is for a continuously illuminated single contrasting edge, and actual eye movements are less than 1°.

As the period of intervening darkness was lengthened, percentage following by the eye progressively fell off. Results up to 10 min are shown in Fig. 3, but a directional response could still be obtained when a movement of a few degrees was made before the light was switched on

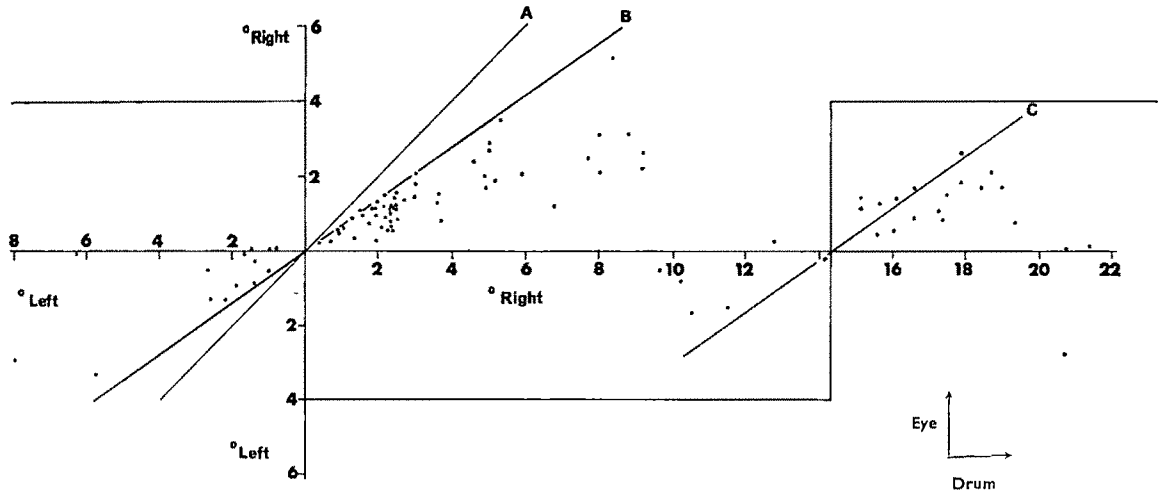


Fig. 1. Eye movements, to right and left plotted vertically, as a response to drum movements which were made during a period of darkness of 1 min through different angles as plotted horizontally. The line *A* shows where eye movement would equal drum movement, which is never realized. The line *B* shows the maximum observed percentage following for a 1-min period of darkness (69 per cent). The line *C* is drawn with the same slope through the point at the half period of the pattern

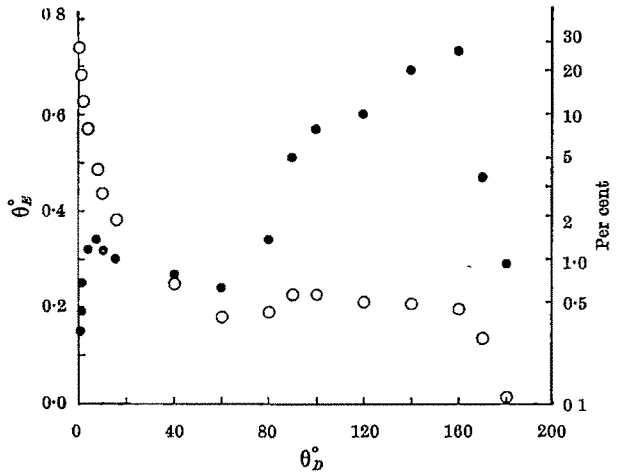


Fig. 2. Responses to a movement, which is made during a dark period of 1 min, of a single black–white edge through various angles θ_D up to 180°. The solid circles, with linear scale on the left, show actual angles θ_E of the eye response, averaged for left and right movements. The open circles, with logarithmic scale on the right, show the ratio of eye movement to drum movement (converted to a percentage)

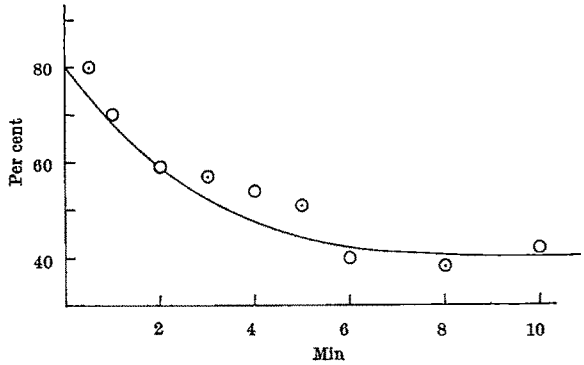


Fig. 3. The percentage following by the eye of a striped drum which is re-illuminated after different periods of darkness as shown, during which time the drum is moved through any angle up to 5°. The curve shows the exponential decay from 80 per cent to 40 per cent with a time constant of 150 sec

after a period of 40 min in the dark. Quantitatively consistent results, however, were not obtained over periods in the dark of longer than 10 min.

In a previous paper[1], it was shown that the eyestalk of the crab would give a directional response which was a reasonably accurate measure of an observed drum speed comparable with that of the Moon across the sky. Besides eliminating models of the mechanism of movement perception which have small time constants, the present results show that the crab can perceive movements at this speed of a contrasting object which is obscured for up to 10 min.

A second experiment which we carried out showed that movements of large contrasting areas rather than their edges predominated in governing a response, so that the response could be in a direction opposite to that in which a continuously illuminated edge moved.

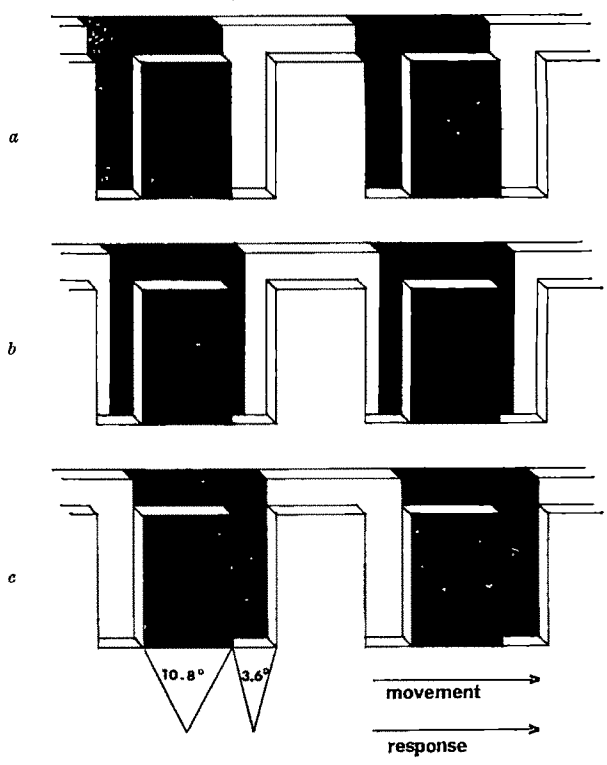


Fig. 4. An outer striped drum moves to the right around the outside of a fixed drum which has slots between each white and black stripe. All edges seen by the crab move to the right and the response is in that direction also. The drums are drawn in three dimensions for clarity

An outer movable drum of equal stripes closely surrounded an inner movable drum which was slotted as well as striped. Both drums had repeat patterns which subtended the same angle at the eye, in our case 28·7°. The first arrangement is shown in Fig. 4. Under continuous illumination the outer drum was moved from position (*a*) to position (*c*) in a controlled period of time, passing through position (*b*). Each black stripe of the inner drum thus lost a band of black on its left and gained one on its right. The time over which the movement occurred was varied between 1 and 900 sec, and the angle of response by the eye in the same direction as the outer drum is shown in Fig. 5*A*. After the end of the movement, 3 min was allowed for the eye to reach equilibrium. Clearly, the movement was well perceived over a wide range of drum velocity.

The experiment was repeated with the black and white stripes of the outer drum reversed in position (Fig. 6). While illuminated, the outer drum moved from (*a*) to (*c*) in the same range of controlled periods. Each inner black stripe now had a diminishing black area moving away from its right and a different growing black area moving towards its left side. At the half-way stage (*b*) there was a symmetrical situation with half the edges moving to the right and half stationary. The curious fact was that, from the beginning, the response was in the opposite direction to the movement, although in other experiments the movements of edges which were separated by these distances were clearly resolved with a sensitivity down to 0·1° of movement.

The eye movements, in the opposite direction to the drum movements, were plotted as percentage following in Fig. 5*B*. In the range 1–10 sec for a 3·6° movement, the form of the response and the percentage following was the same as in Fig. 5*A*; indeed, the human eye would respond to instantaneous movements of Figs. 4 and 6 in the same way as did the crab. The experiment of Fig. 6 showed that each of the inner stripes together with its adjacent slot could be considered as a unit. While the outer drum moved to the right, the 'centre of whiteness' of the white inner stripes, and the 'centre of blackness' of the black inner ones moved at an equal rate to the left. At low speeds, the eye of the crab started off in the opposite direction to the outer drum with a short latency as the drum began to move. Evidently, therefore, it followed the centres of large contrasting areas and had no need to compare Fig. 6*c* when it came to rest with the memory of Fig. 6*a*, although this must have been the mechanism at high drum speeds. At low drum speeds the crab eye movements fell off in Fig. 5*B* more than in Fig. 5*A*, perhaps because the moving or stationary edges interfered with the response, or because the response depended on a memory which faded.

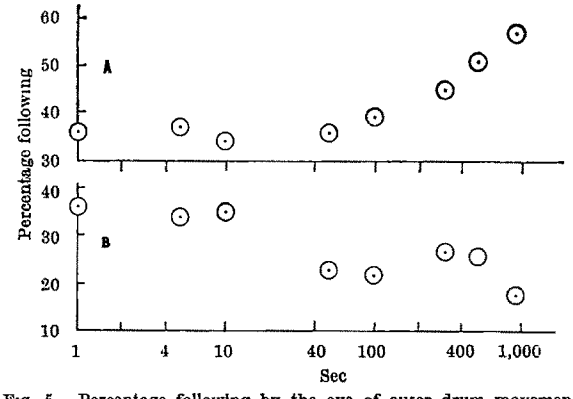


Fig. 5. Percentage following by the eye of outer drum movements occurring over different periods of time (*A*) Movements as in Fig. 4; (*B*) movements as in Fig. 6

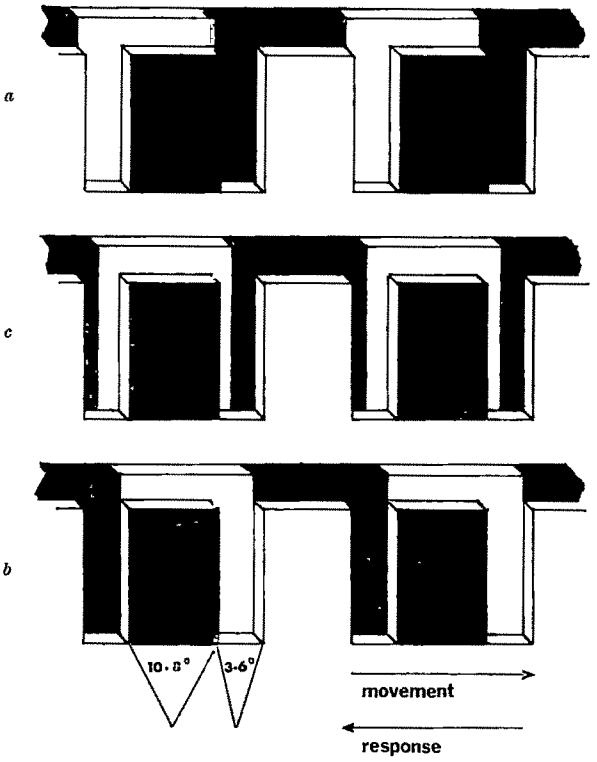


Fig. 6. As in Fig. 4, but with the black and white stripes of the outer drum reversed. Half the contrasting edges move to the right but the centres of the black and white areas (including all within the adjoining slots) move at an equal rate to the left, which is the direction of the eye response

Summarizing, our first experiment showed that the time could be long over which intensity changes were integrated before an inference of movement was made, and that intervening large changes in general illumination were of no consequence. The second experiment suggested that the eye follows not edges, but centres, of large areas. Both experiments were difficult to reconcile with present-day views on vision by the compound eye

[1] Horridge, G. A., and Sandeman, D. C., *Proc. Roy. Soc.*, B, **161**, 216 (1964).

INTESTINAL ABSORPTION OF PRALIDOXIME AND OTHER ALDOXIMES

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CORRELATIONS have been demonstrated between degree of lipoid solubility and rate of penetration of various substances across biological barriers[1], including the intestinal barrier[2]. These observations were used, initially, to make inferences concerning the nature of the biological barrier in terms of the penetration of organic compounds[1]. By circuitous reasoning, similar observations have been used to formulate the generalized con-

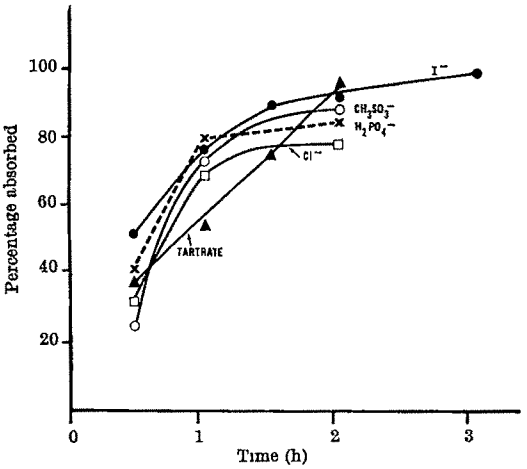


Fig. 1. Absorption of 2-PAM salts in the rat *in vivo* as a function of time. All compounds were administered to *in vivo* intestinal loops of rats in 0·5 ml. of a solution of 8×10^{-3} M concentration of 2-PAM (2-PAM-tartrate contains 2 moles of PAM to 1 mole of tartrate). ●, 2-PAM-I, ○, 2-PAM-CH_3SO_4; ×, 2-PAM-H_2PO_4; □, 2-PAM-Cl; ▲, 2-PAM-tartrate

clusion that organic compounds would readily traverse a barrier, such as the intestinal barrier, only if the compounds were lipophilic; as acids and bases in aqueous solution are involved in protonic equilibrium between a charged species which is considered to be lipophobic and an uncharged species which is considered to be lipophilic, this generalization was extended to exclude from the readily traversing compounds those which exist largely in the charged form at physiological *p*H (ref. 2). (*Note* 1. Various compounds, such as thiopental, aniline and salicylic acid, were judged lipoid soluble on the basis of chloroform/water partition coefficients of 2·6 to >100 and were found to be absorbed to 50 per cent or more from the small intestine; compounds, such as barbituric acid and sulphaguanidine, with chloroform/water partition coefficients of 0·008 to < 0·002, were judged lipoid insoluble and were absorbed to less than 5 per cent.) However, many strongly acidic compounds and strongly basic compounds, as well as some quaternary ammonium compounds, all of which exist entirely or almost entirely in the charged form at physiological *p*H, are known to be absorbed from the gastrointestinal tract, albeit inefficiently[3].

This article presents results of investigations of intestinal absorption of a number of mono- and *bis*-quaternary pyridinium aldoxime salts, including pralidoxime (2-formyl-1-methylpyridinium chloride oxime, 2-PAM). The mono-quaternary members of the group are unique among charged compounds of non-biological origin in their rapid and near-complete absorption from the small intestine.

The degree of intestinal absorption was determined *in vivo* using a single-loop preparation in non-fasted, male rats of the Sprague–Dawley strain, weighing 180–210 g (ref. 3). Each compound was injected quantitatively into the intestinal loop in a total volume of 0·5 ml. of experimental solution. At the end of an experiment the entire loop of gut and its contents were prepared for chemical analysis and determination of the quantity of unabsorbed aldoxime. Before any compound was used in the absorption studies, investigations were undertaken *in vitro* to determine whether or not any degradation of the compound occurred within the intestinal loop[3]. None of the compounds was found to be degraded; this permitted equating loss from the intestinal loop with amount absorbed from the loop.

The ultra-violet spectrographic method used for the determination of the various aldoximes was that of Creasey and Green[4], adapted for each different compound. The specificity of each adaptation was verified by ultra-violet spectral analysis of acid, neutral and alkaline solutions of compound present in the gut compared with corresponding standards. For each experiment, both reagent and tissue blanks were run and the spectrophotometric readings were corrected for these blanks.

The results of *in vivo* absorption investigations with five salts of 2-PAM are shown in Fig. 1. The percentages of absorption of the methanesulphonate, phosphate, chloride and iodide salt were not significantly different from each other (by Student's *t*-test) at any time-interval. However, the percentage absorption of the tartrate salt was significantly lower than that of the other four salts at 1 h and significantly lower than that of the iodide at 1·5 h; there were no significant differences in absorption among any of the salts at 2 h. Thus, 2-PAM tartrate was absorbed more slowly, although ultimately as completely as the other four salts.

To determine whether the observed difference in rate was specific for the molecular species, 2-PAM tartrate, or was the effect of the presence of the tartrate ion *per se*, absorption rates were measured for several combinations of 2-PAM iodide or tartrate with added iodide-ion or tartrate-ion. 1-h absorption periods were used for the latter investigations, since this was the time of greatest difference between the amount of absorption of the iodide and tartrate salts of 2-PAM. Fig. 2 shows the regression lines calculated for each of four different sets of experimental conditions. The calculations were made on the basis of individual values, that is, the percentage absorbed in 4–8 animals at each of five concentrations (between 0 and 64×10^{-3} M) of added tartrate or iodide-ion. All regression lines were found by analysis of variance to be significantly different from straight lines ($P < 0{\cdot}05$). The slope of each of the regression lines was significantly different from zero ($P < 0{\cdot}05$). The two lines of negative slope (*C* and *D*) represent the results of adding potassium tartrate to 2-PAM-iodide and 2-PAM-tartrate; the addition of potassium tartrate progressively

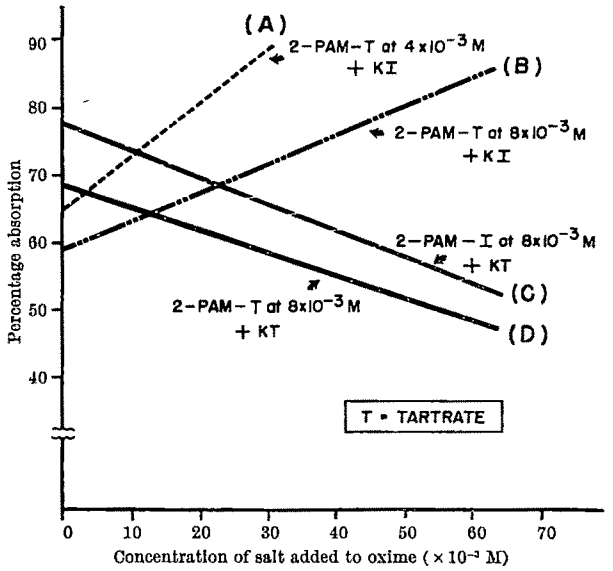


Fig. 2. Effect of added salt on absorption of 2-PAM-I and 2-PAM-tartrate in 1 h. Regression lines calculated for each set of conditions. All compounds were administered to *in vivo* intestinal loops of rats in 0·5 ml. of solution. Concentration of 2-PAM salts in terms of molarity of 2-PAM ----, 2-PAM-tartrate, 4×10^{-3} M, with added potassium iodide, —··—··, 2-PAM-tartrate, 8×10^{-3} M, with added potassium iodide, — — —, 2-PAM-I, 8×10^{-3} M, with added potassium tartrate; ———, 2-PAM-tartrate, 8×10^{-3} M, with added potassium tartrate

PARAMETERS OF REGRESSION LINES OF THE PERCENTAGE ABSORPTION OF 2-PAM ON CONCENTRATION OF SALT ADDED TO OXIME

$Y = bX + a$

Line	*n*	$b \pm S_b$	$a \pm S_a$
A	20	6·24 ± 2·24	64·1 ± 4·62
B	24	3·2 ± 0 956	58·8 ± 3·57
C	20	−3·22 ± 0 795	77·3 ± 3 59
D	24	−2 74 ± 1·21	68·2 ± 4·17

b, Slope of linear regression line; S_b, standard error of the slope; *a*, intercept of line on *Y* axis; S_a, standard error of the intercept; *n*, No. of animals

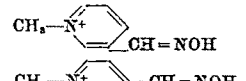
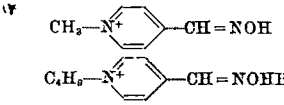
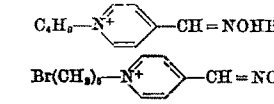
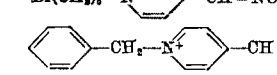
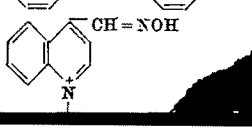
depressed the rate of absorption of both the iodide and tartrate salts of 2-PAM. In Fig. 2, the upper two lines (*A* and *B*) represent the results obtained on addition of potassium iodide to two different concentrations of 2-PAM-tartrate; the addition of iodide-ion progressively increased the absorption of 2-PAM-tartrate, the iodide-ion overcoming the adverse effect of tartrate. The positive slopes of lines *A* and *B* were found to be insignificantly different from each other, as were the negative slopes of lines *C* and *D*. This latter finding coupled with the relative positions of the lines suggests that: (1) there is a common ion effect of tartrate acting independently to adversely affect, in some unknown fashion, the transfer system for 2-PAM, and (2) there is a competitive effect of iodide-ion to overcome the effect of tartrate-ion. The slopes of regression lines (not shown in Fig. 2) for the absorption of 2-PAM-iodide in the presence of increasing concentration of the common anion, iodide (administered as potassium or sodium iodide), were found to be insignificantly different from zero. Any influence of the presence of increased concentrations of inorganic cation was also eliminated from consideration since the addition of sodium or potassium chloride, at 64 × 10^{-3} M, had no effect on the absorption of 2-PAM-iodide. Therefore, the observed differences in rate of absorption of 2-PAM when it was administered as an iodide or tartrate salt can be directly related to the presence of the respective anion.

Investigations carried out to determine the effects of dicarboxylic and hydroxycarboxylic acids have shown that addition of succinate, malonate, lactate, malate and citrate did not alter the absorption of 2-PAM-iodide. Therefore, the effect of tartrate on the absorption of 2-PAM cannot be attributed to any fragment of its structure and would appear to be specific. In at least one other case, the presence of organic anions has been shown to influence the absorption of a quaternary ammonium compound: butyrate, propionate and acetate increased both final percentage and initial rate of absorption of the non-aldoxime, benzomethamine, from *in vivo* intestinal loops of rats[5].

The degree of intestinal absorption of other mono-aldoximes, in addition to 2-PAM, and of a number of *bis*-aldoximes also has been determined. The results of some of these investigations are summarized in Table 1. All the mono-aldoximes, including 3-PAM and 4-PAM, were extremely well absorbed. Time-course investigations with these compounds indicated that their absorption was like that of 2-PAM, that is, rapid initial absorption within the first hour and then a continuous decrease in rate between 1 and 3 h. On the other hand, the *bis*-aldoximes (including TMB-3) were incompletely absorbed compared with the mono-aldoximes. The total amount absorbed and rate of absorption of the *bis*-aldoximes were similar to those of another *bis*-quaternary ammonium compound, hexamethonium, that is, the rates of absorption of both the *bis*-aldoximes and hexamethonium were relatively constant over the 3-h period studied[6]. Partition coefficients were calculated for each aldoxime from the distribution of the compound after shaking an aqueous solution of the aldoxime, buffered at *p*H 6·5, with chloroform for 30 min at 22° C. Using this distribution as a model of lipoid solubility, all the aldoximes were judged to be lipoid insoluble since all the partition coefficients, in the chloroform/buffer system, were less than 0·002; in this system, no marked differences were noted among the various compounds[2] (see *Note* 1 above).

Table 1. Intestinal Absorption of Aldoximes in the Rat

All compounds administered to *in vivo* intestinal loops of rats in 0·5 ml. of a solution containing 8 × 10^{-3} M aldoxime

Structure	*p*K*a*	Percentage absorbed in 3 h: Mean ± standard deviation
 I-(3-PAM)	9·2	85 ± 3
 I⁻ (4-PAM)	8·5	90 ± 2
	8·1	70 ± 4
	8·3	59 ± 8
		

We thank Drs. A. Kondritzer and B. E. Hackley, jun., for supplying samples of test compounds.

This investigation was supported in part by research grant *DA*-18-108-405-*cml*-902 from the Department of the Army and by research grant *B*1966 from the National Institutes of Neurological Diseases and Blindness of the National Institutes of Health, U.S. Public Health Service. A preliminary report of this work was presented at the autumn meeting of the American Society for Pharmacology and Experimental Therapeutics, San Francisco, [illegible]nia, in August 1963. In conducting the research the investigators adhered to the *Principles* [illegible]*eral Care* as established by the National

ANTI-FOULING COMPOSITIONS

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AND
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DURING and immediately after the Second World War extensive programmes of anti-fouling research were carried out, notably in the United States and in the United Kingdom[1-3]. The results of the research, especially those of American investigators[1], have provided a fertile source of information on the economics of the fouling problem, the biology of fouling organisms, the chemistry and physics of anti-fouling paint ingredients, and the prevention of fouling on ships. The research also enabled an insight to be gained of the mechanism of action of anti-fouling compositions and the liberation of poisons from the films.

The theory, derived from the research, for the mode of action of anti-fouling compositions can be summarized as follows: (1) An anti-fouling composition (containing a copper compound as the poison) will prevent fouling so long as the leaching rate is greater than 10 μg copper/cm^2/day. (2) The liberation of poisons can be explained by assuming the existence of two types of anti-fouling compositions—soluble and insoluble-matrix types.

In practice, however, the fouling problem was not solved efficaciously. It has since been shown that the theory cannot be used to explain many of the phenomena[4,5] observed in the study of anti-fouling compositions.

The evidence presented in this article is considered to show the necessity of amending both parts of the theory.

A critical evaluation of testing methods for anti-fouling compositions used by American and British investigators shows that the usefulness of the results obtained is more limited than was originally thought.

The results of raft trials, for example, have a definite value, but only for comparative tests. The advantages are the controlled conditions of exposure and the accessibility of test panels. A disadvantage is that the ageing proceeds in a way which differs quantitatively and qualitatively from practice—on the hull of a ship[6]. This difference may be explained by the greater rate of flow of seawater along the hulls of ships compared with the flow at raft sites.

On the other hand, patch tests on hulls of ships also have disadvantages. Reproducibility is almost impossible since conditions of test vary from one ship to another, for example, weather conditions during application and drying of compositions, the substrate and its pre-treatment, sailing routes, speeds of ships, fouling organisms encountered [illegible] and the number of days spent in port. Inaccessib[illegible] the patches as soon as the ship is out [illegible] another disadvantage, and the environ[illegible] is not always conduc[illegible]

Laboratory tests[6,11] offer the possibility of obtaining quantitative data under carefully controlled conditions; an ideal test should lead to a quick evaluation of the potential behaviour of a composition. A suitable method would consist of accelerated ageing preceding a biological method for an evaluation of the remaining anti-fouling action. The first requisite for accelerated ageing is that it should be comparable, qualitatively, to practice. The methods used hitherto for chemical ageing—the glycine test and acid-alkali test—do not meet this stipulation[12]. Leaching rate determinations cannot give more than a superficial indication of the anti-fouling properties of a composition at the moment of determination[6,13]. Any biological test should also be comparable to conditions in practice.

The results from two types of experiments carried out at the T.N.O. Laboratories indicate the necessity of amending the foregoing theory. The first series of results were obtained with a rotor apparatus for the accelerated ageing of anti-fouling compositions[6,14] used in conjunction with methods for investigating quantitatively the progressive ageing of compositions. The rotor apparatus consists essentially of a cylinder on which test panels are fastened. The cylinder can be rotated at a peripheral speed of up to 40 knots. The second series of results were obtained in experiments in which arbitrary scales of rating for fouling organisms were not used. The method of assessment consisted of measuring and counting accurately the numbers of organisms per unit area. This method could be used with ease in raft tests. For ship tests, separate panels were fastened on to bilge keels; this method has the advantages that in addition to being available for examination in the laboratory, the panels can be prepared under carefully controlled conditions and can be collected, if necessary by a diver during the voyage.

When using the rotor apparatus for the ageing of compositions, it was shown that they are affected in the same way as in practice but at an accelerated rate[6,14]. Compositions of moderate quality very soon—after one day—showed defects such as cold flow, inter-coat flaking or flaking of the wh[illegible] paint system from the substrate.

Quantitativ[illegible]on on the release of copper from the [illegible]ns was obtained in two ways [illegible]paratus and on ships: by [illegible]t of copper before and [illegible] examination of cross- [illegible] copper in the film [illegible]ht and formula-

of the tests demonstrated that the erosion, if any, of a composition by water flow was very limited even when the water speed was as high as 40 knots[6,29]. Also, that the difference between the release of poisons from soluble- and insoluble-matrix types of compositions were not so pronounced as was previously thought[17].

In both types of compositions, copper was liberated in the sea-water mainly by diffusion through the matrix. Further, it was shown that the differences in the lives of the two types of compositions could be explained by the formation of a copper-containing surface deposit in the exhausted matrix of an insoluble-matrix composition. The surface deposit was not detected in the soluble-matrix compositions. The bluish-green deposit which forms on many anti-fouling compositions after ageing may consist of a very insoluble product from the reaction of the paint-ingredients and sea-water; its exact composition is still unknown, although many authors consider it to be basic copper carbonate. It was not possible, however, to detect any carbonate group in the surface deposit when using attenuated total reflexion. The surface deposit is considered to have a very favourable effect on the life of an anti-fouling composition because of its regulating effect on the release of copper from the film.

Studies were also made of the relationship between the composition of the paint film and the release of copper[6,14,30]. It appeared that a better understanding of this relationship could be obtained by introducing the concept of 'total soluble'—the total amount of all sea-water soluble or otherwise sea-water-sensitive materials in the dry paint, expressed as a percentage of the volume of the dry film. The closer the percentage of 'total soluble' approached the volume of a continuous contact structure, the better was the release of poison from the interior of the film. The concept of 'total soluble' is very useful in formulating anti-fouling compositions based on copper or other poisons. For a full understanding of these phenomena, additional investigations into the solubilities of the ingredients in sea-water are necessary.

Wisely[15] considers there are three distinct mechanisms by which an anti-fouling composition can prevent fouling: (*a*) the fouling larvae are repelled and move away; (*b*) the fouling larvae become moribund before attachment and leave no trace on the paint (pre-attachment mortality); (*c*) the fouling larvae attach themselves to the paint and die during or shortly after metamorphosis (post-attachment mortality).

Wisely[16–18] has given examples of organisms which are repelled or which show pre-attachment mortality, while post-attachment mortality has been described by Crisp and Austin[19] for *Balanus balanoides* (L.) and *Elminius modestus* Darwin, and by De Wolf[20] for *Balanus crenatus* Bruguière and *Balanus amphitrite* Broch. These three observations only differ as a result of the differences in the rate of loss of copper from an ageing anti-fouling composition; in the case of *Balanus crenatus*, for example, a very high rate of release of copper acts either as a repellent or by means of pre-attachment mortality, while at lower rates of release, post-attachment mortality prevails.

Many authors state that, as a result of their experimental work, cuprous oxide anti-fouling compositions will prevent fouling so long as copper leaches from the film at a rate greater than 10 μg/cm²/day (refs. 1, 3 and 15). This statement is open to criticism, however, since the leaching rate is determined under well-defined conditions of temperature, flow, aeration, salinity and surface-to-volume ratio. On the other hand, fouling occurs under conditions which may differ considerably from the conditions of the leaching rate determinations in the laboratory. A leaching rate of 10 μg/cm²/day can only be used as an indication of conditions for the prevention of fouling[20].

Results published previously[6] have shown that a value of 10 μg/cm²/day is not always a reliable guide. Table 1 demonstrates that even at very low leaching rates, a considerable proportion of compositions do not foul.

An explanation of these phenomena has been proposed by De Wolf[20] using the assumption that the release of copper from an anti-fouling composition during leaching rate determinations or in raft tests is not homogeneous over the surface of the test panel. Indications that this assumption is correct are to be found by the examination of cross-sections of films. In an aged anti-fouling composition the layer of residual cuprous oxide shows considerable variations in thickness; the thickness of the layers is not constant if the film is applied by brush. This effect will be exaggerated still further if the composition is applied by roller on a relatively rough surface such as a ship's hull. This could explain, at least in part, why an anti-fouling composition on a ship's hull is always superior in performance when applied on a specially prepared patch of relatively small size compared with its performance over a whole ship. Results obtained by the accurate counting of barnacles on aged anti-fouling test panels and controls also support this assumption.

Over-dispersion of various species of young metamorphosed barnacles on non-toxic surfaces has been noted by several authors[22–24], and Wisely[25] has described the same effect of spacing-out of individuals from each other for the polychaetous worm *Spirorbis borealis* Daudin. In all these cases the dispersion of the animals is significantly more even than a dispersion according to a Poisson distribution with the same mean value.

Adult barnacles on a relatively smooth non-toxic surface may also be overdispersed; it has been observed that dispersion of adult barnacles on smooth aged anti-fouling compositions is nearly always significantly more uneven than a dispersion according to the corresponding Poisson distribution. This was found for *Balanus amphitrite* on bilge keel panels and for *Balanus crenatus* on raft panels. This under-dispersion cannot be explained by an inter-individual variation in sensitivity to copper since this would require the assumption that, during the process of settlement, a barnacle cyprid would be influenced by its neighbour's sensitivity to copper.

A variation in the release of copper over the surface, on the other hand, can easily explain the occurrence of under-dispersion in the barnacle population. The assumption in this case is that post-attachment mortality of barnacles will be most pronounced at places where the release of poison is highest. The barnacles present will then serve as indicators for locating where the release of copper is lowest—a kind of biological chromatography. The result would be that the final distribution of barnacles would be governed both by the original distribution of the settled larvae and the distribution of the poison over the surface of the aged composition.

It has been shown that barnacle cypris larvae are very insensitive to copper[26–28], and post-attachment mortality of barnacles is possible by contact poisoning; the copper diffuses directly from the anti-fouling composition into the metamorphosing barnacles[20], and not via the sea-water. This fact is in good agreement with the hypothesis formulated here since poisoning by direct contact stresses the importance of distribution of poison.

Table 1. Relation Between Leaching Rate of Paints and Fouling with *Balanus crenatus* in Raft Tests

Leaching rate of copper in μg/cm²/day	Total No. of panels exposed	No. of panels fouled	No. of panels not fouled
<1	16	11	5
1	17	7	0
2	6	4	2
3	11	8	3
4	6	6	0
5	3	1	2
6	8	3	5
7	3	1	2
8	5	0	5
9	6	4	2
10	4	3	1
11	2	1	1
>12	22	0	22
non-toxic controls	41	41	0

The work referred to in this report forms part of a programme on the fouling and corrosion of the hulls of ships, executed by the Paint Research Institute T.N.O. and the Central Laboratory T.N.O. (Biology Department) in collaboration with the Netherlands Research Centre T.N.O. for Shipbuilding and Navigation. The work is being sponsored by a number of composition manufacturers, the shipowners and the shipyards in The Netherlands.

We thank Mr. A. Partington for correcting the English text.

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MEASURING THE DENSITY AND DIRECTION OF MOLECULAR FLOW IN HIGH VACUUM USING AN IONIZATION GAUGE

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THE unit of pressure, that is force per unit area, is widely used to express the state of the residual gas in a high vacuum system. However, the most commonly used vacuum instruments are ionization devices which respond to gas density and are 'pressure' calibrated at a given temperature. Vacuum workers are concerned with kinetic systems in which gas is continuously evolved and removed from an envelope and the density must vary with position (even when the gas temperature is uniform) and the true pressure at any place will depend on direction. A single 'pressure' reading is usually insufficient to express the true state of the gas in a vacuum vessel, particularly if gas is evolved from a localized source. The important characteristics of a high vacuum for most uses are the gas composition, molecular mean free path and surface impingement rate. To determine the latter for a known gas one must measure its density, preferred flow, if any, and temperature.

There has been a tendency to treat the gas in a kinetic vacuum system as if it were static for measurement purposes and therefore vacuum workers have generally ignored the effect of gas beams on the response of their gauges. We have recently been measuring gas density and flow within a vacuum vessel using a Bayard–Alpert (B.A.) ionization gauge, and the effect of the gauge tubulation and orientation on its response is discussed here.

Gas molecules with a density n in an element of volume may be composed of a number n_c with a chaotic distribution and a balance n_s with a preferred direction of flow. If an ionization gauge is exposed to both components then a total ion current will be indicated given by $I = I_c + I_s$. If the gauge has been calibrated in terms of pressure then one reads a total pressure $P = P_c + P_s^*$, where P_s^* is a hypothetical quantity equal to that a molecular beam of density n_s would have if its preferred direction was replaced by chaos.

Should a gas beam impinge on a plane, then the true pressure P_B due to the beam is a function of direction and composed of two components. The impact of the beam at normal incidence produces a pressure $n_s m v_R^2$, where m is the mass of the gas molecules and v_R their root mean square velocity. As the re-emitted molecules leave in random directions their reaction thrust on a receiver at the gas temperature is $n_s m v_R{}^2 2/3$. Therefore, $P_B = n_s m v_R{}^2 5/3$, and as $P_s^* = n_s m v_R{}^2/3$ we obtain $5P_s^* = P_B$.

When an ionization gauge is used to measure beam density its response will depend on the shape and conductance of its tubulation. If the gauge envelope has a small opening in a thin plate of area A normal to a parallel molecular beam then gas will stream directly into the head, but to leave, it must find the opening by a random process. Thus, the gas density n_e of the beam within the enclosure is given by the equilibrium equation $An_s\bar{v}_s = An_e\bar{v}_e/4$, where $\bar{v}_s$ and $\bar{v}_e$ are the average molecular velocities; when the temperature is constant $P_e = 4\ P_s^*$. This effect was used by Stern[1] to enhance the detection of a molecular beam by a pressure manometer. Later, Frisch and Stern[2] used a canal to obtain a higher pressure factor.

Huntoon and Ellett[3] have used an ionization gauge for detecting weak mercury-beams with a long canal aperture. However, in general vacuum work the gauge tube must usually have a full bore opening to avoid sorption within the head affecting the pressure reading, and it is necessary to determine the enhancement factor of such a head before it can be used to measure beam density. Molecular flow arising from localized desorption in high

vacuum systems may occur in any direction over a large solid angle. Thus the beam detector must be capable of translation and rotation.

The high vacuum system used by us had a vessel (19 in. diam., 24 in. high) made of stainless steel exhausted by a diffusion pump, charged with silicone *DC*.704 and fitted with a water-cooled baffle valve. The baseplate pumping aperture was 12 in. diam. and the horizontal valve plate 9·5 in. diam. The baffled pump speed was 690 l./sec. A circular plate was inserted in the vessel to divide it into two compartments and the air pressure in the upper compartment controlled by a needle valve. The lower compartment had a height which was about equal to its diameter (as shown in Fig. 1). Pressures were measured by a Penning gauge P_1 in the high-pressure compartment and a B.A. gauge P_2 in the low-pressure compartment; both gauges were calibrated against a McLeod gauge. The beam detector was mounted in the lower compartment with its filament electrodes always normal to its plane of movement to preserve symmetry when varying the incidence angle.

The B.A. electrodes of the beam detector were mounted in a stainless-steel tube of $1\frac{5}{8}$ in. diam. by $2\frac{1}{4}$ in. length, as shown in Fig. 1. The grid was $\frac{3}{4}$ in. diam. and because ion collection was limited to ions formed within this region the resolution of transverse density variation was determined by the grid rather than the tube diameter. The sensitivity of the beam detector in terms of ion to electron current ratio was 15/torr and equal to that of the ion gauge P_2 which had the same electrode arrangement. The amplifier used with the beam detector had a backing-off facility allowing the measurement of ion currents of about 10^{-10} amp on a background level of 10^{-8} amp; values of $P_g^* = 10^{-7}$ torr could be measured in the presence of $P_c = 10^{-5}$ torr.

Initial measurements were made of the density at different incidence angles produced by gas effusing from the high-pressure vessel through an aperture (2·26 cm diam.) in a thin plate. The beam intensity for free molecular flow at a distance l from the source is given by $n_s\bar{v} = N \cos\theta \cos\varphi/\pi l^2$, where N is rate of molecular effusion and θ and φ are the angles of incidence at the emitter and receiver planes, respectively. $N = A\, n_1\, \bar{v}/4$, where n_1 is the density of gas in the upper vessel and A is the aperture area.

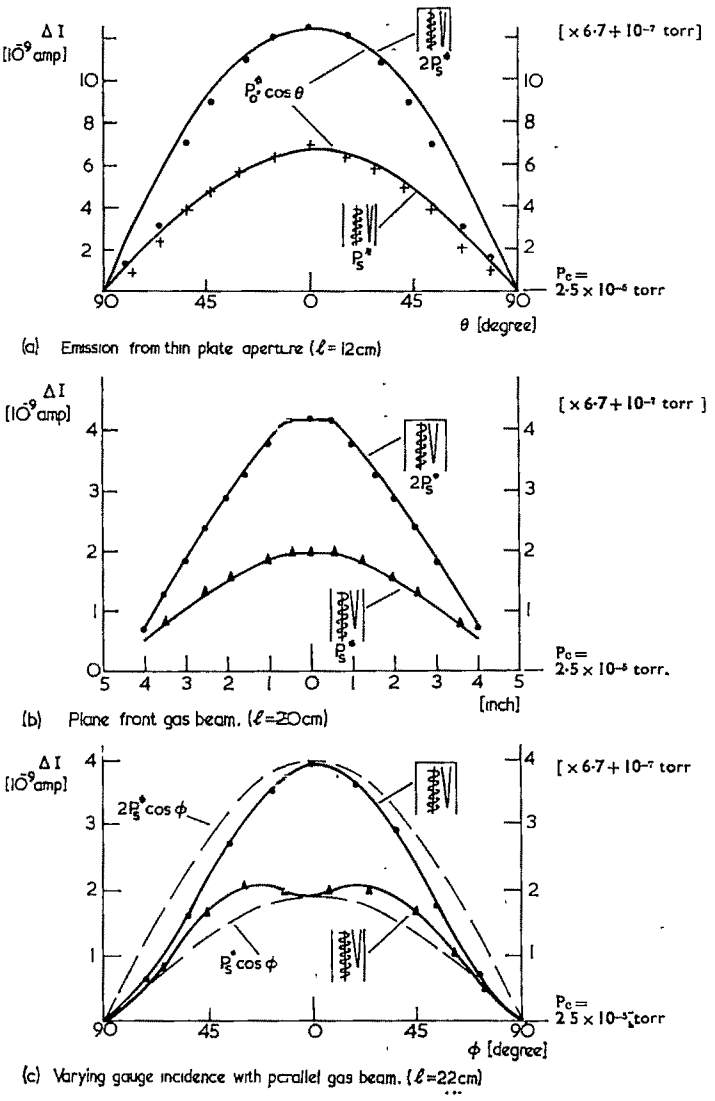


Fig. 2

With $P_1 = 10^{-3}$ torr, $P_2 = 5 \times 10^{-5}$ torr, and the beam detector at $\theta_u = 90°$, the beam current could be set at zero as cos 90° = 0. When the detector was turned around the gas source the expected cosine dependence of ion current on incidence angle was obtained (Fig. 2*a*). Using the same beam conditions the tubular detector was closed at one end to deflect the streaming gas back into the head and this doubled the ion current as shown in Fig. 2*a*. The gas molecules beaming into the detector at $\varphi = 0$ are scattered by collision with the end-cover and pass, on average, once more through the ionizing region. P_c did not vary with the detector position because of the apparatus design. P_c was equal to the equilibrium pressure P_2 given by the relation $Q = P_2 S$, where Q was the quantity of gas entering the system in unit time and S its volumetric rate of removal.

Having established that the beam detector gave an ion current proportional to n_s for $\varphi = 0$, experiments were made at a constant beam density and varying detector incidence. To provide a

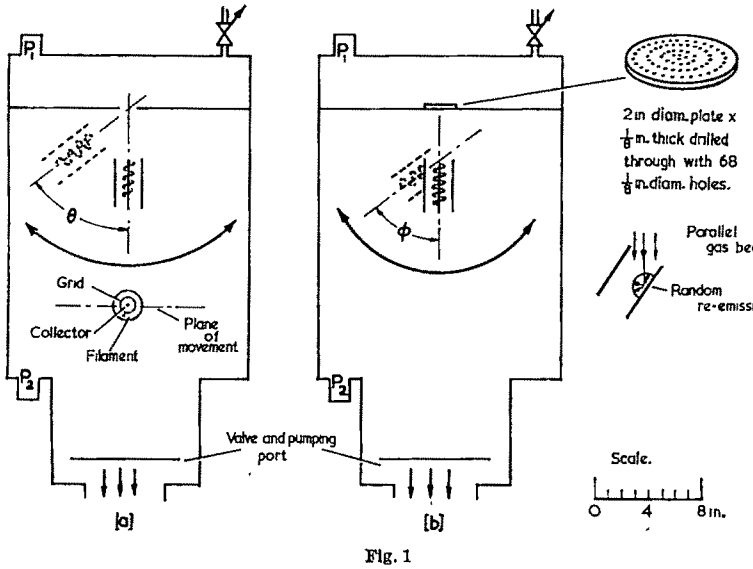


Fig. 1

parallel beam of uniform intensity a plate $\frac{1}{8}$ in. thick by 2 in. diam. was drilled with 68 holes $\frac{1}{8}$ in. diam. so that the individual gas streams formed a single uniform base. The ionization detector was initially traversed at normal incidence across the molecular beam with l constant and this showed a central region of uniform beam density of 1·5 in. diam. comparable with the diameter of the detector tube.

The detector was then rotated in the uniform beam region to vary φ with l constant. The results plotted in Fig. 2c show that the closed gauge gave readings only approximating to a cosine function of φ. When the detector end-cover was removed the sensitivity in terms of beam density decreased as expected, but the I_s versus φ curve had a maximum either side of $\varphi = 0$. When the beam was incident normally the stream passed directly through the open tube, but for $\varphi > 0$ the molecular beam struck the tube wall and was re-emitted into the ionizing region. When the detector was turned on its cylindrical axis to bring the filament into the plane of incidence the I_s versus φ curve became asymmetric.

It follows from the foregoing that a B.A. gauge with a tubular envelope of high conductance can be used to find the components of gas density with a random distribution and preferred direction, that is, n_c, n_s and φ, respectively. However, it is first necessary to determine the beam enhancement factor as a function of the inlet incidence φ angle for the particular gauge used. The instrument cannot show whether a direction of greatest density flow arises from a single beam or a combination of beams with different directions, because it does not measure molecular velocity in any direction. However, the device may find use in systems exhausted at high pumping speeds, for example, by a cryopump, or in apparatus used for measuring the speed of pumps or calibrating vacuum gauges.

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A SYSTEM OF MESOPIC PHOTOMETRY

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To the dark-adapted or scotopic eye, lights of different spectral power distributions, say $E_1(\nu)$ and $E_2(\nu)$ per unit wavenumber interval, will have the same luminosity if:

$$\int E_1(\nu)\, V'(\nu)d\nu = \int E_2(\nu)V'(\nu)d\nu$$

where ν is the wavenumber of monochromatic radiation, and $V'(\nu)$ is the scotopic luminosity function, such as that defined by the Commission Internationale de l'Éclairage (C.I.E.) for the 'standard eye'. A similar equation holds for matches with the light-adapted, or photopic eye, except that $V'(\nu)$ is replaced by $V(\nu)$, the appropriate C.I.E. function for this condition. V and V' are analogous to the response functions of photoelectric cells, and may be defined as the reciprocal of the power of monochromatic radiation of wavenumber ν, required to produce a certain degree of visual sensation under the appropriate conditions. The scale is usually such that V or $V' = 1$ at the wavenumber for which the function is greatest[1]. However, in what follows, the functions will be normalized so that their integrals will give a direct measure of the equivalent luminance of the test source when compared with the standard full radiator source at 2,042° K. That is, two integrals may be defined:

$$S = \int E(\nu)\, v'(\nu)d\nu$$
$$P = \int E(\nu)\, v(\nu)d\nu$$

where S is called the 'scotopic equivalent luminance', P the 'photopic equivalent luminance' of the source, and $v'(\nu)$ and $v(\nu)$ are the normalized scotopic and photopic functions.

Now in general, because of the difference in shape between v' and v, a light of spectral power distribution other than that of the standard white itself may not have the same values for S and P. For example, a red light, originally matching the standard at a low level, may look some hundred times brighter than the standard when both lights are increased in the same physical proportions so as to be in the photopic condition. This is an example of the Purkinje effect[1]. The problem is to predict the photometric relation between lights in the mesopic range, that is, between true scotopic and true photopic conditions.

Solutions to this problem have been bedevilled by the notion that there ought to be a series of functions appropriate to the mesopic eye, intermediate in shape between v' and v, and of the same integrating properties. That this applies approximately in some cases at least has been reported by the C.I.E. committee on mesopic vision[2], but in general the method is not satisfactory (see, for example, the comments of Bridgman[3] and Wright[4]). Even if this approach works perfectly, the appropriate function cannot be precisely known until after measurement of the light source, and must therefore be found by a tedious iterative method of successive approximations.

Another approach which seems to work within an observer's experimental precision may be illustrated by

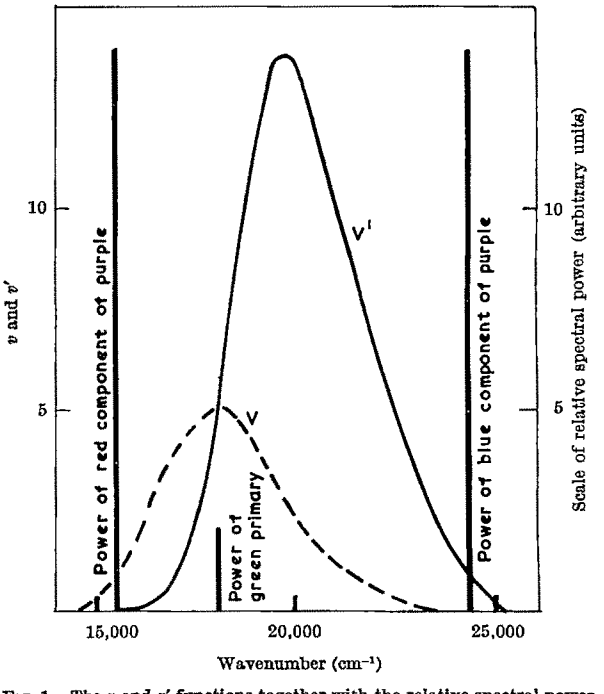


Fig. 1. The v and v' functions together with the relative spectral power distributions of a green and a purple test light of the same scotopic and photopic luminances, to illustrate the proposed method of mesopic photometry

Fig. 1, which represents the v' and v functions. Consider a monochromatic green light taken at the wavenumber where the curves intersect. Obviously the light has the same scotopic and photopic luminances S and P. That is, $S = P = L$, where L is the luminance of the matching standard. Consider also the purple light composed of two monochromatic radiations, a red at a wavenumber where v is much greater than v', and a blue at a wavelength where v' is much greater than v. Obviously the components may be adjusted to give this purple the same values for S and P as the green light, the blue component contributing most of the scotopic luminance and the red most of the photopic luminance. Thus these purple and green lights, together with the white standard illuminant itself, if adjusted to match each other at scotopic levels, will also match at photopic levels when they are all increased physically in the same proportion. It seems not unlikely that they might also keep in match in the mesopic range, approximately at least. If this were so, then by extension the same property would probably be found in a huge family of purples, greens and whites of various spectral types, monochromatic and continuous, all characterized by $S = P$. The next obvious generalization is that the photometric behaviour of lights in the mesopic range could possibly be characterized by their pairs of integrals S and P (or by other pairs of functions derived from these) where, unlike the above examples, S has not necessarily the same value as P. Experiments were therefore made to test this hypothesis.

An immediate difficulty in the design of the experiments was that the C.I.E. V' function is defined for a 20° field eccentrically fixated as in Crawford's[5] original work, while the V function is defined for a 2° field centrally viewed. Hence, an experimenter wishing to bridge these conditions must decide how to change his method of viewing systematically through the mesopic range. Fortunately, the C.I.E. has recently suggested a photopic function for a 10° field centrally viewed[2], so it was decided to use a similar field throughout, in the hope that the V' function would not be totally inappropriate to this condition. This choice was underlined by the use of the National Physical Laboratory's trichromator[6,7] on which, indeed, Stiles had measured the new 10° photopic function.

The next problem was the choice of conditions for heterochromatic photometry. As is well known, heterochromatic matching by direct comparison is not particularly additive at photopic levels[4], and additivity, or something like it, is desirable in all photometry. On the other hand, flicker photometry, although additive at photopic levels, is not satisfactory in the mesopic range[4] because the flicker is then difficult to see. Therefore to compare these conditions it was decided that one observer (D. A. P.) should make both flicker and direct comparison matches at several levels (see later).

The central tier of the trichromator was used to provide thirty different test lights, which included fourteen monochromatic radiations of the purity reported by Stiles[6,7], and eight combinations of two or more of these. The remaining eight test lights had continuous spectral distributions and were obtained by removing the central slit and mask and inserting coloured filters. Since the trichromator is an excellent double monochromator, the relative spectral power distributions of the test lights could be measured by running a slit through the spectrum, while recording the output on a photomultiplier whose spectral response had been measured in the Radiometry Section of the National Physical Laboratory.

The relative spectral power distributions per unit wavenumber interval of the test colours together with the numerals used in the diagrams are given in Table 1 (the colour names are intended only as rough guides to the appearance of the lights). It was convenient to express the radiation in wavenumbers because Stiles had already

Table 1. Spectral Specifications of the Test Lights

The fourth digit in each value is an index representing the multiplying power of 10. Where appropriate, the values are normalized to 100 (1·00 + 2) at 15,500 cm^{-1}

Monochromatic test lights							
Light No.	1	2	3	4	5	6	7
Wavenumber, cm^{-1}	13,750	14,625	15,500	16,375	17,250	18,125	19,000
Light No.	8	9	10	11	12	13	14
Wavenumber, cm^{-1}	19,875	20,750	21,625	22,500	23,375	24,250	25,125

Test lights with more than one monochromatic component
Relative powers of components

Component wavenumber, cm^{-1}	Light No. and colour name							
	15 green	16 yellow	17 yellow	18 red	19 purple	20 purple	21 purple	22 purple
15,500	—	1·00 + 2	1·00 + 2	1 00 + 2	1 00 + 2	1 00 + 2	—	—
17,250	1·00 + 2	—	—	—	—	—	1·00 + 2	1·00 + 2
19,000	—	1 16 + 1	1·16 + 1	4 22 + 0	—	—	—	—
20,750	1·73 + 1	6·00 + 0	—	—	—	—	—	—
22,500	—	—	—	—	1·46 + 1	5·50 + 1	5·09 + 1	2·36 + 2

Test lights with continuous spectral distributions
Relative spectral powers per unit wavenumber interval

Wavenumber, cm^{-1}	Light No. and colour name							
	23 red	24 purple	25 yellow	26 green	27 blue	28 white	29 blue	30 standard
13,750	2 97 + 2	5·65 + 2	2·46 + 2	2 17 + 3	1·44 + 3	2·62 + 2	3·19 + 5	2·46 + 2
14,625	1·77 + 2	3·31 + 2	1·58 + 2	7·42 + 2	5 39 + 2	1·51 + 2	5·33 + 4	1·61 + 2
15,500	1·00 + 2	1·00 + 2	1·00 + 2	1·00 + 2	1·00 + 2	1·00 + 2	1·00 + 2	1·00 + 2
16,375	3·03 + 1	1·03 + 1	6·28 + 1	1·89 + 2	2·74 + 0	4·31 + 1	0·00 + 0	6·38 + 1
17,250	1·07 + 0	6·08 + 0	3·33 + 1	8·71 + 2	1·89 + 0	2·56 + 1	0·00 + 0	3·62 + 1
18,125	0·06 + 0	4·68 + 0	1·90 + 1	1·07 + 3	2·09 + 1	2·71 + 1	0·07 + 0	2·49 + 1
19,000	0·02 + 0	7·30 + 0	7·88 + 0	8·49 + 2	8·44 + 1	2·35 + 1	1·88 + 3	1·65 + 1
19,875	0·00 + 0	1·47 + 1	2·29 + 0	5·21 + 2	1·37 + 2	1·66 + 1	2·59 + 4	1·04 + 1
20,750	0·00 + 0	3·05 + 1	0·77 + 0	1·91 + 2	1·25 + 2	1·08 + 1	6·67 + 4	6·32 + 0
21,625	0·00 + 0	3·88 + 1	0·01 + 0	3·15 + 1	8·65 + 1	6·61 + 0	7·43 + 4	3·67 + 0
22,500	0·00 + 0	2·72 + 1	0·00 + 0	3·61 + 0	4·76 + 1	3·10 + 0	3·97 + 4	1·81 + 0
23,375	0·00 + 0	1·50 + 1	0·00 + 0	0 36 + 0	1·76 + 1	1·57 + 0	1·07 + 4	1·02 + 0
24,250	0·00 + 0	8·15 + 0	0 00 + 0	0·07 + 0	7·38 + 0	0·73 + 0	1·53 + 3	0·36 + 0
25,125	0 00 + 0	2·10 + 0	0·00 + 0	0 00 + 0	1·39 + 0	0 23 + 0	1 67 + 2	0·11 + 0

used the instrument in this way[6,7]. Some of the chosen wavenumbers are Stiles's primaries, the rest are interpolated to give fourteen evenly spaced points through the visible spectrum. Included among the lights is a white (light no. 30) corresponding as closely as possible to a full radiator at 2,042° K. This is the standard source recommended by the C.I.E. for photometry[2], so that physical measurements of its power may be taken as a direct measure of its luminance. In fact, the actual source had an equivalent colour temperature of about 2,060° K, and its chromaticity ($x = 0{\cdot}520$, $y = 0{\cdot}417$, $z = 0{\cdot}063$ in the C.I.E. 2° system) fell on the green side of the locus, the red and blue regions of the spectrum being slightly deficient. This slight variation from the ideal standard does not invalidate the principle of the method described here, and a correction can be applied to relate the data to the ideal radiator (see later).

The luminance L of the standard field was measured at several levels with a Macbeth illuminometer previously calibrated against a standard lamp at 2,042° K. The corresponding outputs from the photomultiplier were measured to establish a scale for L. The photocell and associated equipment had been found to be linear to within ±2 per cent over the large range used. In the data, the field brightness will be expressed in trolands entering the eye from the aerial pupil, the dimensions of which were about 1·0 mm horizontally by 1·5 mm vertically.

The standard and other test lights could be attenuated neutrally over the enormous range required by means of gauze screens and an iris, through which the light from the lamp fell on to a translucent diffusing screen in front of the entrance slit of the instrument. Some stray light at low levels, no great problem in a good double monochromator, was removed with baffles.

The field was circular, 10° in subtense, with a dark surround. It was divided horizontally for the direct comparison matches, the test lights, including the standard white, appearing in the upper half of the field. They were matched in turn against another white light in the lower half, of nearly the same chromaticity as the standard, but the spectral distribution of which was not so closely known. However, this knowledge was not strictly necessary, as the lower white field served merely as an intermediary for the comparison of the test colours with the standard white. By this substitution technique, the test colours were in fact compared with a standard which occupied the same part of the visual field as themselves. For flicker photometry a similar substitution method was used except that the test lights and the intermediary white now occupied the whole field successively. After each match had been made, the observer removed his head from the dental impression and forehead rest, to permit measurements of the amounts of light on both sides of the match with the calibrated photomultiplier in the manner described by Crawford[8].

From these measurements and from the photomultiplier response, the spectral power distributions being known, it was possible to calculate the values of the integrals S and P for each light in match with the standard of luminance, L. The C.I.E. scotopic function was used to compute S, and the proposed C.I.E. 10° photopic function was used to compute P.

The observers made matches at levels selected from the following: (1) 620 trolands; (2) 62 trolands; (3) 6·2 trolands; (4) 0·62 trolands; (5) 0·062 trolands; (6) 0·0062 trolands; (7) 0·00062 trolands. At the lowest level, which is only some three times greater than the visual threshold, and at levels 5 and 6, the observers were dark-adapted for 1 h before making matches. At level 4, half an hour's dark adaptation was deemed sufficient, and for the other levels there was no adaptation from the normal room illumination. Even at the lowest levels, the deep monochromatic reds, below 16,375 cm^{-1}, still retained their reddish appearance. Only a selection of the test lights was used at the highest and lowest levels, which were done merely to ensure that true photopic and scotopic conditions had been reached. Miss B. J. W. used only a selection throughout.

The observers' ages were; Miss B. J. W., twenty-one years; J. F. C., twenty-three years; D. A. P., thirty years.

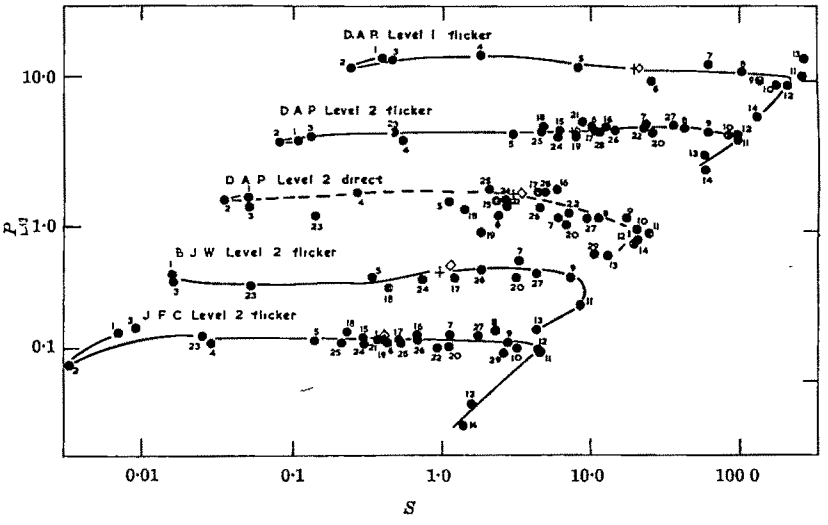


Fig. 2. Logarithmic plots of the photopic luminance, P, against the scotopic luminance, S, for each test light matching the standard at level 1, 620 trolands, and at level 2, 62 trolands

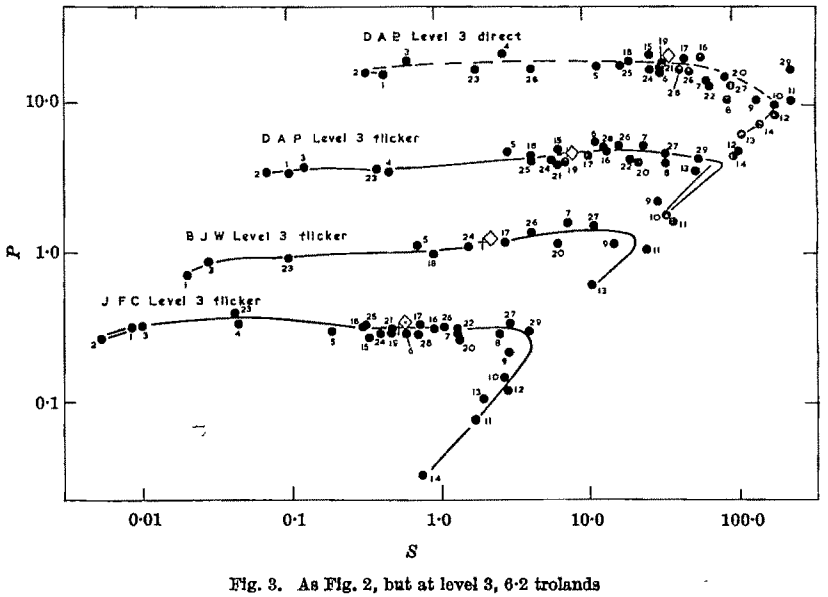


Fig. 3. As Fig. 2, but at level 3, 6·2 trolands

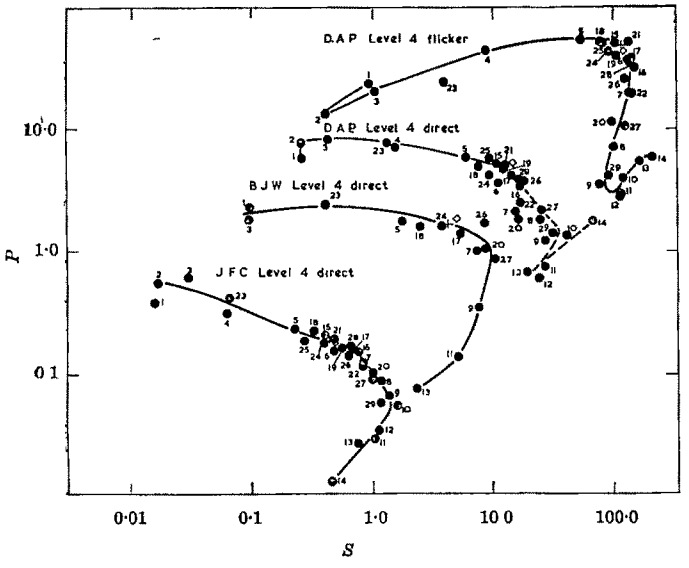


Fig. 4. As Fig. 2, but at level 4, 0·62 trolands

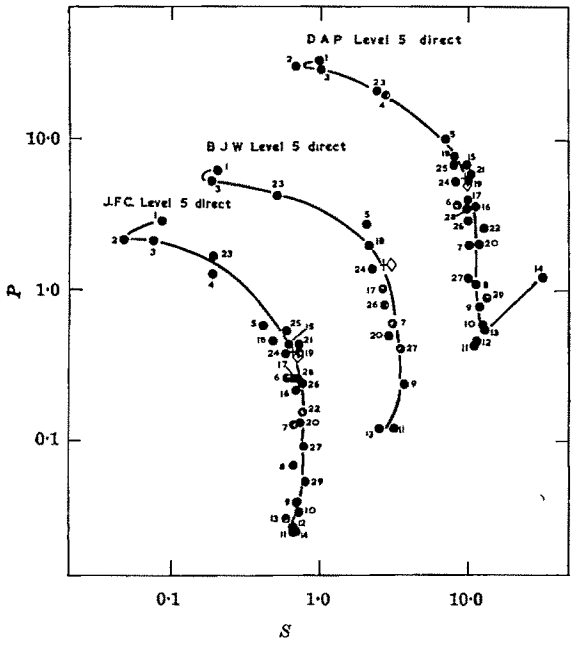


Fig. 5. As Fig. 2, but at level 5, 0·062 trolands

The observers allowed their eyes to roam freely, but made their final matches while looking towards the centre of the field, judging it as a whole. They were not troubled by any apparent non-uniformity in the field. Fifteen to twenty matches were made for the important comparisons between the standard white and the intermediary, and three to five matches for the other test lights. The arithmetical average of each set was used to plot the diagrams.

A convenient and descriptive way of displaying the results is to plot log P against log S for the set of test lights matching the standard at a given luminance, L. If the hypothesis holds, then lights matching the standard and having the same ratio between S and P should all fall together in the diagram at the same point. In other words, the locus of log P against log S for a given value of L should be unique.

The experimentally determined points are shown in Figs. 2–6. The luminance-level and conditions of match, whether by flicker or direct comparison, are given in the diagrams. The curves have been shifted along the 1 in 1 line by an arbitrary amount in order to display each observer's results separately. This may be done with a logarithmic scale, of course, without distorting the shape of the graphs. The point where $S = P = L$, representing the luminance of the standard white at which each curve was obtained, is shown as a lozenge (◈).

If the hypothesis holds, the loci should pass through this point, as indeed they generally do. The loci are very distinct with no great spread of points about themselves. The exceptions, the direct comparison results (D. A. P.) at the highest levels, apparently display non-additivity. The more saturated the colour, the less is required for a match against the standard white than would be expected from the computations from the $v(\nu)$ curve (the Helmholtz–Kohlrausch effect).

In general, however, the standard deviations of all the points about the mean loci, averaged for the three observers, are ± 7·6 per cent at level 2 (flicker, photopic), ± 7·1 per cent at level 3 (flicker, mesopic), ± 8·8 per cent at level 4 (direct comparison, mesopic), ± 6·6 per cent at level 5 (direct comparison, mesopic), ± 8·5 per cent at level 6 (direct comparison, scotopic). These figures illustrate that the precision of this method in specifying luminances in the mesopic range is comparable with what is found in photopic (flicker) and scotopic (direct comparison) photometry. Indeed, the deviations must largely reflect the precision of the observer since there is no systematic tendency to set particular matches above or below the mean locus, with the exceptions just noted.

On the other hand, if 'mesopic luminosity functions' are derived from the monochromatic matches, and used to predict the matches for the test lights of more than one

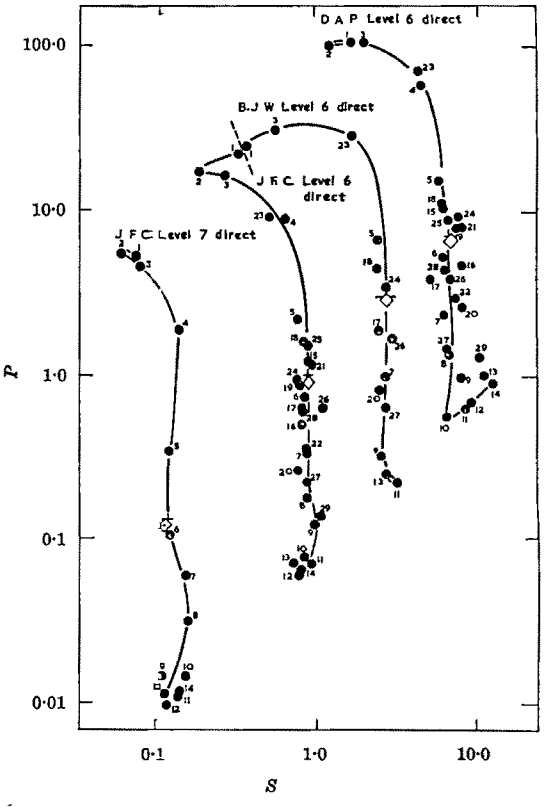


Fig. 6. As Fig. 2, but at level 6, 0·0062 trolands, and at level 7, 0·00062 trolands

primary, then the standard deviations of the errors become much larger, for example, some ±20 per cent at level 4. The purples fare particularly badly with this method. For example, from J. F. C.'s results at level 4, the discrepancies are −15 per cent for light No. 19, +27 per cent for No. 20, +22 per cent for No. 21, +29 per cent for No. 22, −28 per cent for No. 24, compared with discrepancies of 0, +3, +6, −2 and −4 per cent for the same matches plotted on the S, P diagram.

The expected shape of the loci will now be discussed. For an 'additive' eye, scotopic conditions should produce a series of rectilinear loci parallel to the log P axis, and similarly, photopic conditions should produce rectilinear loci parallel to the log S axis. Approximations to such loci are found, but there are important deviations from the ideal scheme. The scotopic curves, for example, are still curled at the red end, even at the lowest level (see, for example, J. F. C.'s level 7), which is lower than that at which Crawford[5] determined the scotopic function. The effect is almost certainly due to the observers' viewing the field centrally, thus using more cones than did Crawford's observers. However, the loci are well defined in the red end (for example, the deep red test light No. 23 with a continuous spectral distribution fits well with its neighbouring monochromatic lights), and in any event it is fruitless to search for a series of parallel lines in the reds at scotopic levels, as the deep reds, notoriously, never appear to settle down to true scotopic conditions[4]. Another peculiarity is the tail on each locus at the violet end, which means that the photopic function is too low there by a factor of two or more. Probably since the present observers are younger than the mean age of Stiles's[6] observers (to whom the photopic function is due) they have not yet acquired so much yellow pigment in their ocular tissues, and hence are more sensitive to violet light. If the photopic function were adjusted to eliminate the tails, the loci would still double back on themselves (similar loops are found at the red end). These are because the v' function at first falls less quickly than v, but then starts to catch up, with the roles of the functions reversed at the red end. The loops are found on most loci, whether photopic, mesopic or scotopic, but as they affect only the extremities of the spectrum they are probably not very important practically.

In short, the C.I.E. scotopic function and the proposed 10° photopic function serve well for the computation of S and P for the three observers, with the reservations just noted. The agreement between direct comparison and flicker photometry is not perfect, especially at the ends of the spectrum (for example, D. A. P.'s results in Fig. 4), but for most test lights the discrepancies are not great.

In the mesopic range the shape of the loci can be found only by experiment. The main point of the experiments is that mesopic loci can indeed be determined, as definite as those for photopic and scotopic conditions. A system of mesopic photometry based on the S, P diagram is therefore possible.

In this connexion, the most interesting families of lights are the greens, purples and whites which lie so close to each other in the diagram, showing that colours of different chromaticities may behave similarly in photometry, provided that their S, P co-ordinates are similar.

The reds, oranges and yellows also show this, together with the complementary phenomenon that colours of the same chromaticity need not lie near to each other in the S, P diagram. For example, the yellow light No. 25 falls some distance from the monochromatic yellow No. 5 of similar chromaticity, while No. 18, of almost the same chromaticity as the red No. 23, falls closer to the yellow No. 25.

These observations illustrate that the integrals $S = \int v'(\nu)\, E(\nu)d\nu$ and $P = \int v(\nu)\, E(\nu)d\nu$ are not the same as their mathematical analogues in colorimetry:

$$X = \int \bar{x}(\nu)\, E(\nu)d\nu;\quad Y = \int \bar{y}(\nu)\, E(\nu)d\nu;\quad Z = \int \bar{z}(\nu)\, E(\nu)d\nu$$

although of course $v(\nu) = \bar{y}(\nu)$ in some systems. These resemblances and differences between S, P and X, Y, Z were pointed out by Mr. J. N. Greene.

To sum up, the experiments suggest a simple and accurate system of mesopic photometry, which corresponds to what observers actually see, and which takes in the scotopic and photopic conditions as special cases. As an example of the system in practice, only two photocells would be required, corrected to appropriate scotopic and photopic luminosity functions. The equivalent luminance of any source could then be measured by comparing responses on the cells with those for the standard light-source at 2,042° K. These measurements would yield S and P, which would then be plotted as a point on an S, P diagram with loci determined for the 'average' observer. The equivalent luminance L of the unknown source would then be read off directly by interpolation between the loci of constant L, whether L lay in the scotopic, mesopic or photopic ranges.

As mentioned earlier, it may not be necessary to have a perfectly ideal source at 2,042° K in order to make the measurements for the 'standard observer'. Provided that he obeys the laws apparently in evidence here, it would be permissible to use the results to compute the luminance of the ideal radiator when compared with the standard actually used, and from this computation, to refer all other sources to the ideal. This mathematical process is like a transformation to ideal primaries in colorimetry. The points representing the ideal source computed from the present data are shown as crosses (+) in the S, P diagrams.

A better theoretical argument than the heuristic approach sketched out in the introduction will now be attempted. The sufficiency of only three integrals to define chromaticity has always been taken as strong evidence for three types of retinal receptor mediating in the perception of colour. Thus the present requirement of only two integrals S and P to define equivalent luminance, which are mathematically similar to the chromaticity integrals, might by analogy imply the existence of only two types of receptor to mediate in the perception of luminosity, even if not more were known. But of course the scotopic and photopic functions are already assumed, from other evidence, to represent the activities of the rods and the cones—very plausible candidates for the role of luminance detectors. The integrals S and P could therefore be regarded as representing the basic responses of the rods and cones, which are combined by the eye in a proportion depending on the level, to give the perceived luminosity. At low levels the rods alone would contribute, and at high levels only the cones, with both types effective at mesopic levels in the proportions implied by the loci of constant luminance in the S, P diagram. It follows that lights stimulating the rods and the cones in the same ratio before the summation process is considered would exhibit identical photometric behaviour at all levels. From this point of view, the phantom mesopic luminosity functions vanish.

Of course the scheme is complicated by the existence of three types of cone, which presumably causes the discrepancies already noted in direct comparison matches at high levels. However, the present photopic results, measured by flicker photometry, agree well (except at the violet end) with Stiles's proposed 10° function derived from colorimetry which is, after all, another form of direct comparison matching. This suggests that an additive photopic luminosity function may be independent of the method of its measurement. Extensions of the present experiments could probably yield information about summation processes from pure cone and pure rod responses, but it is not intended to pursue this further here, but merely to demonstrate and to justify a practical method of mesopic photometry.

Of course, several questions remain unsettled. For example, is 10° the best field size? Can the difference

between flicker and direct comparison matching be bridged more satisfactorily? Is there sufficient agreement between normal observers to justify setting up a defined average? Should the measurements be done monocularly with a restricted artificial pupil, as in the present experiments, or should more natural binocular vision with unrestricted pupils be specified? The latter case involves, of course, the effect of pupillary contraction, and the great differences known to occur between observers in this respect. Do the scotopic and photopic luminosity functions need revision? It would be absurd, for example, to define a standard mesopic observer who was youthful scotopically and middle-aged photopically. It is hoped that further experiments will resolve these questions.

This work was undertaken as part of the official research programme of the National Physical Laboratory. I thank Mr. J. N. Greene for his help.

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WIND DRAINAGE OFF THE HIGH PLATEAU OF EASTERN ANTARCTICA

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By K. B. MATHER and G. S. MILLER
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It is well known that coastal stations on or close to the steep ice slopes of Antarctica experience strong downslope (katabatic) winds, the direction of which accords with the direction of maximum slope of the ice surface if allowance is made for the easterly component introduced by Coriolis acceleration. The mechanics of the air layer on a sloping surface under conditions of temperature inversion have been discussed by various authors, notably by Ball[1-3]. Quantitatively, the accelerations involved have the magnitudes $A_k \approx g\alpha\ \Delta T/T$ and $A_c = 2\Omega V \sin\varphi$, respectively, where g is acceleration due to gravity, α the surface slope, T the potential temperature, ΔT the inversion strength, Ω the Earth's angular velocity, V the wind speed and φ the latitude of the observing station. Superimposed on the katabatic component, in general, will be the acceleration due to the horizontal pressure gradient, $A_p = \Delta P/\rho$. The fact that most, if not all, coastal stations located at the foot of the steepest slopes record consistently downslope drainage during most of the year means that A_k is considerably greater than A_p. Moreover, it can readily be shown[3] that this result is quite plausible by making numerical estimates of A_k and A_p using reasonable values of ΔT and for the synoptic gradient.

However, coastal stations present a rather special case, with conditions weighted heavily in favour of the katabatic component because of the steep slope; α tends to average between 1 in 20 and 1 in 30 for the first 10 km inland, and may reach 1 in 10 within a kilometre or so of the shore. Further inland, the slope generally declines to 10^{-2} well within the first 100 km, although notable variations occur. (The most extensive fetch of steep slope is the hinterland of the Princess Astrid Coast, where α apparently averages about 10^{-2} for 400 km inland.) Across much of the central plateau of Eastern Antarctica α approaches 10^{-3}. For these much smaller values of slope, A_k can still be significant and possibly comparable with A_p, especially as severe inversions can be expected, but it has not been known whether the katabatic component is sufficient to manifest itself in the wind pattern in some recognizable form when cyclonic variations are superimposed. For instance, is there a general drainage off the high plateau to the coast and low-lying areas?

A previous attempt to answer this question[4-6] was based on an examination of wind data from around the vast depression in the plateau caused by the Lambert Glacier and Amery Ice Shelf. The argument ran as follows: the direction of maximum slope around the Lambert Glacier changes radically within a dimension of 500 km, which is small compared with the size of the continent. Whereas a 'prevailing anticyclone' over eastern Antarctica would tend to produce an anticlockwise circulation (not altogether inconsistent with what had been observed at a number of stations), it would be difficult to concoct a synoptic pattern that required winds to blow from the south at the south of the Lambert, from the west at the west of it, and from the east at the east of it, that is, a synoptic pattern which would match the expected gravity drainage. In fact, all available observations have confirmed that the Lambert Glacier is a 'sink' into which cold air drains from the surrounding plateau to the east, south and west, including slopes for which α was much less than 10^{-2}. The results established that the katabatic regime encompassed a much more substantial area of Antarctica than a narrow coastal strip.

Now, however, as a result of more numerous station and traverse-party observations during and since the IGY, there is much more evidence bearing on the katabatic question. The purpose of this article is mainly to present accumulated data on the gross drainage pattern, with emphasis on the high plateau of Eastern Antarctica, though not exclusively so. Data have been analysed from McMurdo, Hallett, d'Urville, Charcot, Wilkes, Mirny, Pionerskaya, Komsomolskaya, Vostok, Sovietskaya, Davis (and precincts), Mawson (and precincts), Syowa and the vicinity of Lutzow-Holm Bay, Norway Base, Maudheim, Molodezhnaya, Lazarev, Novolazarevskaya, Halley Bay, Shackleton, Ellsworth, South Ice, Amundsen-Scott, Little America, Byrd, Roi Baudouin, and from various traverse parties and a few ship's observations offshore. For the most part the data consist of surface meteorological observations, supplemented on the traverses by sastrugi directions, which are useful indications of prevailing winds[4].

Fig. 1 shows wind roses (frequency of occurrence of wind as a function of direction) in relation to plateau contours. The short arrows elsewhere on the chart represent traverse data obtained in passage; they are not long-term averages. Concerning the coastal stations, it is essential to know the exact siting of the observation point with respect to the foot of the ice slope. Some stations are on islands offshore, some are on ice shelves, others are on ice-free expanses of rock. As the katabatic wind peters out a few kilometres from the foot of the ice slope, such stations may not register the downslope drainage even though it is well developed on the ice slopes along that same stretch of coast. (By the same token, of

course, these stations are rather more useful for synoptic analyses.) Examples are McMurdo, Davis, Lazarev, Maudheim, Halley Bay, Shackleton and Little America, all of which can be disregarded in katabatic studies. Syowa, situated on East Ongul Island 4 km from the nearest ice slope, presents an interesting borderline case[7]. Hallett is also a special case because of its location near the tip of the long Hallett Peninsula, together with a complicated drainage zone due to the peaks of the Admiralty Range. Wilkes is about 3 km from the ice slope and the station is partly sheltered by low hills; the gravity drainage seems to be considerably impeded[8].

The broad conclusion from Fig. 1 is that the wind régime of most of Eastern Antarctic is consistent with an interpretation in terms of an appreciable katabatic contribution. With the exception of one station, nowhere are the most frequent station winds contrary to the downslope direction, modified for Coriolis deflexion. The best-documented portion of the inland is that from the Mirny-Wilkes coast to the Soviet stations on the plateau, and this affords another remarkable example, like that of the Lambert depression, in which gross changes in contour directions are matched by a complete reorientation of the prevailing wind. On the line from Komsomolskaya via Pionerskaya to Mirny, the drainage is downwards towards Mirny. The three stations Vostok, Komsomolskaya and Sovietskaya, all within 540 km of one another, are dispersed around the eastern end of the (approximately) 3,500-m contour of the high plateau in such a way that the direction of maximum surface slope changes by nearly 180°. As shown in Fig. 1, the wind swings correspondingly from approximately west at Vostok to south at Komsomolskaya and to east at Sovietskaya. The essentially opposite but collinear wind vectors at Vostok and Sovietskaya, on opposite sides of the ridge, have been noted also by Russian meteorologists[9], who interpreted the behaviour as due to katabatic flow. Even more interesting, perhaps, is the fact that Vostok and Sovietskaya are only 420 km apart, which would seem to imply that katabatic winds can develop in quite a short stretch of slope, that is, the flow at any point reflects only the very local conditions of slope and inversion. Notice also the shift in direction in the course of a very short distance from Komsomolskaya on the traverse to Sovietskaya.

Although there is still little information about the winds on the western part of the plateau, for example, between the South Pole and the Princess Astrid and Princess Ragnhild Coasts, it now seems fairly certain that wind drainage must occur outwards from all parts of Eastern Antarctica, even where the slopes are $\sim 10^{-3}$. This alone does not prove that the flow is due to gravity but the pattern is strongly suggestive of a gravity contribution in many areas.

Some examples of inland slopes are: Pionerskaya $2{\cdot}9 \times 10^{-3}$, Komsomolskaya $1{\cdot}7 \times 10^{-3}$, Sovietskaya and Vostok about $1{\cdot}5 \times 10^{-3}$. These figures are all

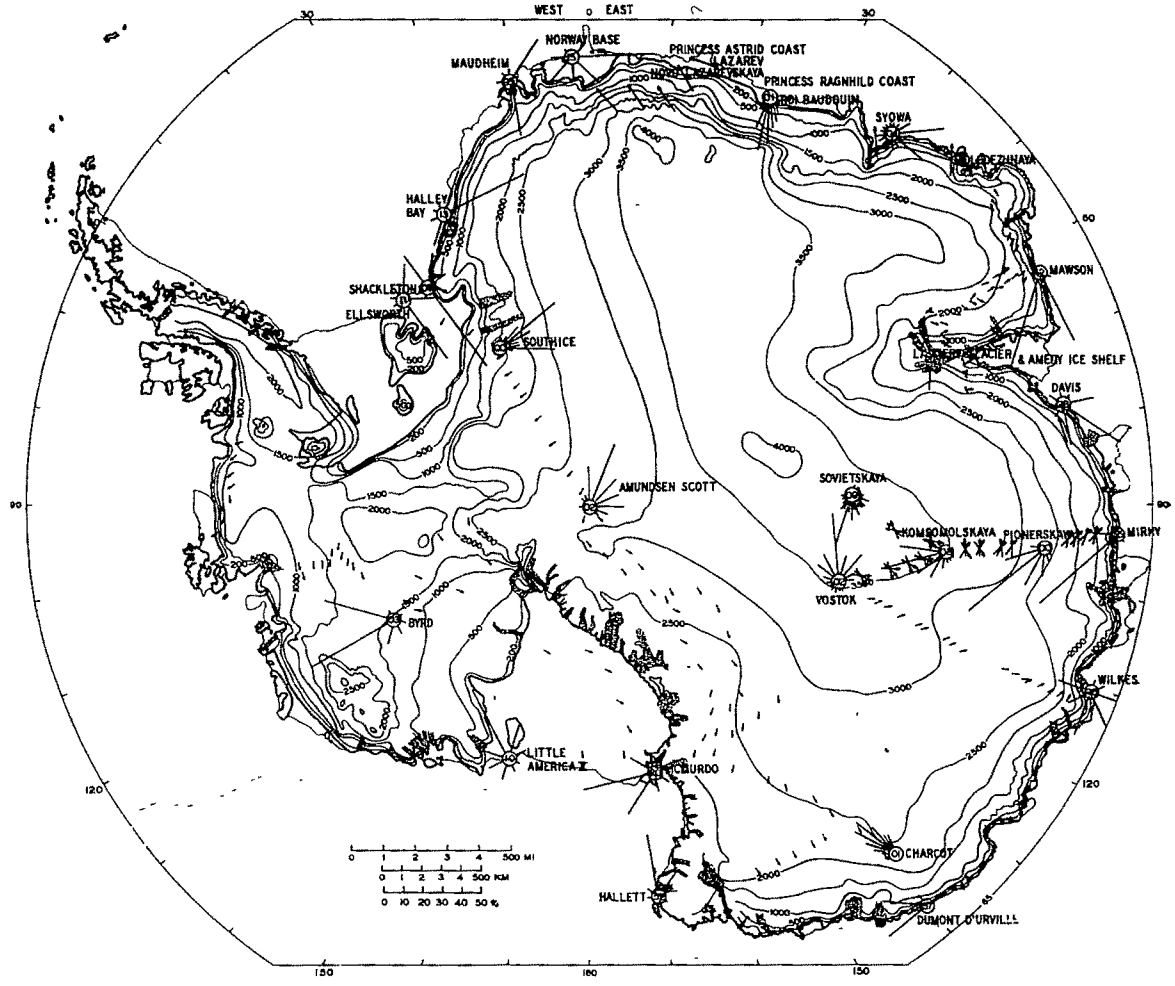


Fig. 1. Wind roses and sastrugi orientation. The orientation of snow dunes has been included on some traverses, for example, Mirny–Vostok. Since dunes are the result of cyclonic winds, their orientation varies widely

rather uncertain; the Antarctica contours are not sufficiently reliable to allow accurate interpolation. However, it is unlikely that the error would exceed 50 per cent for any one of the stations named, and this is sufficiently accurate to show that a gravity component of the wind can rival the pressure gradient component. Assuming an inversion strength $\Delta T = 10°$ C, which is quite moderate for the plateau, and a temperature $T = -30°$ C (that is, summer conditions; the annual mean temperature on the high plateau is about $-55°$ C), then for $\alpha = 1{\cdot}5 \times 10^{-3}$ we obtain $A_k = 6 \times 10^{-4}$ m sec^{-2}. This would be equalled by a pressure gradient of 6×10^{-3} mb km^{-1} at the 700 mb level, which is approximately the pressure height of the Soviet inland stations. Such a pressure gradient is not particularly strong, but one can certainly draw the conclusion that even for very strong gradients the katabatic term cannot be ignored so long as there is a surface inversion of typical strength. Notice that the slope will, for some inland stations, be twice the value adopted above. Moreover, the inversion ΔT can be much larger at times, especially during winter when it may lie between 20° and 30°. During mid-summer months it may be only a few degrees, and the probability of its being destroyed temporarily by cyclonic intrusions is greater, but throughout most of the year there is a well-developed surface inversion for which 10° is a fair representation in the first few hundred metres of height.

If A_k and A_p are comparable, the direction of inland winds can be expected to vary widely from day to day according to cyclonic conditions. A low-pressure movement across the plateau generally brings steeper pressure gradients and also more cloud cover which inhibits the temperature inversion. The wind direction will then tend towards the geostrophic pattern. The wind roses of Fig. 1 show that the inland stations do indeed experience a wide range of wind directions. However, the predominance of the downslope direction means that, averaged over the year, A_k is significantly greater than A_p across the inland regions.

The one exception mentioned earlier is Amundsen-Scott, where the most frequent wind is obviously not in agreement with the general picture. The wind direction appears to be cross-slope or even up-slope, rather than down-slope. According to Dalrymple *et al.*[10] the mean slope of the terrain surrounding the Pole is $(1{\cdot}76 \pm 0{\cdot}26) \times 10^{-3}$, with an up-slope bearing of about 152°. Strong inversions have been registered at the South Pole and it would therefore be phenomenal if a gravity component played no part in the equation of motion. However, it is quite possible that a katabatic wind is obscured by larger influences. Dalrymple *et al.* have given an interesting interpretation of the cross-slope flow at the Pole, summarizing their conclusions thus: "... the air motion in the lower atmosphere is controlled by surface friction, and by geostrophic motion in the free atmosphere above the inversion layer, modified by thermal winds due to horizontal temperature gradients which result from the general slope of the terrain".

The proximity of Amundsen-Scott to the Ross Ice Shelf and the persistent strong cyclone over the Shelf and the Ross Sea are probably important in this case. Comparing the traverse data (Fig. 1, small arrows) from South Ice to the Pole and thence to McMurdo, it seems that a fairly sharp change in wind direction occurs in the region of the Pole. Moreover, the winds on the Shelf are not very different in direction from those on the plateau to the west of the Shelf where they tend to be cross-slope. We are inclined to the view that Amundsen-Scott shares in the influence of the Ross Shelf cyclone, that is, $A_p > A_k$ in this area.

In principle, one should instrument a station to obtain accurate and frequent monitoring of the temperature and wind profiles in and above the surface inversion, along with other meteorological parameters. One could then determine the magnitudes of the several force terms in the equation and observe the actual change in wind speed and direction with respect to the relative magnitudes of the terms. Rarely, if ever, has the full instrumentation been maintained so that a systematic study can be conducted. In our analysis, using chiefly surface data collected at Antarctic stations without much anticipation for the present problem, the approach in pursuing the matter further has been to look for characteristic 'signatures' of a katabatic component, for example, diurnal cycles, sudden onsets accompanied by surging, and correlations of wind characteristics with temperature, pressure and cloud cover. We have transferred much of the available meteorological data to punched cards for computer processing. One result from this approach is described here.

The diurnal variation of katabatic winds at coastal stations is a familiar experience. To see whether this same cyclic behaviour could be detected at inland stations, power spectral analyses have been obtained from wind speed data. The spectral programme was modified from that of Munk *et al.*[11] and computations conducted on an IBM 1620; 32 periods were used for 0·5–16 days. To test the programme it was run on a year's data (6-hourly observations, 1958) from Mirny, which is a known katabatic-prone environment.

Fig. 2 shows the power spectrum for Mirny for a range of periods 0·5–5·5 days. The dominant broad peak centred at about 3·5–4·0 days presumably represents the average time lapse between successive cyclonic events and is not of interest in the present analysis. The Pionerskaya spectrum shown in the same figure, which is also based on a full year of 6-hourly data, shows the same 'cyclonic' peak.

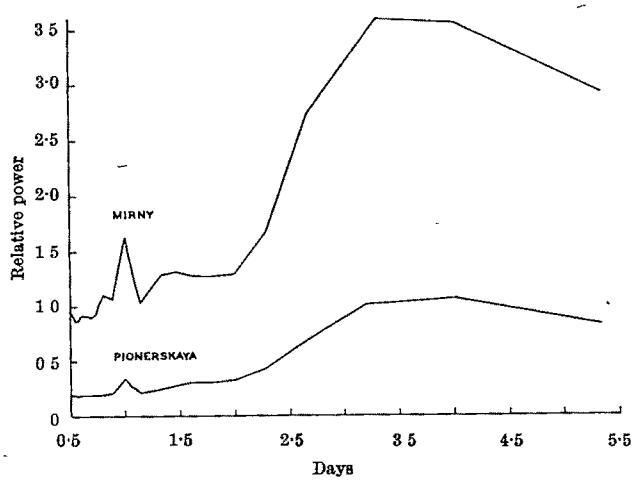


Fig. 2. Spectra of one year's data (6-h observations, 1958)

More important is the occurrence of the 1-day peak on both the Mirny and Pionerskaya spectra. Its amplitude relative to background is about the same for both stations, but its amplitude relative to the cyclonic peak is rather smaller for Pionerskaya, that is, the cyclonic influence on wind speed is relatively more important at the inland station, as would be expected.

Thinking that the diurnal variation inland ought to be more pronounced for near-summer months, as it certainly is on the coast, we have also derived power spectra for either the three months January–March or October–December, 1958. (This is not an ideal choice for 'summer'; it was made because of the availability of an unbroken sequence of data.) The amplitude of the daily cycle of insolation decreases as winter approaches, and in fact falls essentially to zero for stations within the Antarctic Circle. The incidence of cloud and storm is also greater in winter, emphasizing the cyclonic peak in the spectrum. For these reasons, to obtain the most sensitive detection of any

diurnal component which exists far inland, we have used only the summer data for Komsomolskaya, Vostok and Sovietskaya.

The summer spectra for four stations are shown in Fig. 3 on an expanded time-scale covering the periods 0·5–2·0 days. As anticipated, the 1-day peak is now more pronounced on the Pionerskaya spectrum, compared with background. Komsomolskaya shows an unmistakable diurnal cycle, and in the case of Sovietskaya some diurnal effect is at least credible. Vostok is the least convincing. There is a slight hump at about 1 day but a similar hump occurs at about 1·4 days. (Actually several of the spectra show minor peaks at ~1·3–1·6 days. It is not known whether this is fortuitous, an artifact introduced by the particular power spectral programme used, or real. The spectrum from Roi Baudouin, among others, also shows this.) If Vostok has a genuine diurnal variation it would probably be revealed by concentrating on strictly summer months, say December and January. All the spectra, when extended beyond 2 days, show the cyclonic peak.

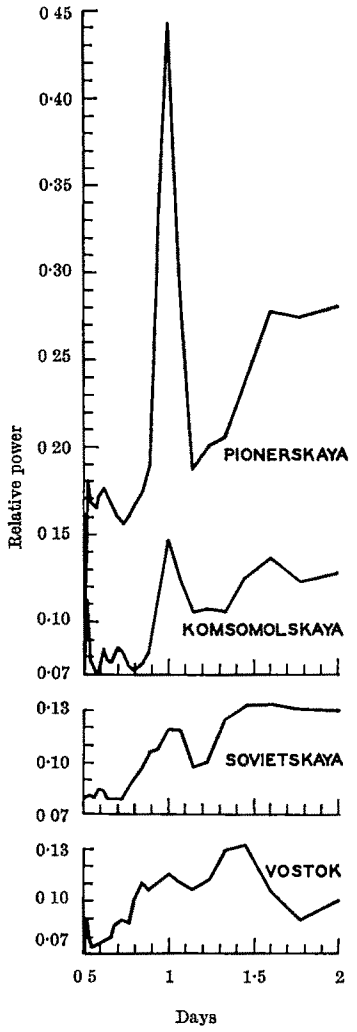


Fig. 3. Summer spectra (January–March or October–December, 1958). (Note that Komsomolskaya and Pionerskaya are on the same scale)

There is, of course, one point in Antarctica where no daily cycle of insolation occurs (ignoring any preferred direction due to the plateau surface slope). This is the South Pole itself, where the Sun maintains the same elevation throughout any one day. We have derived the power spectrum of winds recorded at Amundsen-Scott but have failed to find any trace of a 1-day peak, even though (1) the average wind speed considerably exceeds that at Komsomolskaya, Vostok and Sovietskaya; and (2) the cyclonic peak (~4 days) is again clearly evident. This implies that, not only is the South Pole lacking its own 'cyclic driving function', it is also uninfluenced by diurnal variations which must exist in the drainage of wind from other regions, for example, between the Pole and the 4,000 m summit of the plateau.

The significance of the above observation is that one should not regard the high plateau as a 'breeding ground' from which gravity winds drain radially outwards across the rest of the continent. Rather, the wind is generated locally, as in the case of glacier and valley winds, according to local conditions of slope and inversion, and its continued flow downstream depends wholly on the existence of similar favourable generating conditions downstream, not on the inertia of the air mass. Other observations, such as the very limited offshore penetration of the coastal katabatic wind (< 10 km) and the sensitive response of wind direction to small-scale changes in surface contours, also indicate that the scale of the katabatic process is quite small. We have also found that variations in the wind speed at the inland stations, Komsomolskaya, Vostok and Sovietskaya, show very little interdependence. These investigations will be discussed more fully elsewhere; for the present it is sufficient to note that the absence at the South Pole of a diurnal influence from other parts of the plateau is quite plausible. (It would be interesting to determine where, between Amundsen-Scott and the plateau summit, a diurnal component begins to manifest itself.)

The main conclusions from this work are: (1) a compilation of all available surface wind data from eastern Antarctica shows that the predominant direction of wind drainage is generally consistent with an interpretation in terms of katabatic flow, even where surface slopes are $\sim 10^{-3}$. Remaining uncertainties are due chiefly to poor information on surface contours. (2) Notable exceptions to downslope flow, all coastal, are due to the location of stations offshore, beyond the range of significant katabatic effect. (3) Drainage into the Lambert Glacier and away from the high ridge where the Soviet inland stations are located are two notable examples of localized flow specific to the pattern of surface contours. (4) The effect of pressure gradients due to cyclonic development is very marked, especially inland, but it is not sufficient to obscure the downslope flow over a prolonged period of observations. (5) The diurnal cycle of wind speed, which is a characteristic feature of coastal katabatics, can be traced to inland stations also, using methods of power spectral analyses. (6) No diurnal variation has been detected at the South Pole.

Meteorological data have been drawn from many sources, too numerous to quote individually. We thank the various national Antarctic expeditions represented by the stations on Fig. 1 for their help. The co-operation of the National Weather Records Center, Asheville, N.C., is particularly appreciated. This work was supported by the Office of Antarctic Programs, National Science Foundation, grant *GA*–28.

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LETTERS TO THE EDITOR

ASTRONOMY

Prediction of the Dates and Intensities of the Next Two Sunspot Maxima

THERE is still no satisfactory method of predicting sunspot activity beyond the current cycle, but two recent papers have provided new evidence that sunspot activity may be controlled by the positions of the planets. This possibility was discussed more than 50 years ago by Brown[1], and later by Schuster[2], and was adopted as a basis for prediction by others. The recent evidence has been provided by Jose[3] and by Wood and Wood[4]. Jose[3] has shown that the rate of change of the angular momentum of the Sun about the centre of mass of the solar system exhibits a 178-year periodicity which can plausibly be correlated with sunspot activity. Jupiter and Saturn are the planets mainly responsible. Wood and Wood[4] have suggested that the rate of change of angular acceleration of the Sun, which is controlled mainly by the inner planets, shows resemblances in detail to the sunspot numbers. These new results, although not immediately useful in predicting solar activity, do point towards the conclusion that variations in solar activity have a causal basis. If so, an empirical law for the variation of sunspot numbers should eventually be found.

Dates of sunspot maxima and minima. In an article in 1963 I suggested predicting the dates of solar maxima and minima by means of a seven-cycle recurrence tendency in the rise-time t_R from minimum to maximum activity, and a similar recurrence tendency in the quantity t_R/T, where T is the total cycle-length.

The 'official' date of the 1964 sunspot minimum is not yet known because it depends on 12-monthly running means, but it seems very likely to have been in August or September 1964. This is reasonably close to the value predicted by the seven-cycle recurrence method[5]; so the method has not been invalidated, and can be applied again to give revised dates for the next sunspot maxima and minima.

The values found by this method for the rise-time t_R in each of the next two cycles (cycles 20 and 21) are 3·4 years and 3·8 years respectively, and the corresponding values for t_R/T are 0·34 and 0·36 respectively. If the 1964 minimum is taken at 1964·7, the predicted dates for the next two sunspot maxima are 1968·1 and 1978·5, and for the next two minima, 1974·7 and 1985·3.

Although Jose's paper[3] is primarily concerned with long-term solar activity, he gives predictions for the dates of the next two maxima, namely 1967 and 1984. The predictions in the previous paragraph give no indication of the very lengthy cycle implied by Jose's date of 1984 for the maximum of cycle 21. In another recent paper, Bell and Wolbach[6] apply Danjon's formula, based on the brightness of the eclipsed Moon, for predicting the dates of sunspot minima. They obtain 1974·9 and 1985·7 as the dates for the next minima: these agree well with the predictions in the preceding paragraph, 1974·7 and 1985·3.

Intensities of sunspot maxima. The maximum smoothed monthly sunspot number in each cycle, R_m, shows wide variations with little sign of regularity[7]. We require to find a parameter which depends on R_m, but remains fairly constant. In the previous paper[5], the parameter chosen was $R_m\, t_R^2$, which usually has a value near 1750 (if t_R is in years); and Xanthakis[8] has adopted rather a similar approach, suggesting that $R_m \simeq 81 + 0{\cdot}1\,(t_R - 6{\cdot}25)^2$, with some corrections which he specifies. In both these treatments, however, the exceptionally strong maximum in 1957 has to be excluded as a freak. It seems better to devise an expression which includes the 1957 maximum.

The relation previously suggested, namely $R_m\, t_R^2 \simeq 1750$, has therefore been modified by subtracting a term strongly dependent on R_m, so as to destroy the uniqueness of the 1957 maximum, and by changing $R_m\, t_R^2$ to $(R_m - 16)\, t_R^2$, which keeps the numerical value within narrower limits. On inserting values of R_m and t_R for the past ten cycles, back to the year 1860, we find.

$$(R_m - 16)\, t_R^2 - 10^{-5}\,(R_m - 100)^4 \simeq 1401 \qquad (1)$$

with a maximum error of only 70, that is 5 per cent, on the right-hand side. Equation (1) is satisfied by all the ten sunspot maxima since Wolf introduced the concept of sunspot relative number in 1848. Some of the earlier cycles do not satisfy equation (1), but the values of t_R and R_m (particularly the latter) are of doubtful accuracy in the years prior to 1848. Nevertheless the success of equation (1) in future cycles cannot be guaranteed.

Fig. 1 shows the variation of R_m with t_R given by equation (1), with the values from the past ten cycles[7] plotted as circles. The observed values are remarkably close to the curve in Fig. 1, thus strongly indicating a causal link between t_R and R_m. Indeed, Fig. 1 may be just as good as many of the empirical relationships which have in other subjects served as forerunners to the development of true physical laws.

Fig. 1 is not ideal for use in predicting R_m, because two values of R_m are possible if t_R is between 3·3 and 3·6. However, since only one of the ten observed values lies on the upper branch of the curve (above $R_m = 170$), the lower part can be regarded as normal and is to be preferred for prediction. If the value of t_R already obtained for the present cycle, 3·4 years, is correct, Fig. 1 suggests that the highest monthly smoothed sunspot number at the maximum in 1968 will be about 140 (with $R_m = 190$ as a less likely alternative if the upper part of the curve applies). If $R_m = 140$, the present cycle may resemble the cycles with maxima in 1870 and 1947, and solar activity may, at maximum, reach the level prevailing in late 1959.

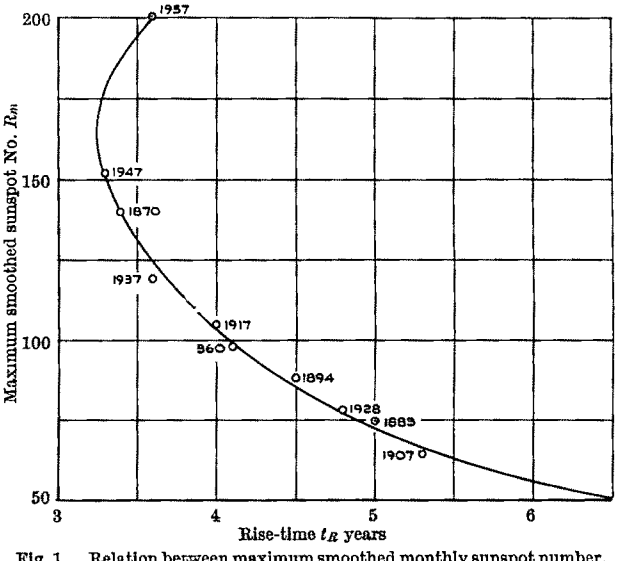


Fig. 1. Relation between maximum smoothed monthly sunspot number, R_m, and rise-time, t_R. ———, Values given by equation (1); ○, actual values in year shown

If the rise-time in the subsequent cycle is 3·8 years, the maximum R_m in 1978 should be about 110.

The predictions may therefore be summarized as follows:

	Cycle 20	Cycle 21
Date of maximum sunspot number	1968·1	1978·5
Intensity of maximum, R_m	140	110
Date of subsequent minimum	1974·7	1985 3

The validity of the prediction methods used here is of course disputable, and there can be no certainty that the predictions are even approximately correct. However, it is certain that the problem of predicting future sunspot numbers cannot be shirked, because the lifetimes of near-Earth satellites[9] are controlled by upper-atmosphere density, which is strongly affected by solar activity. The theory of satellite lifetime estimation cannot be placed on a sound basis until better predictions of solar activity are available, and the prediction of the date and intensity of the next sunspot maximum is also a matter of practical importance. For example, the U.K.3 satellite is intended to fly in a circular orbit at a height of 500 km, and its lifetime in orbit would be reduced by a factor of up to 10 if, at the time of launching (probably 1967), sunspot numbers were very high rather than slowly climbing towards a low maximum. As a second example, the *Echo* 1 satellite would probably not survive a vigorous sunspot maximum, but could last for 10 years longer if the next maximum is very weak. Plans are being made for geodetic triangulation programmes utilizing *Echo* 1, and the date of its decay affects the planning. For these and various other practical reasons (see, for example, refs. 5 and 10), the prediction of sunspot numbers must be attempted, and out of the chaos of discredited methods a correct one may well emerge.

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ASTROPHYSICS

Lightning, Solar Flares and Radio Galaxies

In 1925, when it was still believed that a potential of $n \times 10^9$ V was necessary to cause a lightning flash[1a], C. T. R. Wilson put forward the suggestion that the soft cosmic rays up to about 10^9 eV might be accounted for by runaway electrons in thundercloud fields[1b]. When it was pointed out that a lightning flash can occur with a total thundercloud potential of $n \times 10^7$ V (refs. 2[a,b]), the suggestion ceased to have much significance as applied to thunderclouds. However, in presenting a paper on these long leader strokes at the Belfast meeting of the British Association in 1952, I pointed out[2c] that the Wilson mechanism might apply in the much longer cosmic electrical discharges, in which relatively large electric fields must be built up by the short-circuiting of these much more extensive electric fields by the leader stroke of such discharges as solar flares.

In an earlier communication[2d], it was emphasized that temperatures of the order of 10^8 °K must be reached in these last electrical discharges, and in a later communication[2e] it was pointed out that observational evidence had actually been obtained for the association of such temperatures with outbursts of solar flares.

The velocity of the jets of gas generated by these discharges is of the same order as that of the thermal velocities of the ions, namely, about 10^8 cm/sec, and thus accounted for the delay of about 1 day between the solar outburst and the main magnetic storm at the Earth. The associated electron velocities will be about 40 times this value or about 4×10^9 cm/sec, and it is now suggested that some of these electrons become the runaway electrons which account for the arrival at the Earth of a soft cosmic ray component about half an hour after the observed solar outburst. Their average speed is thus about 10^{10} cm/sec, so they would only require to be accelerated by a factor of about 2 in the solar electric field.

This consideration would confirm the view arrived at from an investigation of cometary phenomena[2f] that the polarity of the solar field is the same as that of the thunderstorm field; that is, the regions farthest out in the gravitational field become positive.

This view of the existence of two major velocities of propagation of two different types of activity, a high-speed electron component and a plasma jet, has interesting possible applications in the investigation of radio galaxies, in which the same two components may be expected to occur. Observations on the gas velocities made by Seyfert[3] of about 4×10^8 cm/sec suggest the existence of plasma jets at temperatures of about 4×10^8 °K (ref. 2[g]). Associated with these we should expect to find active haloes due to the runaway electrons from the main discharges and reaching dimensions of the order of ten times the latter. This is just what is observed, and in the case of Virgo *A* (*NGC* 4486) "the general direction of the extension is the same as that of the 'jet' which optical observations have shown issuing from the nucleus of *NGC* 4486" (ref. 4*a*). Shain writes in the same contribution that "when angular sizes of a number of radio galaxies are determined, any optical search for associated galaxies must extend to galaxies having one-tenth or less of the radio diameters"[4b].

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PHYSICS

Formation of Straight Whiskers on Sublimating Single Potassium Chloride Crystal

Nannichi has reported on the formation of silicon whiskers on a sublimating surface[1]. The specimen had in this case been kept at least 200 h at 1,000°–1,250° C in an initial vacuum of less than 10^{-8} torr. The whiskers were bent and whirled but their tips were at the height of the original surface. Matter is transported away from the neighbourhood of certain places by thermal erosion so that what is left is thin enough to be called a 'whisker'.

In an investigation of the effect of annealing on the cleavage surface, straight whiskers were found on single potassium chloride crystals annealed at 600° C for 5–23 h in a vacuum of about 0·1–1 torr. They were first found lying on the crystal surface (Fig. 1). A closer investigation revealed that there were also whiskers at right angles to the (100) surfaces (Fig. 2). The white dots marked with arrows in Fig. 1 are due to upright whiskers.

Different surface features are formed during the heat treatment[2,3]. Abnormal features, for example plateaux,

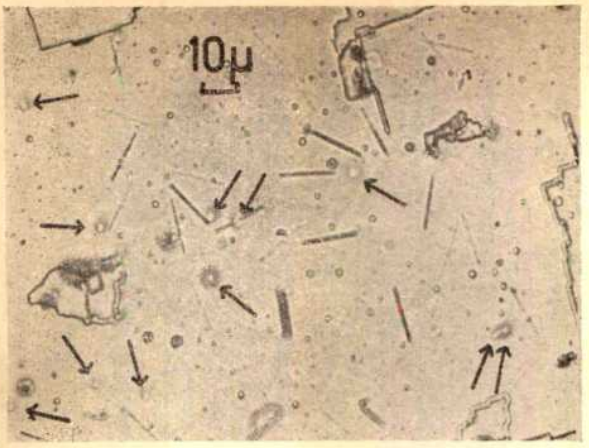


Fig. 1. Whiskers of about the same length, but of different thickness, lying on an annealed (5 h at 600° C) potassium chloride crystal. The white spots marked with arrows are upright whiskers

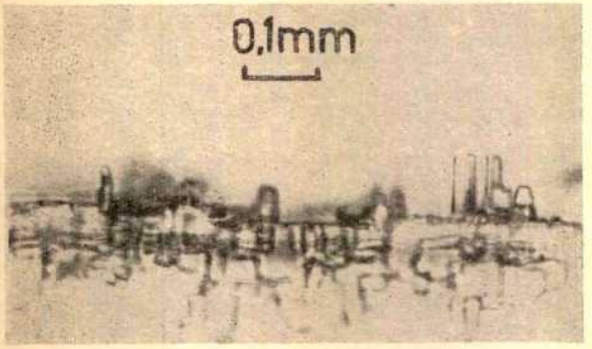


Fig. 2. Whiskers and pillars on an annealed (23 h at 600° C) potassium chloride crystal

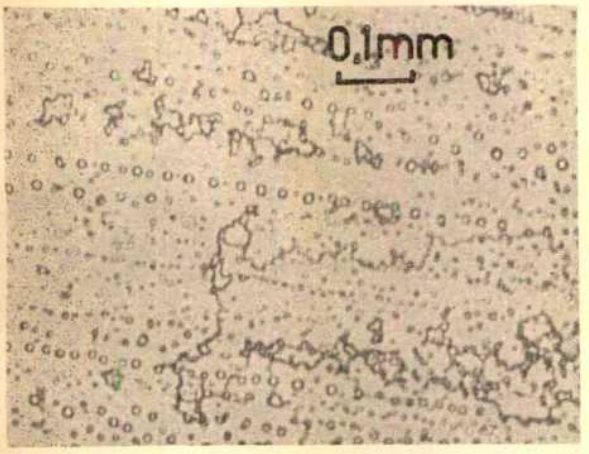


Fig. 3. Pillars formed in rows parallel to the cleavage steps on an annealed (5 h at 600° C) potassium chloride crystal

are traceable to impurities like silica compounds[4]. These cover the original surface in some places and inhibit sublimation. The widening of thermal etch pits is in certain places slowed down by these impurities. When a pit deepens the sublimation steps reach the edge, thus forming steep sides. When the pits combine around a poisoned place, pillars of different cross-sections are formed. The pillars are sometimes formed in rows parallel to cleavage steps (Fig. 3). This indicates that the impurities may easily attach themselves to some critical places formed in the cleavage process. The sides of the pillars are not perfect and thus sublimation takes place on them. The less perfect sides sublimate more rapidly and thus the thinning pillar seeks for itself a position with more perfect sides. Whiskers are known to sublimate slowly[5,6] because of their perfect sides. Thus the pillars formed with better sides are left on the sublimating surface, becoming longer and thinner until totally sublimated. Because the whiskers in Fig. 1 are nearly of equal length they have fallen down after the heat treatment.

The part played by impurities (higher carbohydrates originating from the vacuum pump and greased joints[7]), adsorbed on advancing sublimation steps, which could govern the steepening of the sides of the pits, is not clear. It is probable that they may be disregarded; all that is needed is for poisoned places to lie on the original cleavage surface, and for the whiskers formed to have a high thermal strength.

I thank Prof. M. Kantola for extending the facilities of the laboratory. This work was supported by Suomen Kulttuurirahasto.

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Estimation of Moisture Content by Neutron Scattering: Effect of Sample Density and Composition

SINCE it was first developed about 1950[1,2] the technique of estimating moisture content by neutron scattering has been extensively used in the determination of soil moisture. In recent years, workers in several countries have extended the method to other materials[3], utilizing in some instances the surface probe technique developed by Pawliw and Spinks for concrete[4]. The basic assumptions in all these measurements have been (*a*) that the thermalizing power of hydrogen is so great in comparison with other elements that the slow neutron flux measured by the instrument can be ascribed solely to the hydrogen present in the sample[5], and (*b*) that there is a unique relationship between hydrogen content and slow neutron flux except when elements of high capture cross-section for neutrons are present[3a].

We have examined the response of a commercial surface-type instrument to changes of moisture content in a number of building materials. Fig. 1 shows the results obtained with four specimens. These were (*a*) a concrete made with Portland cement, sand and basalt, with oven-dry density 2·23 g/c.c., (*b*) a concrete made with Portland cement, sand and expanded shale, with oven dry density 1·41 g/c.c., (*c*) foamed Portland cement; oven dry density 0·99 g/c.c., (*d*) foamed Portland cement; oven dry density 0·50 g/c.c. The thermal neutron count is expressed as a fraction of that obtained on a standard paraffin wax block ('relative thermal neutron count'). The total water content includes bound water. It can be seen that the curves are essentially parallel, but are displaced from one another to such an extent that the same neutron count corresponds to much more water in specimen *d* than in specimen *a*; for example, at a relative neutron count of 0·25, the water contents vary almost in the ratio of 2 : 1. The relative positions of these curves suggest that the density of the material has a large effect on the response, although the coincidence of the curves for samples *b* and *c* shows that density alone is not the deciding factor. A similar density effect has been reported recently by

Lachaud in connexion with an attempt to use neutron scattering to estimate the bitumen content of road surfacing mixtures[6].

From these observations it appears that the thermalizing power of atoms other than hydrogen cannot be neglected and that it is necessary to consider both the density and the type of the other atoms present.

It seems that the assumption of the overwhelming thermalizing power of hydrogen has arisen from considerations at thermal and near-thermal levels where the microscopic slowing-down power (defined as $\xi\ \sigma_s$ where ξ is the average logarithmic energy loss of the neutron per collision and σ_s the scattering cross-section of the nucleus concerned) is very much greater for hydrogen than for other atoms. For example, at 0·025 eV the ratio of the microscopic slowing-down powers of hydrogen and oxygen is 72 : 1 and of hydrogen and carbon 45 : 1. However, σ_s varies with neutron energy and, although ξ is approximately constant for each atom below 100 keV, above this energy there is a reduction in its effective value as the scattering becomes anisotropic. The percentage change in ξ is greatest for hydrogen but is of a similar order of magnitude for all of the atoms with which we are concerned here, that is, the differences in this latter effect for the various atoms are sufficiently small for them to be neglected in calculations such as those which follow. Fig. 2 shows the variation of the total cross-sections of hydrogen and of oxygen with neutron energy. For these elements the total cross-section is essentially the scattering cross-section. Although the cross-section of hydrogen is much larger than that of oxygen at very low neutron energies, the difference diminishes as the neutron energy increases until, above 3 MeV, the cross-section of oxygen actually exceeds that of hydrogen.

In these higher energy ranges, where the effect of atoms other than hydrogen is appreciable, a significant proportion of the total number of collisions needed to cause thermalization occurs; slightly more than 20 per cent occur between 5 MeV and 100 keV. From the data in Table 1 it appears that over this energy range the hydrogen content of a concrete specimen (*b*) may be responsible for only about half the total slowing down. At lower energies the hydrogen will have a proportionately greater effect, but the contribution of the other atoms will still not be zero. For example, between 100 keV and 1 eV the oxygen content of this specimen should produce about one-fifth of the slowing down produced by hydrogen. Consequently it is only to be expected that any major change in density or in the composition of the non-aqueous part of the specimen will change the slow neutron flux corresponding to a given moisture content.

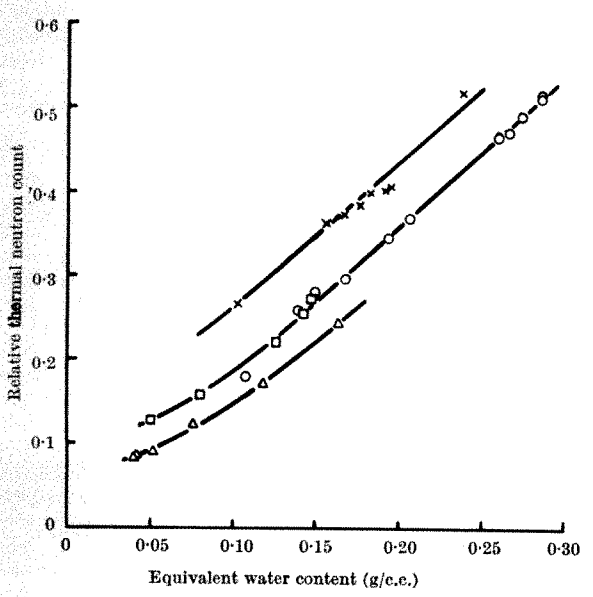


Fig. 1. Relationship of neutron count to water content for different materials. ×, basalt concrete, 2·23 g/c.c., *a*; □, shale concrete, 1·41 g/c.c., *b*; ○, foamed cement, 0·99 g/c.c., *c*; △, foamed cement, 0·50 g/c.c., *d*

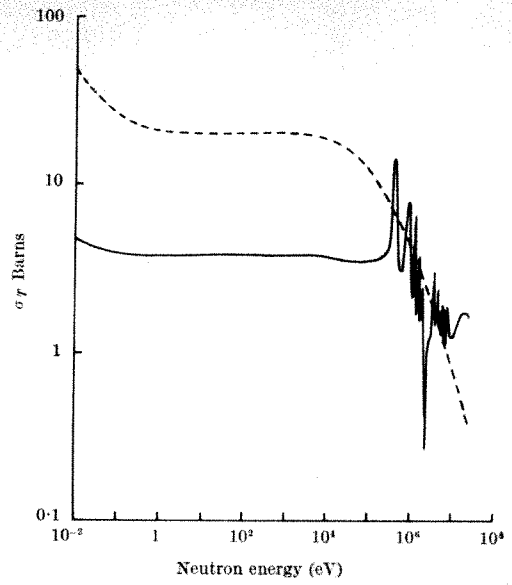


Fig. 2. Variation of total cross-section of hydrogen (broken line) and oxygen (solid line) with neutron energy

Our experimental data, however, suggest that the initial collisions of the fast neutrons (for example, between 5 MeV and 100 keV) may have an even greater influence on the thermal neutron flux than would be expected from the foregoing argument. The slow neutron flux from a given source should be directly proportional to the sum of the microscopic slowing-down powers of the components of the test material suitably averaged over the whole energy range from emission (5 MeV) to thermal energies (say 0·025 eV).

Table 1. Contributions of the Various Components of Specimen *b* (Expanded Shale Concrete, Water Content 0·05 g/c.c.) to Neutron Energy Loss Between 5 MeV and 100 keV

Element	Atoms per c.c. × 10^{-20} (N)	ξ	Mean scattering cross-section 5 MeV–100 keV (σ_s) barns	Microscopic slowing-down power 5 MeV–100 keV $\xi \times \sigma_s$	Macroscopic slowing-down power 5 MeV–100 keV $N \times \xi \times \sigma_s$
H	34·4	1·000	5·7	5·7	196
C	2·3	0·158	2·8	0·44	1·0
O	261·5	0·120	5·2	0·62	162
Al	9·7	0·072	3·4	0·25	2·4
Si	94·2	0·070	3·3	0·23	22
Ca	22·7	0·049	2·3	0·11	2·5

Table 2. Observed and Calculated Relative Thermal Neutron Counts at Different Moisture Levels

Observed	Calculated		
	Specimen *b*	Specimen *c*	Specimen *d*
0·127	0·127	—	0·128
0·179	—	0·172	0·179
0·266	0·259	0·226	0·250

We have calculated the theoretical instrument response on this basis using the mean scattering cross-section for the range 5–100 keV, that is, calculating as if the relative cross-sections remain at their average value over the first 20 per cent of the thermalizing collisions for all subsequent collisions. Since the constant of proportionality is not known, one was selected arbitrarily so that the calculated value for specimen *b* at a moisture content of 0·05 g/c.c. agreed with the observed value. The results are given in Table 2, from which it can be seen that the correspondence between observed and calculated values is good, that is to say, the observed slow neutron flux varies as if it were greatly influenced by the initial collisions of the fast neutrons during which the scattering cross-section of

hydrogen is of the same order of magnitude as that of many of the common atoms.

Agreement to within 20 per cent has been obtained between the calculated and observed responses from a number of other materials containing various amounts of water or other source of hydrogen, namely, wood, sand, sand–sugar mixtures, and gypsum, and hence the same dependence of the thermal neutron flux on atoms other than hydrogen has been found over a range of composition and density. Allowance must, therefore, be made for this if neutron scattering is to be successfully used for moisture estimations in materials of density or composition different from that with which the instrument was calibrated.

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Propagation of Stress Pulses across the Interface between Two Immiscible Liquids

Previous experiments have been described by me and others[1,2] in which pulses of compression and of tension have been propagated through a liquid column. This communication reports some experiments in which stress pulses were propagated across the plane boundary between two liquids which do not mix.

For this purpose, a cylindrical steel tube was used to contain the liquids. This tube was 3 ft. long, internal diameter 7/8 in. and external diameter 1·25 in. It was fitted with a steel piston at its lower end and a pressure pulse was generated in the liquid by firing a lead bullet of 0·22 in. calibre so as to strike the lower end of the piston normally at its centre. The pressure changes in the liquid were measured by means of a horizontal pressure bar mounted in a 'porthole' below the liquid surface.

In this series of experiments, water occupied the steel tube to a level of 5 cm or so above the pressure bar; about 50 cm³ of transformer oil were then added. If we refer to the water and the oil as media (1) and (2), respectively, their acoustic impedances are $R_1 = 1{\cdot}49 \times 10^5$ g cm^{-2} sec^{-1} and $R_2 = 1{\cdot}28 \times 10^5$ g cm^{-2} sec^{-1}. Thus, for a stress pulse incident on the water–oil boundary from the water below we have the results:

$$\text{Transmissivity, } t_{12} = \frac{2R_1}{R_1+R_2} = 1{\cdot}076$$

$$\text{Reflectivity, } r_{12} = \frac{R_2-R_1}{R_1+R_2} = -0{\cdot}076$$

Similarly, for a pulse incident on the boundary from the oil, we have:

$$t_{21} = \frac{2R_2}{R_1+R_2} = 0{\cdot}924$$

$$r_{21} = \frac{R_1-R_2}{R_1+R_2} = 0{\cdot}076$$

Let us now consider what happens when a compressional pulse, of peak pressure P_t, is incident on the interface from the water. The pulse transmitted into the oil then has a peak pressure of $P_t = 1{\cdot}076\ P_t$, and at the upper free surface of the oil it is reflected as a tension pulse of peak value $P'_t = -P_t = -1{\cdot}076\ P_t$. When this downgoing pulse in the oil reaches the interface a tension pulse of peak value $P''_t = 0{\cdot}924\ P'_t = -0{\cdot}995\ P_t$ is propagated down into the water. In these experiments P_t is of the order of 100 atm. so that the peak tension P''_t to be expected in the water is of the same order. All this reasoning is based, of course, on the assumption that no cavitation occurs.

In a number of records taken the maximum tension recorded in the water was about 7 atm. This is the same as the value obtained when the steel tube contained water only and reported earlier by Rees and me. The most surprising thing is that it is possible for a tension wave to be propagated at all across a well-defined plane interface like that between these two liquids. Certainly the result is surprising from a 'macroscopic' view of stress-wave propagation and brings to mind Hopkinson's[3] celebrated work on the pressure-bar. In these experiments Hopkinson used a pressure-bar which had a detachable end-piece wrung to one end and he found that compressional pulses could be transmitted across the plane boundary between the bar and end-piece but that tension pulses could not. The present arrangement is rather similar in the sense that the water column is like the pressure-bar and the oil column like the end-piece in contact with it. These experimental results certainly emphasize the fact that pulse propagation in liquids is more accurately described by means of a 'microscopic' approach, that is, in terms of the motion of the individual particles of the medium.

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GEOLOGY

Pumice from the South Sandwich Eruption of March 1962 reaches New Zealand

On March 14, 1962, H.M.S. *Protector* encountered an extensive raft of large and small pumice fragments in the neighbourhood of Zavodovski Island in the South Sandwich Islands[1]. It is believed that this pumice was erupted on March 5 from a submarine volcano which forms a shoal, minimum depth 15 fathoms, 55·9° S, 28·1° W., lying some 35 miles north-west of Zavodovski Island. Towards the end of 1963, fragments of pumice up to 3 ft. in diameter were noticed washed up on the south-west coast of Tasmania and these have been tentatively correlated with the South Sandwich occurrence[2].

On January 24, 1964, H.M.N.Z.S. *Pukaki* collected pea- and marble-sized pieces of pumice floating in abundance south of Macquarie Island at 60° S., 160° E., and these, together with a larger block carrying barnacles, were forwarded to the University of Otago. During the southern autumn and winter of 1964 abundant blocks of pumice were noticed at Mason Bay, on the west coast of Stewart Island off the south tip of South Island of New Zealand and in the general area of Foveaux Strait, which separates Stewart Island from South Island. In August of the same year abundant blocks of pumice up to 1 ft. or more in diameter were observed by us on the western shores of Bluff Peninsula in Foveaux Strait.

Since then we have been given numerous specimens of identical pumice that were collected at similar or even earlier dates, for example, one large block said to have been collected 20 miles south of Dunedin in January, 1964, and two large blocks, representative of many found during March 1964, in Lyttleton Harbour, Banks Peninsula, about 330 miles north-east of Bluff on the east coast

of the South Island. Furthermore, a reliable observer reports pumice on Foveaux Strait beaches as early as August 1963. Late in September 1964 many pieces were collected on the west coast of the South Island near Greymouth, 340 miles north of Foveaux Strait. No pumice had been found on the same beach previously, and furthermore, like the Bluff Peninsula blocks, those from Greymouth, carried live colonies of the barnacle *Lepas anatifera* (identified by Dr. E. Batham of the Portobello Marine Biological Station, University of Otago). The approximate date of arrival on the beach is thus clear.

By December 1964 fragments, often carrying live colonies of *Lepas*, began to arrive in abundance on many South Island beaches, both on the east and west coasts (for example, Waimairi Beach near Christchurch; Spit, Warrington and Akatore beaches near Dunedin; Big Bay, Jackson Bay and Karamea on the West Coast) while a block of about 1·5 ft. diameter was observed on D'Urville Island at the western entrance of Cook Strait separating the North and South Islands of New Zealand. Previous searches of Warrington Beach had failed to yield any pumice although considerable quantities of pumice had been reported from Waimairi Beach about one year previously—a statement not incompatible with the collection from Lyttleton Harbour. In places, for example, Akatore, January 1965, pea-size and smaller (very coarse sand) grains have been noticed floating like scum on the water.

Like the South Sandwich materials the New Zealand specimens are pale grey to whitish in colour, with occasional darker streaks and inclusions. On being broken open, a number of them give off a distinct sulphurous smell, as was also recorded by Gass *et al.*[1]. The pumice is intensely vesicular consisting of a fine, delicate mesh of glass with larger cavities that not uncommonly contain a filigree of glass fibres. They contain small scattered crystals of several minerals.

The refractive index of the pumice in the South Island and *Pukaki* collections shows little variation. Determinations on about 20 fragments (for example, *OU* 19796–197808) range from 1·493 to 1·496 ± 0·002, though usually near 1·493. This is in fair agreement with the figure of 1·496 ± 0·002 given by Gass *et al.* for floating pumice collected near Zavodovsky Island in March 1962. Specimens from near the original site, forwarded to us by Dr. P. G. Harris, have refractive index 1·494 ± 0·002.

The commonest phenocrysts and microphenocrysts are plagioclase which often shows oscillatory zoning, the maximum variation observed being An_{70} to An_{40}. Hexagonal bipyramids of quartz, 1–2 mm in diameter, are not uncommon in the pumice grounded on New Zealand beaches, and although not mentioned in the account of Gass *et al.*[1], they do occur in the specimens forwarded to us by Dr. Harris. The most abundant mafic mineral is greenish-brown hypersthene in prisms up to 2 mm long containing fluid inclusions and acicular apatite. The refractive index, γ, is commonly in the range 1·720–1·733 ± 0·002, the crystals sometimes being noticeably zoned. Augite (β 1·704–1·706 ± 0·002) is subordinate to hypersthene and occurs as scattered green prisms. Magnetite is also present as scattered grains, often partially included in the microphenocrysts of pyroxene. The assemblage of mafic minerals in the drifted pumice is indistinguishable from that in the South Sandwich pumice provided by Dr. Harris. Small xenoliths, including one of hornblende hornfels, were observed. Another feature common to the South Sandwich and South Islands specimens is the relative abundance of halite cubes and other soluble salts even in the interior of quite large blocks.

The data presented here firmly establish the correlation of the drift pumice with that of the South Sandwich eruption. In contrast it may be noted that the refractive index of the glass, the relatively anorthite-rich range of plagioclase compositions and the presence of quartz, contrast rather sharply with the sub-recent pumices of the North Island of New Zealand[3].

To reach Foveaux Strait the first flush of pumice to arrive in abundance has travelled a net distance of about 7,000 nautical miles in 850 days in the West Wind Drift at an average net rate of about 8 nautical miles per day. The earliest South Island drift pumice we have checked microscopically has travelled the distance in 680 days at a rate of 10 miles per day, while some pumice, presumably from the same eruption, would appear to have completed the journey in about 530 days at an average net rate of about 13 nautical miles per day. The drift rate for the coarse and fine pumice encountered by H.M.N.Z.S. *Pukaki* and for the 1963 groundings on Tasmania is also about 10 miles per day. Since then pumice has been more slowly distributed to east coast beaches by the coastal Southland current[4].

Previous data on the velocity of the West Wind Drift appear to be scanty, but Deacon[5] notes the eastward drift of bottles and of jetsam at about 8 miles a day. Russell[6] gives a list of recent papers that reported a rapid daily drift; he notes numerous examples from the southern seas with drift rates of 11–13 miles a day. It may be noted, in comparison, that Richards[7] has shown that pumice from the 1952 eruption of Isla San Benedicto, off the coast of Mexico, travelled to points as far westward as the Marshall Islands at rates ranging from an average of 4·7–14·7 nautical miles a day, while Krakatoa pumice drifted westwards across the Indian Ocean at rather similar rates.

In addition to the light they throw on the drift of materials floating in the surface layers of the sea, these instances provide graphic evidence of the manner in which certain pyroclastic materials can be transported for very great distances, ultimately no doubt to be incorporated in sedimentary rocks deposited remote from the site of any volcanic activity.

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Changes in Level of Lake Naivasha, Kenya, during Postglacial Times

Strandlines high above the present levels of three small lakes in Kenya provide some of the earliest-recognized evidence for past climatic changes in tropical Africa. The terraces above Lakes Nakuru, Naivasha and Elmenteita were studied intensively by Nilsson[1,2] more than 30 years ago, and are one of the major bases on which the pluvial theory of Pleistocene climates in Africa was constructed. Despite later disagreements on the number and interpretation of these strandlines, it is still generally accepted that they provide good evidence for at least one pluvial period (the Gamblian) and two lesser post-pluvial wet phases (the Makalian and Nakuran)[3,4].

The three lakes lie in adjacent closed basins in the Eastern Rift Valley, between latitudes 0° 15′ and 0° 50′ S., and longitudes 36° 0′ and 36° 30′ E. They have fluctuated sensitively during the past 50 years in accordance with

both yearly changes and more long-term trends of rainfall.

In December 1960 and January 1961, Robert Kendall and I raised a 28-m core from the deepest part of Lake Naivasha, a submerged crater near the eastern shore. At that time the crater was 13 m deep, and was almost cut off from the main body of the lake by its slightly elevated outer rim (Crescent Island). During past periods of lower water, the crater must have been completely isolated.

Core analyses of diatoms, pollen, chemistry and mineralogy have been carried out in collaboration with Dr. D. A. Livingstone, and three carbon dates have been provided by the Yale Radiocarbon Laboratory. An extremely rapid average rate of sedimentation has characterized that portion of the history of the Lake covered by the core, the total age of the section being 9,200 ± 160 years. This is insufficient to provide evidence regarding the Gamblian Pluvial, which is believed to have ended 10^3 or 12×10^3 years ago[5], but the core has supplied a very detailed record of changes in this basin during most of the supposed time-span of the Makalian and Nakuran wet phases.

The lower half of the section was much more homogeneous than the upper in gross appearance, chemistry and mineralogy; and the diatom assemblages of this region, though variable, always contained euplanktonic species (for example, *Strephanodiscus cstraea*) as important constituents, together with more littoral forms. These lines of evidence indicate a lake of medium size in which the crater was freely confluent with the main lake. A date of 5,650 ± 120 years before 1950 fixes the end of this relatively stable phase in the history of the Lake.

Metres 14–8·20 represent a second phase, during which the Lake shrank and the crater portion was reduced to a weedy pond. Percentages of organic matter and carbonate were higher in this part of the core, and sharp changes in the diatom assemblages suggested that the crater was isolated from the main lake as far back as the middle of metre 12. This phase was terminated abruptly by a complete drying of the crater basin, as indicated by a partially weathered, impermeable, sandy layer extending from metre 8·20–7·80. The sharp interface between this layer and the underlying lake mud suggests that during this dry period the water table never lay more than 40 or 50 cm beneath the surface (that is, approximately 21·7 m below the water level at the end of 1960). A date of 3,000 ± 60 B.P. has been obtained for the portion of metre 8 just above the dry zone.

The remaining few upper metres of the core were, when wet, very colourfully banded in many parts, and all the analyses suggest a lake the conditions of which were rapidly fluctuating, often more concentrated chemically than it is to-day. This lake was usually (if not always) smaller than it has been in historic times.

These stages of Lake Naivasha are compared in Table 1 with a recent chronology[4] of the post-pluvial wet phases, in which the dates were approximated from cultural evidence and from a presumed relationship between these phases and postglacial climatic events in Europe. The new evidence from Naivasha corresponds quite closely with the previously established picture, but indicates that the latter must be modified in one important respect. The Nakuran wet phase was described from a strandline at Lake Nakuru which Nilsson and others believed equivalent to a Naivasha strandline 23 m above the 1960-level of the Lake. If the core interpretation is correct, the Lake could not have been this high for almost 5,000 years, and the Nakuran wet phase must thus be back-dated considerably. The present investigation produced results which agree with suggestions that there has been a return to wetter conditions in the past 3,000 years, but this shift has been more modest than hitherto supposed.

Table 1. PRESUMED HOLOCENE CLIMATIC SEQUENCE IN EAST AFRICA WITH ESTIMATED DATES (ADAPTED FROM COLE[4]), COMPARED WITH CARBON-14 DATED STAGES OF LAKE NAIVASHA

Climatic phase	Years before 1950 (est.)	Naivasha	Years before 1950 (C^{14})
	0		(0)
Nakuran wet phase		Small, fluctuating lake	
	2,750		3,000 ± 60
Dry interval		Dry	
	4,450	Shrinking	
			5,650 ± 120
Makalian wet phase (2)		Lake larger than at present	
	7,450		
Drier			9,200 ± 160
	9,450		
Makalian wet phase (1)?		(No evidence)	

The core has also provided evidence that more sharply seasonal climates characterized the Naivasha watershed until about 2,500 years ago. Varves were found through most of the section below metre 7, but not above. In most of East Africa to-day the climate is characterized by one rainy and one dry season yearly, but a narrow equatorial belt that includes Naivasha exhibits a more muted seasonality, with two moderately rainy and two moderately dry periods per annum. The more seasonal rainfall pattern at present occurs within 65 km of Naivasha, and no great migration of climatic belts—probably just a slight accentuation of the monsoon, in fact—need be postulated for periods when this more seasonal régime occurred at Naivasha.

Modern trends of total yearly rainfall have not been uniform throughout East Africa. It has been shown that various parts of Kenya alone can follow persistent opposing rainfall trends over periods of 30 years or more[6]. Therefore the possibility cannot be dismissed that the relatively moderate changes in size of Lake Naivasha during the past 9,000 years reflect quite local climatic shifts. Evidence from other investigations in Africa, however, makes this seem unlikely. Two sharp changes in a pollen curve from Mount Kenya[6], 140 km north-east of the Lake, seem clearly to coincide with the onset of the shrinking stage and of the dry period at Naivasha. Changes in the level of the Lake cannot be correlated so closely with a pollen profile from the Cherangani Hills[7], 240 km north-west of Naivasha, but the latter investigation is not contradictory. Farther afield, climates deduced for dated levels at Kalambo Falls[8] and in north-eastern Angola[9], 1,000 and 1,850 km south-west respectively, are consistent with those that could be inferred contemporaneously at Naivasha. Broadly similar climatic trends, although perhaps asynchronous by a thousand years or more, may even be traced during this period in the Chad basin[10], far to the west. These comparisons suggest that widespread climatic phenomena, particularly the warmer temperatures of the climatic optimum, were at least partly responsible for the changes in size of Lake Naivasha.

I thank Dr. S. D. Heron and the Department of Geology at Duke University for assistance with the mineralogical investigations; Dr. Minze Stuiver and his colleagues at the Yale Radiocarbon Laboratory for the valuable absolute dates; and the National Science Foundation for providing financial support for Dr. D. A. Livingstone and myself. This material formed part of a doctoral thesis submitted to the Graduate School of Arts and Sciences at Duke University.

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CHEMISTRY

Tetrakis (Triphenyl Phosphite) Nickel (0) as an Initiator of Polymerization at Room Temperature

Organo-metallic compounds in which the metal is in a low (preferably zero) oxidation state, in the presence of a suitable organic halide, can initiate the free-radical polymerization of vinyl monomers[1]. The metal carbonyls and their derivatives, and the hexaphenylisocyano[2] complexes of group VI metals have been most studied from this point of view. Recently we have studied the compound tetrakis (triphenyl phosphite) nickel (0) (TTPN), described in the literature earlier this year[3]; our observations on this material, which shows unusually high activity as an initiator, are reported here. From a practical point of view this derivative has the advantage of possessing a comparatively high stability in the solid state (it melts fairly sharply at 146° C)—an unusual attribute for a material which is an effective initiator at room temperature.

As a ligand, triphenyl phosphite belongs to the same class as carbon monoxide and phenyl isocyanide; however, the double-bond character of the phosphorus to metal bond arising from the back-donation of electrons from the *d* orbitals of the metal to the ligand is much less and consequently the ligand is more readily displaced.

Tetrakis (triphenyl phosphite) nickel (0) was prepared by the reaction of the pure ligand and freshly sublimed nickelocene in boiling benzene under a nitrogen atmosphere, according to the method of Olechowski, McAlister and Clark[3], and purified as described by these authors.

The derivative alone is not an effective initiator of the polymerization of methyl methacrylate at 25° C, but it initiates readily in the presence of a low concentration of a suitable organic halide such as carbon tetrachloride. The dependence of rate of polymerization on carbon tetrachloride concentration is remarkably similar to that observed with the metal carbonyl[1] and phenyl isocyanide derivatives[2]; the rate, almost zero for $[CCl_4] = 0$, at first rises rapidly with increasing $[CCl_4]$ but ultimately reaches a plateau value, so that for $[CCl_4] > 0{\cdot}02$ mole $l.^{-1}$ it is independent of $[CCl_4]$. With nickel carbonyl the limiting rate is reached only at higher values of $[CCl_4]$ (ref. 4).

In bulk methyl methacrylate at 25° C the rate of polymerization is given by:

$$-\frac{d[M]}{dt} = 1{\cdot}02 \times 10^{-2}\,[\mathrm{TTPN}]^{1/2}\ \text{mole}\ l.^{-1}\ \text{sec}^{-1} \quad (1)$$

(M represents the monomer). The free-radical nature of the reaction is indicated by the half-order in initiator concentration in (1) and also by the fact that the kinetic parameter $k_p k_t^{-\frac{1}{2}}$ (k_p, k_t being propagation and termination coefficients, respectively) calculated from mean rates and degrees of polymerization has the normal value for the free-radical polymerization of methyl methacrylate at 25° C. The order in monomer concentration is 1·5 (with benzene as diluent), showing that the monomer enters into the initiation step; this has been found with some carbonyls, for example, molybdenum carbonyl[5]. We believe that a similar mechanism holds in all these systems—the metal derivative reacting with monomer to form a complex which afterwards yields radicals on reaction with the halide.

Tetrakis (triphenyl phosphite) nickel (0) does not show the marked inhibition observed with nickel carbonyl[4], so that the relationship (1) is obeyed over the whole range of concentrations investigated (up to $2{\cdot}9 \times 10^{-3}$ mole $l.^{-1}$). Initiation at 25° C is much more rapid under comparable conditions of concentration than with nickel carbonyl particularly at high initiator concentrations when inhibition in the nickel carbonyl system is very marked. Even at low concentrations the difference amounts to a factor of nearly 100 in the rate of initiation. These results, therefore, are consistent with the view that triphenyl phosphite is a more labile ligand.

Triphenyl phosphite acts as a mild inhibitor of the polymerization, the effect being very small compared to the inhibition by carbon monoxide encountered in the corresponding carbonyl system. (Triphenyl phosphite is not a retarder in these reactions, nor does it affect the free-radical polymerization of methyl methacrylate initiated by azo-*bis*-isobutyronitrile.) This may indicate that the initial reaction of the nickel derivative with monomer is not readily reversible, or that the greater part of the process involves addition of monomer without scission of a triphenyl phosphite ligand.

Use of polyvinyl trichloracetate (PVTCA) as the halide in place of carbon tetrachloride leads to very rapid gelation at 25° C. Thus with $[\mathrm{PVTCA}] = 3{\cdot}84 \times 10^{-2}$ base mole $l.^{-1}$ (weight average molecular weight 88,000) and $[\mathrm{TTPN}] = 6{\cdot}15 \times 10^{-4}$ mole $l.^{-1}$ the gel time is 10 min, approximately. As already shown[6], this implies that radical formation takes place by abstraction of a halogen atom from the halide by the metal derivative, giving an organic radical (for example, $\cdot CCl_3$ from CCl_4) capable of initiating polymerization.

Work is in progress to investigate the effect of changes in the structure of the organic ligand on the activity of these initiating systems.

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Effect of Zinc Chloride on the Velocity Coefficients in the Polymerization of Methyl Methacrylate

It is well known that the reactivity of the propagating species in ionic polymerization is greatly influenced by the proximity and character of the gegenions and by complex formation. As a rule, free radical reactions are comparatively insensitive to the nature of their environment, although there are references in the literature to changes in the rate of free-radical polymerizations, and the nature of the polymers formed, which have been attributed to the participation of radicals in complexed forms. The investigation of the structure and reactivity of radical complexes in polymerization is a field which has not been surveyed systematically, but which, nevertheless, may contain interesting features. We have embarked on an investigation of the absolute rate coefficients in the free-radical polymerization of vinyl monomers in the presence of possible complexing agents and report here some results for the methyl methacrylate–zinc chloride system. The only measurements of a similar type recorded in the literature appear to be those of Bamford, Jenkins and Johnston[1], on acrylonitrile in *NN*-dimethylformamide solution in the presence of lithium salts. Kargin, Kabanov and Zubov[2] considered that the presence of zinc chloride during the polymerization of methyl methacrylate affects the microstructure of the polymer, but this finding was not confirmed by Bovey[3]. More recently, Imoto, Otsu and Harada[4] have

investigated the steady-state polymerization of methyl methacrylate in the presence of zinc chloride and have shown that at 60° C the rate of the reaction initiated by azo-*bis*-isobutyronitrile is increased by addition of zinc chloride, the ratio $k_p/\sqrt{k_t}$ also being increased (k_p, k_t are the velocity coefficients for propagation and total bimolecular termination, respectively).

In our work, k_p/k_t was measured at 25° C by the rotating sector technique, with light of wave-length 3650 Å and azo-*bis*-isobutyronitrile as photosensitizer. The geometry of the sector was such that the dark periods had twice the duration of the light periods. Rates of polymerization were determined dilatometrically; it was shown that the relation between the contraction in volume and the extent of conversion is not changed by the presence of zinc chloride. In all cases less than 10 per cent of the incident light was absorbed.

The treatment of Matheson *et al.*[5], in which allowance is made for a finite rate of initiation in the dark, was used in evaluating k_p/k_t. (Note that we define k_t so that the rate of mutual destruction of radicals is $k_t[R\cdot]^2$, where $[R\cdot]$ is the radical concentration. Matheson *et al.*[5] write $2k_t[R\cdot]^2$ for the same quantity.) An approximate estimate of $R_p^s/(k_tI)^{\frac{1}{2}}$ was made by comparing plots of $\sqrt{3}.\bar{R}_p/R_p^s$ against $\log_{10}R_p^st_1$ with the appropriate theoretical curve. Values of $\log_{10}[R_p^s/(k_tI)^{\frac{1}{2}}]$ corresponding to each experimental point in the steeply sloping region of the curve were then found, and hence the mean value and the standard error of $\log_{10}[R_p^s/(k_tI)^{\frac{1}{2}}]$. ($R_p^s$, $\bar{R}_p$ are the rates of polymerization under constant illumination and intermittent illumination with the duration of a light period t_1, respectively.) Fig. 1 shows the plots obtained in the presence and absence of zinc chloride; clearly the salt changes the value of $R_p^s/(k_tI)^{\frac{1}{2}}$. The derived values of k_p/k_t in Table 1 indicate that the presence of the salt at a concentration of 1·42 mole l.$^{-1}$ increases the ratio by a factor of approximately 2·5.

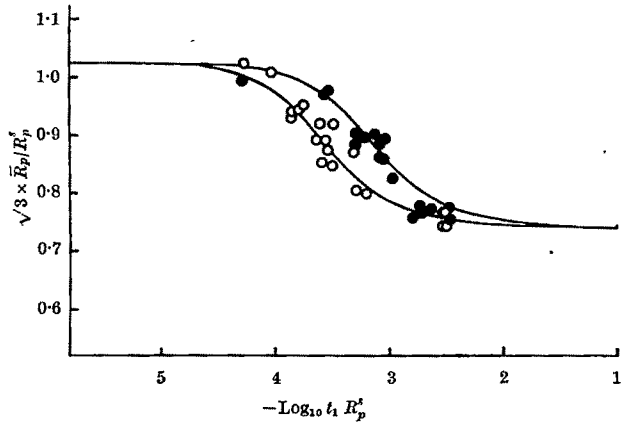


Fig. 1. Results obtained by the rotating sector technique. The ratio of the dark rate to R_p^s is 0·14. ○, Zinc chloride absent; ●, 1·42 mole l.$^{-1}$ zinc chloride

The magnitude of the ratio $k_p/\sqrt{k_t}$ (required to evaluate the rate coefficients separately) was obtained from molecular weight and rate determinations. Number average molecular weights were estimated from intrinsic viscosities. For this purpose allowance was made for the fact that termination occurs both by disproportionation and combination. If, with ordinary nomenclature, the relation:

$$[\eta] = K\bar{M}_n^a \qquad (1)$$

applies to a 'disproportionation' polymer, it may be shown that, if y is the ratio of combination to disproportionation:

$$[\eta] = K\bar{M}_n^a\left(\frac{2+y}{2(1+y)}\right)^a\left(\frac{1+y\,(1+\alpha/2)}{1+y}\right) \qquad (2)$$

Equation (2) was used to evaluate $\bar{M}_n$ from $[\eta]$, using the values of K, α given by Fox *et al.*[6], and taking $y = 0{\cdot}47$ at 25° C (Bevington *et al.*[7]). Finally, $k_p/\sqrt{k_t}$ was evaluated from equation (3).

$$\frac{k_p}{\sqrt{k_t}} = \frac{1}{[M]}\left(\bar{P}_nR_p\,\frac{2+y}{2(1+y)}\right)^{\frac{1}{2}} \qquad (3)$$

in which R_p is the rate of polymerization corresponding to a number average degree of polymerization $\bar{P}_n$.

Equations (2) and (3) are only valid if transfer is negligible. Imoto *et al.*[4], using the Mayo equation, derived a value of $2{\cdot}78 \times 10^{-3}$ for the transfer constant of zinc chloride at 60° C. However, the Mayo relation should not be applied to systems in which the rate of polymerization or the velocity coefficients are functions of the concentration of the transfer agent. A plot of the reciprocal of $\bar{P}_n$ (determined from equation (2)) against R_p at constant $[ZnCl_2]$ is linear and passes close to the origin, and is, therefore, consistent with the occurrence of only a very small amount of transfer. The transfer constant of zinc chloride must be less than 10^{-4} for $[ZnCl_2] = 1{\cdot}42$ mole l.$^{-1}$; use of equations (2) and (3) is therefore justifiable under our conditions. The degrees of polymerization were corrected to allow for transfer.

Table 1. Effect of Zinc Chloride on the Rate Coefficients in the Polymerization of Methyl Methacrylate at 25° C

Concentrations of the components of the reaction mixture, other than monomer, are shown. Photosensitizer azo-*bis*-isobutyronitrile (AZDN)

$[ZnCl_2]$ (mole l.$^{-1}$)	10^3[AZDN] (mole l.$^{-1}$)	$10^6k_p/k_t$	$k_p/\sqrt{k_t}$ (mole$^{-\frac{1}{2}}$ l$^{\frac{1}{2}}$ sec$^{-\frac{1}{2}}$)	$10^{-3}k_p$ (mole^{-1} l.sec^{-1})	$10^{-7}k_t$ (mole^{-1} l.sec^{-1})
0	1·89	10·6 ± 0·9	0·051 ± 0·001	2·5 ± 0·3	2·3 ± 0·6
0	6·10				
1·42	3·05	27·2 ± 3·0	0·128 ± 0·002	6·1 ± 1·0	2·2 ± 0·6
1·42	6·10				

The values of $k_p/\sqrt{k_t}$, and the individual values of k_p and k_t, are exhibited in Table 1. It is of interest that while k_p is increased markedly by zinc chloride, k_t remains virtually unaffected; in this respect the results resemble those obtained earlier with acrylonitrile and lithium chloride[1]. Our observations in the absence of zinc chloride are in good agreement with those of Matheson *et al.*[5].

The solution of zinc chloride in monomer has a high viscosity—about 73 per cent higher than that of pure methyl methacrylate (MMA) at 30° C. Imoto *et al.* showed from infra-red observations that the salt and monomer form a complex, which they formulated as $ZnCl_2$.MMA. It seemed likely to us that the complex might exist in polymeric form in solution, and thus give rise to the high viscosity. Measurements of the depression of the vapour pressure of the monomer are consistent with this and indicate a mean degree of association of 3·3. Probably some chains considerably longer than this are built up, with both zinc chloride and monomer acting as bifunctional units. Solutions of zinc chloride in ethyl acetate also show a similarly increased viscosity.

The marked increase in the velocity coefficient of chain propagation in the presence of zinc chloride could arise in several ways: (i) the salt might complex with the propagating radicals in such a way as to increase their reactivity to methyl methacrylate; (ii) the monomeric complex $ZnCl_2$.MMA may be more reactive than the monomer; (iii) chains of zinc chloride and MMA units may have the latter units arranged sterically in a manner suitable for polymerization.

Imoto *et al.*[4] carried out copolymerization experiments with vinylidene chloride at 20°–25° C., and, as these workers point out, their measurements of the reactivity ratio r_2 (vinylidene chloride is monomer 2) suggest that (ii) is important. However, the decrease in r_1 produced by the presence of the salt is larger than would be anticipated from our measurements of k_p alone; this may indicate complexing of the MMA radicals, which might well decrease k_{p12} in addition to increasing k_{p11}. (It is assumed in this argument that neither the vinylidene monomer nor radical is affected by zinc chloride.)

The constancy of the termination coefficient is consistent with the view that the high viscosity of the solution arises from the presence of a low concentration of polymeric chains.

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Electron Spin Resonance Spectrum of $NH_2^+SO_3^-$

Changes in the electron spin resonance spectra of various carbon[1,2] and nitrogen[3,4] centred π-radicals on cooling to low temperatures prompted us to re-examine the spectra of the radical $NH_2^+SO_3^-$ trapped in irradiated sulphamic acid[5]. From the earlier work of Rowlands and Whiffen[5] it appears that at 300° K there is rapid re-orientation of the NH_2^+ group between two equivalent positions, so that the two protons are equivalent for all orientations of the radical in the magnetic field of the spectrometer. At 77° K a marked increase in the magnitude of the hyperfine interaction of the unpaired electron with the ^{14}N nucleus was noted for the orientation H_0 parallel to the crystallographic *b*-axis. It was suggested that this might be due to changes in the radical geometry.

In order to establish if this were indeed the case we examined the spectra at 4° K, at which temperature rotational motion no longer occurs. Furthermore, in order to improve the resolution of the spectral lines the radical has been generated in sulphamic acid enriched to over 95 per cent in the isotope ^{15}N. This material was purchased from Merck, Sharp and Dohme, Ltd., Montreal. Single crystals of sulphamic acid were grown from aqueous solution; they belong to an orthorhombic system[6] having $a = 8{\cdot}100$ Å, $b = 8{\cdot}049$ Å, and $c = 9{\cdot}228$ Å, and form hexagonal tablets on (100). Both X- and γ-irradiation facilities were used, at 300° K and at 77° K, in an attempt to minimize the formation of SO_3^-, the presence[7] of which partly obscures the spectra of $NH_2^+SO_3^-$. None of these experiments had any effect on the relative concentration of the two radicals. The electron spin resonance spectra were examined with a Varian V–4502 spectrometer previously described[4]. Room temperature data were obtained from a crystal mounted on a two-circle goniometer[8]; the low-temperature data were obtained from crystals mounted on quartz rods and immersed in Dewar vessels containing liquid nitrogen (77° K) or liquid helium[9] (4° K).

The proton and ^{15}N-hyperfine tensors determined from the spectra of $NH_2^+SO_3^-$ at 300° K are given in Table 1.

Table 1. Hyperfine Tensors for $NH_2^+SO_3^-$ in Sulphamic Acid at 300° K

Nucleus	Tensor* in *abc* axis-system (Mc/s)			Principal values	Principal directions
^{15}N	71·8	63·6	3·5	146·6	(+0·631, +0·727, +0·269)
	63·6	78·6	34·6	48·5	(−0·525, +0·146, +0 839)
	3·5	34·6	44·7	0·0	(+0·571, −0 671, +0 474)
Protons	64·1	−6·0	15·9	62·7	(+0·667, +0 715, +0·210)
	−6·0	71·7	−11·4	39·1	(−0·489, +0·207, +0·847)
	15·9	−11·4	51·1	85·1	(+0·562, −0·668, +0·488)

* These tensors refer to one particular site; the tensors for the other three sites are obtained by changing the signs of the off-diagonal elements in pairs, with appropriate sign changes in the fourth column. All four tensors have the same principal values.

The principal values agree well with those previously reported[5], when allowance is made for the different nuclear moments of ^{14}N and ^{15}N ($\gamma_{15}/\gamma_{14} = 1{\cdot}403$). Within the experimental error both tensors are diagonal in an axis-system defined as follows: perpendicular to the radical plane ($t_N = 147$ Mc/s, $t_H = 63$ Mc/s); parallel to the S–N bond ($t_N = 0$ Mc/s, $t_H = 85$ Mc/s) and the mutually perpendicular direction ($t_N = 48$ Mc/s, $t_H = 39$ Mc/s). From the crystal structure of sulphamic acid[6] it may be calculated that an S–N bond in the undamaged crystal has direction cosines (0·537, −0·743, 0·399) and that this vector makes an angle of $\cos^{-1}$ 0·993 or 6·9° with the largest principal value of the proton tensor. It is clear that at 300° K the radical $NH_2^+SO_3^-$ is a typical π-electron radical.

At 77° K the reorientation of the amino group had slowed to a frequency comparable with that of the hyperfine interaction, and consequently lines in the spectra arising from radicals the proton spins of which were antiparallel had broadened. Moreover, because of the anisotropic nature of the hyperfine interaction, this broadening was dependent on orientation. A complete analysis of the spectra at 77° K was not possible for this reason and, since our results add little to those of previous workers[5], no data are presented for 77° K.

At 4° K rotational motion of the amino group has ceased so far as the electron spin resonance experiment is concerned. All the lines in the spectra were sharp, and the two protons were distinguishable. As the magnetic field explored planes perpendicular to the orthorhombic axes two magnetically distinct sites, each giving rise to an eight-line spectrum, were apparent. Analysis of the spectra was made more difficult by the strong, and rather broad, signal from SO_3^-, and by the very small *g*-factor anisotropy in the spectra from $NH_2^+SO_3^-$ radicals. For these reasons no reliable estimate of the off-diagonal elements of the tensors can be offered. In Table 2 the observed hyperfine splittings of the three nuclei are listed for H_0 parallel to the three crystal axes. These splittings enable us to place an upper limit on the isotropic hyperfine interactions. The averages of the three diagonal elements listed in Table 2 are 109·5 Mc/s for the ^{15}N nucleus, and 64·6 and 66·6 Mc/s for the two protons. Since the off-diagonal elements of the ^{15}N tensor are not small, it is estimated that the isotropic hyperfine interaction cannot be greater than 95 Mc/s, or less than 75 Mc/s.

Table 2. Hyperfine Splitting of $NH_2^+SO_3^-$ in Sulphamic Acid at 4° K

Nucleus	Hyperfine splitting (Mc/s) with H_0 along axis		
	a	*b*	*c*
^{15}N	109·0	129·7	89·8
Proton	54·5	82·0	57·3
Proton	93·2	82·0	24·7

With regard to the possibility of a change in radical geometry at low temperatures, it will be recalled that the effect of changing the hybridization at the nitrogen atom from sp^2 to a non-planar configuration in which the hybrids used in bonding remain equivalent is to put *s* character into the orbital containing the unpaired electron. The fractional amount of such *s* character is $2\cot^2\theta$, where θ is the angle between the half-occupied orbital and one of the hybrids used in bonding[10]. Recently tabulated[11] *SCF-AO* data indicate a value for $\psi^2_{2s}(O)$ of 4·770 a.u. for nitrogen, and hence a hyperfine interaction of 2,161 Mc/s for an unpaired electron in an ^{15}N 2*s* orbital. It may be calculated that a distortion of 5° from planarity ($\theta = 85°$) would give a contribution to the isotropic ^{15}N hyperfine interaction of 33 Mc/s by this mechanism, and a distortion of 10° a contribution of 134 Mc/s. Such a contribution would add to that produced by spin polarization.

It is clear, however, that if the present estimate of 85 ± 10 Mc/s for the isotropic interaction at 4° K be accepted, a significant increase over that pertaining at 300° K (65 Mc/s) is apparent, and this may be due to a

distortion of ~5° from a planar skeleton at the nitrogen atom. On the other hand, an increase of comparable magnitude may well be due to the crystal field effects discussed by Rowlands and Whiffen[5].

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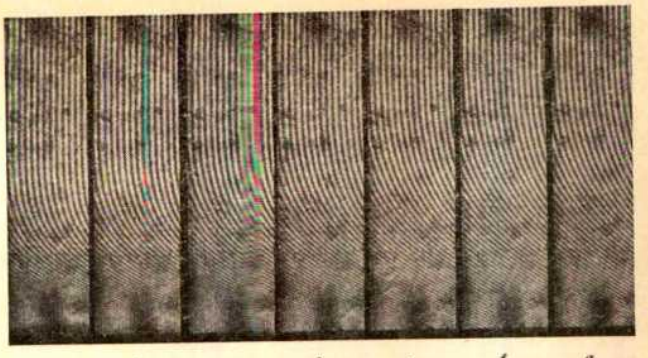
Equilibrium at the Solid–Liquid Interface

Ward and Brooks[1] in 1952 provided the only experimental justification for the much used assumption of concentration equilibrium at the interface during solid dissolution investigations. Using both Lamm scale and Rayleigh interferometer measurements of concentration gradients they calculated interfacial concentrations equal to the saturation values for the dissolution of salicylic acid and of *n*-butyl acetate in water. They also reported probable saturation for the benzoic acid–water system although the calculated concentration at the interface varied somewhat with the experimental method. It appears that a more appropriate instrument for such measurements would be the Mach–Zender interferometer, which permits the direct measurement of concentrations in the vicinity of a solid surface[2]. For the present work an interferometer was constructed similar to that described by Caldwell for diffusion studies[3]. The continuous light source was replaced, however, by a high-intensity, short-duration spark discharge[4] to provide better photographic resolution of the rapidly changing concentrations in the vicinity of the interface. This instrument was then used to investigate the dissolution in water of benzoic acid and of acetanilide.

The diffusion cells constructed for this investigation had two separate compartments arranged so that each was intersected by one of the interferometer light beams. The upper (reference) compartment was then filled with distilled water only, while the lower one contained both the solid under investigation and the water solution in which that solid was slowly dissolving. Powders of the appropriate solids were compressed into blocks in a hydraulic press under an applied pressure of approximately 10,000 lb./in.^{2}. The compressed solids were then carefully machined to fit into the cells and to provide flat, horizontal interfaces. Distilled water was permitted to fill the cell and overflow slowly. The water flow was then halted and a series of interferograms taken at convenient time intervals.

Fig. 1 is a typical set of interferograms. Parallel and vertical fringes on these photographs indicate a region of pure solvent. The number of fringes crossed while traversing downwards along a vertical path towards the interface gives the 'fringe shift' ($F.S.$) at any location. The concentration may be evaluated from the fringe shift at any point and the reference concentration as follows:

$$C - C_r = \frac{\lambda_0}{Lk}(F.S.)$$



a *b* *c* *d* *e* *f* *g*

Fig. 1. Interferograms for dissolution of benzoic acid in water. Time of contact: *a*, 2,340 sec; *b*, 2,820 sec; *c*, 3,360 sec; *d*, 4,020 sec; *e*, 5,040 sec; *f*, 5,820 sec; *g*, 6,300 sec

where λ_0 is the wave-length of the light beam in vacuum, L is the optical path length, and k is the concentration coefficient of refractive index. In this investigation the reference concentration, C_r, was always zero.

Interfacial concentrations were determined by measuring the maximum fringe shift from the undisturbed region to the interface by means of a Hilger microcomparator. Table 1 lists typical interfacial concentrations as well as saturation concentrations and calculated interfacial fluxes for both systems. The fluxes were obtained from the time-dependent concentration profiles measured for each system.

Table 1. Interfacial Concentrations at 25·25° C

System	Measured interfacial concentration (g/l.)	Measured saturation concentration (g/l.)	Interfacial flux (g/cm^2-sec)
Benzoic acid–water	3·273	3·370	$1·9 \times 10^{-7}$
Acetanilide–water	6·366	6·390	$1·83 \times 10^{-7}$

Because the observed interfacial concentrations are slightly less than the saturation values, it should be noted that the measured concentrations are distance averaged values along the optical path. Although care is taken to make the solid surface parallel to the interferometer beam, the camera cannot detect depression of the far side of the solid. The effect of any such surface misalignment or irregularity will be to reduce the apparent interfacial concentration by a slight amount. Thus, the measured deviations from equilibrium must represent a maximum value for this quantity with the possibility that the true deviation is somewhat smaller.

An interfacial transfer coefficient, β, was evaluated in the manner proposed by Drickamer[5]:

$$\text{flux}, J = \beta (C_{satn} - C_i)$$

For the conditions in Table 1, β assumes a value of $1·93 \times 10^{-3}$ cm/sec for benzoic acid in water and $7·51 \times 10^{-3}$ cm/sec for acetanilide in water. Since these parameters are nearly a thousand times greater than the respective diffusion coefficients, they far exceed the limits set by Drickamer for no measurable effect at the interface. Thus it is unlikely that a measurement of net mass flux between phases would detect any interfacial effect for either system, even if the departure from equilibrium reported for benzoic acid is realistic.

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Relationship between the Number of Interacting Particles and Flux Ratio

When the flux ratio does not obey the Ussing relationship[1] in a passive system, interaction must be occurring between molecules of substrate passing through the membrane. By using a thermodynamic argument based on induced transport[2] it is possible to relate the deviation from the Ussing relationship to the minimum number of particles that interact without reference to the mechanism involved.

Consider two chambers I and II separated by a passive membrane. Suppose that the concentration of the substrate in question is C_I in chamber I and C_{II} in chamber II, and that there is a net flux of substrate from I to II. Let a very small quantity of radioactive substrate be added to the chambers. As diffusion of the substrate through the membrane occurs, suppose that there is a device that selectively removes the non-radioactive molecules of substrate from one chamber and returns them to the other so that the concentrations C_I and C_{II} are maintained unchanged. As a consequence of this process the radioactive molecules will eventually become distributed in such a manner that the flux of radioactive molecules in the two directions are equal, or:

$$k_I\, m_{I\to II} = k_{II}\, m_{II\to I} \qquad (1)$$

where k_I is the specific activity of the substrate in chamber I, k_{II} is the specific activity of the substrate in chamber II, $m_{I\to II}$ is the flux of substrate from chamber I to II, and $m_{II\to I}$ is the flux of substrate from chamber II to I.

As equilibrium is approached the free energy required to transport $\Delta m'$ moles of radioactive substrate from chamber I to II becomes:

$$\left(RT \ln \frac{f_{II}\, C_{II}\, k_{II}}{f_I\, C_I\, k_I} - z\,EF\right) \Delta m' \qquad (2)$$

where E is the electrical potential difference between chamber I and II (E positive if chamber I is positive), z is the number of positive electrical charges on one molecule of substrate, f_I is the activity coefficient of substrate in chamber I, f_{II} is the activity coefficient of substrate in chamber II, R is the gas constant and T is the absolute temperature. This expression is derived on the assumption that the energy associated with radioactive and non-radioactive molecules is the same, and from a consideration of the entropy changes based on the probabilities of the systems. The free energy made available when Δm moles of non-radioactive substrate pass from chamber I to II is, on the other hand:

$$\left(RT \ln \frac{f_I\, C_I}{f_{II}\, C_{II}} + z\,EF\right) \Delta m \qquad (3)$$

Since the energy to transport the radioactive substrate molecules must have come from the movement of non-radioactive substrate, it follows that if Δm molecules of non-radioactive substrate transport $\Delta m'$ molecules of radioactive substrate from chambers I to II:

$$\left(RT \ln \frac{f_I\, C_I}{f_{II}\, C_{II}} + zEF\right) \Delta m \geqslant \left(RT \ln \frac{f_{II}\, C_{II}\, k_{II}}{f_I\, C_I\, k_I} - zEF\right) \Delta m' \qquad (4)$$

Let $r = \dfrac{\Delta m + \Delta m'}{\Delta m'}$, then from equations (1) and (4):

$$\frac{m_{I\to II}}{m_{II\to I}} \leqslant \left(\frac{f_I\, C_I}{f_{II}\, C_{II}}\, e^{zEF/RT}\right)^r \qquad (5)$$

Consider the process involved in the interaction (for example, a polyvalent carrier or a long pore). If one radioactive substrate molecule takes part at any instant in the process it is probable that all the other substrate molecules at the same instant are non-radioactive since the radioactive molecules are present in extremely small concentration. Hence r must represent the number of molecules that, on average, interact. Consequently, if an equality is assumed in equation (5) the experimental value of r will give the minimum number of interacting particles.

It is possible for the interaction to be such that the radioactive substrate molecules are induced to travel in the opposite direction to the movement of non-radioactive molecules. In this case, equation (2) has a negative sign and if $\Delta m/\Delta m' = r'$:

$$\frac{m_{I\to II}}{m_{II\to I}} \geqslant \left(\frac{f_I\, C_I}{f_{II}\, C_{II}}\, e^{zEF/RT}\right)^{1-r'} \qquad (6)$$

r' is the average number of non-radioactive molecules associated with the movement of one radioactive molecule in the opposite direction. Again, if an equality is assumed in equation (6) the experimental value of r' will represent the minimum number of particles.

The flux ratio criterion may be applied to chemical reactions[3] and similar arguments may also be used concerning the number of interacting particles.

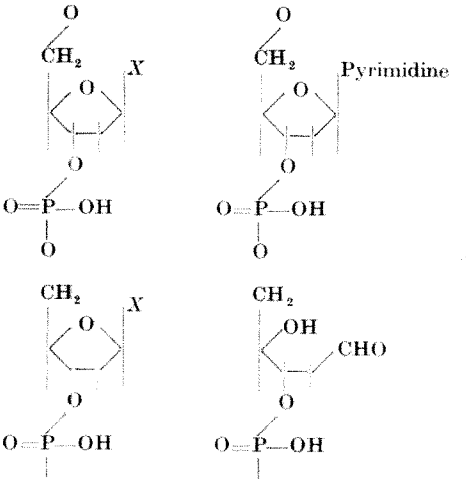
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Use of Acidic Degradation for the Determination of the Distribution of Pyrimidine Nucleotides in Deoxyribonucleic Acids

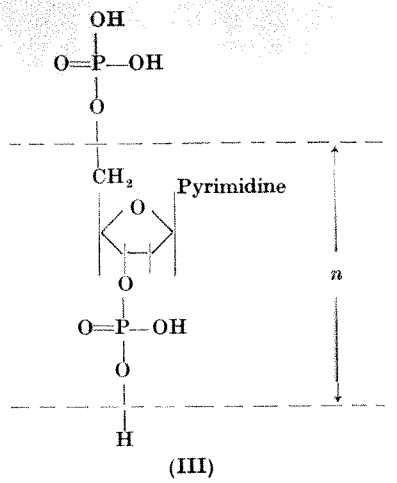
Acidic degradation has been widely used to determine the distribution of the pyrimidine nucleotides in deoxyribonucleic acids (DNA; I). Two main methods have been used; in one the DNA was treated with 0·1 M sulphuric acid at 100° for 35 min, and in the other, 67 per cent formic acid and 2 per cent diphenylamine at 30° for 17 h were used[2]. In both procedures it appears that the first stage is the hydrolysis of the purine glycosidic linkages to give apurinic acid (II).



X is purine or pyrimidine residue

(I) (II)

It is claimed by the authors of the two methods that the next stage is the degradation of the apurinic acid to give products with the structure $(\text{pyrimidine nucleoside})_n$ phosphate_{n+1} (III).



(III)

It is apparent, however, that the results obtained by these two methods are not the same, and also that there is greater degradation of the DNA by formic acid and diphenylamine than by 0·1 M sulphuric acid under the conditions used[3,4]. The question arises as to which, if either, procedure gives the correct result for the distribution of the pyrimidine nucleotide residues in DNA. As we were interested in applying these procedures to some oxidized deoxyribonucleic acids, we have made a detailed comparison of the two methods.

Highly polymerized calf-thymus DNA was treated with 0·1 M sulphuric acid at 100° for 35 min as described elsewhere[1]. The purines were removed from the hydrolysate by absorption on a column of 'Zeo-Karb 225' and the solution of pyrimidine oligonucleotides examined by paper chromatography in propan-2-ol–ammonia (sp. gr. 0·88)–water (70 : 6 : 30) for 18 h. Only very small amounts of free purines could be detected. The whole of the pyrimidine oligonucleotide-containing zones were eluted from the paper and analysed for purines. Only 1·3 per cent of the adenine and 1·8 per cent of the guanine of the original DNA remained in the oligonucleotide fraction. When calf-thymus DNA was treated with 67 per cent formic acid and 2 per cent diphenylamine at 37° for 18 h (the original authors used 30°; we found it more convenient to use 37°, which gave essentially the same degradation) and the products were examined as already outlined, the oligonucleotide fraction contained 0·7 per cent of the adenine and 1·8 per cent of the guanine of the original DNA.

These results showed that in both methods the purine glycosidic linkages were essentially completely hydrolysed.

A large-scale hydrolysis of DNA (320 mg) with 0·1 M sulphuric acid at 100° for 35 min was then carried out and the hydrolysate exhaustively dialysed against distilled water. It was found that 18·4 per cent of the phosphorus did not diffuse through the dialysis membrane and that the material that did not diffuse contained 1·15 moles of cytosine and 1·71 moles of thymine/4 g atoms phosphorus and negligible amounts of purines. On treatment of the material with pure prostatic phosphomonoesterase, 28·7 per cent of the phosphorus was converted into inorganic phosphate, showing that, on the assumption that each oligonucleotide chain carried two terminal phosphate groups, the material had an average chain-length of six nucleotide units (seven phosphate groups).

These results showed that only five bases per seven phosphate groups were present compared with the six bases required by structures of the type (pyrimidine nucleoside)$_n$ phosphate$_{n+1}$ (III). It appeared, therefore, that 2-deoxyribose units were present which were not glycosidically linked to purines or pyrimidines. That this was so was confirmed as follows.

(*a*) The non-diffusible material was treated with the Dische diphenylamine reagent[5] (the solutions were heated for 4 min instead of the 10 min given in ref. 5) when the typical blue colour given by 2-deoxyribose and purine deoxyribonucleosides was obtained. The amount of colour (after allowance had been made for the small amount produced from pyrimidine nucleosides) corresponded with the presence of 1·11 2-deoxyribose residues per seven phosphate groups.

(*b*) The non-diffusible material was treated with intestinal phosphatase (a mixture of phosphomonoesterase and phosphodiesterase) and the mixture examined by paper chromatography in butan-1-ol–ethanol–water (4 : 1 : 5) and the 2-deoxyribose-containing compounds detected by the use of a cysteine-sulphuric acid spray[6]. In addition to thymidine and deoxycytidine, a spot corresponding to 2-deoxyribose was detected.

(*c*) When the non-diffusible material was treated with 67 per cent formic acid and diphenylamine at 37°, the characteristic blue colour (which appears when DNA is treated with the reagent) was produced and 4·7 per cent of the total phosphorus was converted into inorganic phosphate.

It appeared, therefore, that the 'free' 2-deoxyribose units were randomly distributed in the oligonucleotide chains because, if they were all end groups, the liberation of inorganic phosphate would have been about 14 per cent and if they were all single residues within the chain there would have been no inorganic phosphate.

The diffusible material was then examined by paper chromatography and found to contain a small amount (< 1 per cent) of thymidylic acid and deoxycytidylic acid. A small amount of thymine (< 1 per cent) and an appreciable amount of cytosine (5·5 per cent of the cytosine of the DNA) were also present. Small amounts of ultra-violet-absorbing components, which in the propan-2-ol–ammonia–water solvent had R_F values between those of thymidylic acid and thymidine-3′,5′-diphosphate, were also detected. These were not identified, but their spectra indicated that they probably contained pyrimidines. The treatment of the DNA with the 0·1 M sulphuric acid converted 13·1 per cent of the phosphorus into inorganic phosphate.

A similar investigation was then made of the action on DNA of 67 per cent formic acid and 2 per cent diphenylamine at 37° for 18 h. In this case only 10·1 per cent of the phosphorus of the DNA did not diffuse through the dialysis membrane and the non-diffusible material contained 1·38 moles of cytosine and 2·18 moles of thymine/4 g atoms phosphorus. The average chain-length was 6·7 phosphate groups. This showed that in this case no 2-deoxyribose residues were present which were not glycosidically bound to pyrimidines. This was confirmed by the fact that the material gave no reaction for 2-deoxyribose with the Dische diphenylamine reagent; moreover, after treatment with intestinal phosphatase, thymidine and deoxycytidine could be detected but no 2-deoxyribose.

The diffusible material contained a small amount (< 1 per cent) of thymidylic acid and deoxycytidylic acid, no thymine was present and the amount of cytosine present was only 1·2 per cent of the total present in the DNA. There were no ultra-violet-absorbing components running between thymidylic acid and thymidine-3′,5′-diphosphate on the propan-2-ol–water–ammonia chromatograms. The amount of inorganic phosphate produced during the reaction with formic acid and diphenylamine was 28·3 per cent.

These results show that treatment with 0·1 M sulphuric acid at 100° for 35 min does not completely degrade DNA to pyrimidine sequences of the type (pyrimidine nucleoside)$_n$ phosphate$_{n+1}$ (III), whereas with 67 per cent formic acid and 2 per cent diphenylamine at 37° for 18 h it appears that the degradation to (III) is essentially complete and specific. This explains why, in the former procedure, a greater amount of the longer nucleotide sequences was apparently present than with the latter. The degradation of these higher sequences (produced by

the action of 0·1 M sulphuric acid by a subsequent treatment with formic acid and diphenylamine) is therefore a specific degradation and not non-specific as has been claimed[4]. (It should be noted that a sequence of the type (pyrimidine nucleoside)$_n$ phosphate$_{n+1}$ cannot be degraded to two shorter sequences of the same type without liberation of a pyrimidine base or pyrimidine nucleoside.) Furthermore, the treatment with 0·1 M sulphuric acid liberated a considerable amount of cytosine. This may explain why sequences of four or more cytosine units were not found using this method, but were found when the other method was used[3]. This formation of cytosine also indicated that it was unlikely that the procedure could be improved by increasing the time or temperature of the reaction.

It can be concluded, therefore, that the method of choice for the determination of pyrimidine nucleotide distribution in DNA is the use of formic acid and diphenylamine; the results obtained here confirm those of the original authors and show that the method is highly specific.

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BIOPHYSICS

Possible Mechanism of Thrombus Formation

It has often been observed that after trauma (and possibly under other conditions) the thrombi which form in the vascular system consist of a small white head (the white embolus) and a longer red tail[1]. The predominant constituent of the embolus is generally aggregated platelets, and some recent experiments may throw light on the mechanism of formation of the red tail, which consists primarily of red cells.

It is well known that when a suspension of rigid, neutral-density particles flows down a tube the mean velocity of the particles is slightly greater than that of the transporting liquid[2]. Thus when a suspension is first drawn through an empty tube there is an increase in the concentration of particles at the leading interface as it moves along the tube[3]. Blood behaves in a similar way to a suspension of particles in this respect[4], red cells congregating at the leading interface. Moreover, lower transit times (corresponding to higher velocities) have been measured *in vivo* for red cells than for plasma[5].

We have now been able to show that if, into a suspension of rigid particles flowing down a tube, a sphere is introduced the diameter of which is very near to that of the tube the concentration of particles immediately behind it increases, while that immediately ahead falls. Fig. 1 shows the result of introducing a polystyrene sphere of diameter 1·8 mm into a tube of diameter 1·9 mm down which a neutral-density suspension of polystyrene spheres, 0·35–0·42 mm in diameter, is flowing at a mean volume concentration of 5 per cent.

The reason for the concentration changes is that the wall exclusion effect which is the primary cause of the difference in mean velocity between the particles and the liquid is only important over a limited range of particle/tube diameters. It disappears when the particles are very small (that is, less than about 1/30th of the tube diameter)[3] and also when the diameters of the particles and the tube approach equality. The particle sizes for the experiment are chosen so that the small spheres try and overtake the large sphere but are unable to squeeze by it so that their concentration rises immediately behind the sphere and falls immediately ahead of it.

From the observed similarities in the flow behaviour of neutral-density spheres in a liquid and red cells in plasma which was mentioned earlier, there are good grounds for expecting that the concentration changes observed in a suspension adjacent to a close-fitting sphere in a tube would be paralleled by changes in the distribution of red cells near white emboli moving in vessels of appropriate diameters. The differential flow velocity of red cells and plasma should reach a maximum in the range of diameter of human blood vessels of about 15–50μ, so that vessels of this size would be most affected. Vascular occlusion would be most likely in vessels possessing a minimum of branches, and the chances of complete stasis would increase with decreasing elasticity of the vessel wall. The plug would be identified by a reduced concentration of red cells in the plasma immediately ahead of it and by a white head which practically filled the lumen. It would be interesting to discover whether occlusions of this type have, in fact, been observed.

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BIOCHEMISTRY

Separation of Urinary Isoamylases on Cellulose Acetate

A number of electrophoretic investigations have been carried out on human α-amylases (*E.C.* 3.2.1.1.). Paper[1,2], agar[3,4], and polyacrylamide gel[5] have been used as media.



Fig. 1



Fig. 1. Electrophoresis of (*A*) pancreatic extract; (*B*) urine (normal) concentrated twenty times; (*C*) saliva

Norby[4] achieved separation of salivary and pancreatic isoamylases from extracts of their tissue of origin on agar gel and also showed that salivary amylolytic activity is itself complex—a point confirmed by Muus and Vnenchak[5] recently.

The present investigation on urinary amylases made use of cellulose acetate with veronal buffer as the separating system. The substrate, instead of starch-containing agar used by previous investigators[3,4], was a brand of hydrolysed starch of the type used for starch-gel electrophoresis[6] (Starch-hydrolysed Lot 227–1, Connaught Medical Research Laboratories, Toronto, Canada). This starch, made up with buffer in the concentration recommended by the manufacturer, provided an all-starch substrate of sufficient consistency to be cast by thin-layer chromatography apparatus on glass plates. Such a substrate has an advantage over those with an agar base because agar is not digested by α-amylases. The buffer was Michaelis[7] barbital sodium-acetate containing 0·02 M calcium chloride to prevent phosphorolytic digestion of the starch.

Twenty-two specimens of urine were obtained from healthy staff members of this hospital and three urine specimens from infants attending a well-baby clinic. The urines were filtered and concentrated twenty times by ultrafiltration. Extracts were made of salivary glands and pancreatic tissues from post-mortem subjects in the above-mentioned buffer. Diluted saliva was centrifuged to clear its mucin. Suitable dilutions were then made of saliva, salivary gland and pancreatic extracts. The amount applied to the strips was 1 μl. of each of the materials investigated. Electrophoresis was carried out at 0·4 m.amp/cm. width at 200 V for 1 h. The resulting strips were carefully laid on the starch-plates and incubated at 37° C. At the end of 30 min the strips were removed and the plates stained in 0·02 N iodine. The presence of amylolytic activity was revealed by clear areas against a purple background of undigested starch. All the bands of amylolytic activity were on the cathodic side of the origin. The result of one experiment is shown in Fig. 1.

The urine was observed to contain two isoamylases as revealed by their digestion of starch gel. The faster-moving component appeared to be the same as that obtained from saliva and salivary gland extracts. Extracts of pancreatic tissue produced a band with the same mobility as the slower band of the urine. The relative mobilities are similar to those revealed by agar-gel electrophoresis[4]. In another experiment the two bands of activity from urine and the single bands from saliva and pancreas were cut from the electrophoresis strip using portions of the same strips which have been developed on starch plates as markers. The isoamylases were eluted out with buffer, concentrated and re-run on cellulose acetate using *tris*-EDTA-borate buffer of Aronsson and Grönwall[8]. The relative positions of the bands were retained. The amylases of saliva and those present in extracts of sub-lingual, sub-mandibular and parotid glands appear to have the same electrophoretic mobilities. Two patients with symptoms and signs of pancreatitis had serum amylase levels of 2,200 and 320 Somogyi units per 100 ml. respectively. Their urines did not require concentration and were electrophoresed alongside concentrated normal urine. In both cases a distinct band of activity with a mobility similar to that of the pancreatic enzyme was seen (Fig. 2). The salivary band predominated in the urine from a patient with mumps.

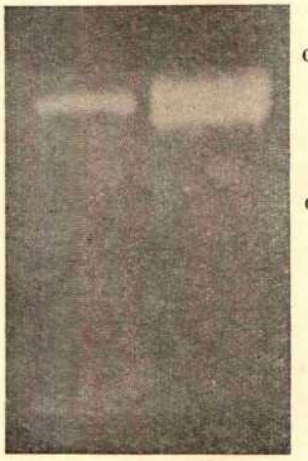


Fig. 2. Electrophoresis of (*P*) patient's urine; (*N*) normal urine concentrated twenty times. Note the absence of salivary amylolytic activity in the patient's urine which was not concentrated by ultrafiltration

The twenty-two adult urines yielded two bands each. Where they appeared well developed they were of equal intensity. This may not be surprising when it is noted that the weights of salivary glands and pancreas are roughly equal. The former apparently make a greater contribution to urinary amylase than previously suspected. Furthermore, in the urine of the babies investigated (all less than 1 year old) the band of pancreatic activity was significantly less marked than that associated with salivary amylase. According to Somogyi[9], plasma amylase, absent in the new-born, only reaches normal level in about 1 year. Pancreatic secretion of amylase is not well established in the first year of life[10].

The isoamylases of salivary glands and pancreas appear to be associated with definite proteins (Fig. 3).

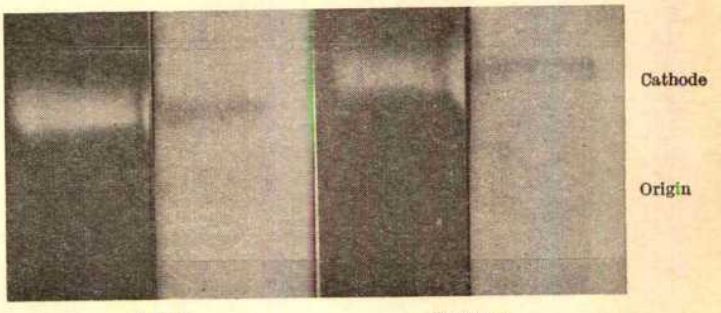


Fig. 3. Amylolytic activity of diluted saliva and pancreatic extract are shown alongside electropherograms of their sources. Protein stained with amidoschwarz 10*B* on the right, developed starch-plates on the left

Cellulose acetate electrophoresis of a urinary concentrate may prove of value in elucidating the nature of abnormal levels of serum amylases.

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Fructose as a Component of the Foetal Blood and Foetal Fluids of the Bush Baby (*Galago senegalensis senegalensis*)

The presence of fructose in substantial concentrations in the foetal blood and fluids appears to be associated with those animals (ungulates and Cetacea) which have either an epitheliochorial or a syndesmochorial type of placenta[1]. The galago, although a member of the Primate Order, has a placenta of the epitheliochorial type[2,3] and is in contrast to many other members of this Order which have placentae of the haemochorial type. On the foregoing generalization, the placenta of *Galago* could be expected to produce fructose.

Three animals which had been mated in captivity at the Royal Veterinary College were anaesthetized intramuscularly with 2 mg/kg body-weight of 'Sernyl' (Parke, Davis and Co.) followed by inhalation of ether. Each animal bore a single foetus which was exposed by Caesarian section; at the same time samples of the foetal fluids were removed. Blood samples were obtained from both foetus and mother by cardiac puncture. The mating dates of the animals were not known. However, the foetuses were covered with a well-developed pelage, and from consideration of their crown-rump lengths and body weights it would appear that they were within a few weeks of the reported 4 months' gestation period[4,5].

All samples were deproteinized with barium hydroxide–zinc sulphate and the filtrates estimated for glucose and fructose by the glucose oxidase[6] and resorcinol[7] methods respectively. The resulting colours were read on a Spekker absorptiometer with a blue (Kodak No. 1) filter. The glycogen concentration in the placenta and foetal liver was estimated by a modification of Pflüger's method[8]. The values obtained in these determinations are shown in Table 1.

It will be seen that the glucose concentration of the maternal blood exceeded that of the foetal blood. Both foetal fluids had relatively low concentrations of glucose and in this respect they resemble the foetal fluids of the sheep[9]. Considerably more glycogen was present in the foetal liver than in the placenta. The fructose concentration of the foetal blood and in particular that of the foetal fluids is high. It was, however, observed that the resulting colour after heating with acid-resorcinol was atypical and indicated the presence of an interfering substance. It thus became important to establish the presence of fructose by other methods. The absorption spectrum of the acid-resorcinol colour produced by amniotic fluid was similar to that of a fructose solution in having peaks at 410 and 480 mμ when read in a Unicam *SP* 600 spectrophotometer. The slower decline of the amniotic fluid curve at the higher wave-lengths suggested that a substance having a broad absorption in the green region of the spectrum was superimposed on the curve. An attempt to reveal the absorption of this substance by the removal of fructose in an initial alkaline hydrolysis[10] proved unsuccessful and the subsequent absorption spectrum was similar to that given by the acid-resorcinol reagents (Fig. 1).

Some of the deproteinized samples were treated with indolyl-3-acetic acid[11] and gave absorption spectra identical to that of fructose in having a single peak at 520 mμ; an example is shown in Fig. 2.

Further confirmation of the presence of fructose in the samples was afforded by descending paper chromatography using amyl alcohol–pyridine–water (4 : 3 : 2) and butanol–acetic acid–water (12 : 3 : 5); the substances were visualized by a silver nitrate method[12]. In each animal fructose was detected in the foetal blood and in the amniotic and allantoic fluids. An example of a chromatogram is seen in Fig. 3.

It would seem, therefore, that fructose is present in a high concentration in the foetal blood and foetal fluids of

Table 1. Concentration of Glucose, Fructose and Glycogen in Samples obtained from Pregnant Bush Babies (*Galago senegalensis senegalensis*)

Values expressed in mg/100 ml. or mg/100 g of wet tissue

Foetal wt. (g)		9·71	11·17	14·32
Crown-rump lengths (cm)		—	5·6	6·0
Maternal blood:	glucose	178	255	225
	fructose	10	15	3
Foetal blood:	glucose	73	79	55
	fructose	218	236	87
Amniotic fluid:	glucose	5	13	12
	fructose	555	748	207
Allantoic fluid:	glucose	—	27	19
	fructose	—	738	151
Placental glycogen		168	4	87
Foetal liver glycogen		625	460	3,775

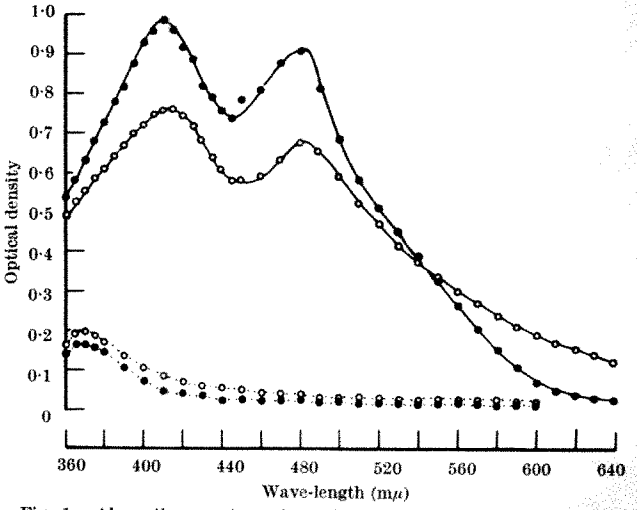


Fig. 1. Absorption spectra using acid-resorcinol. ●—●, Fructose; ○—○, amniotic fluid; ○ - - - ○, amniotic fluid after alkaline hydrolysis; ● - - - ●, blank reagents

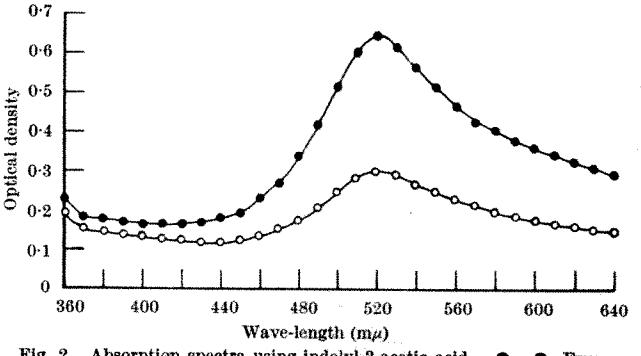


Fig. 2. Absorption spectra using indolyl-3-acetic acid. ●—●, Fructose; ○—○, foetal blood

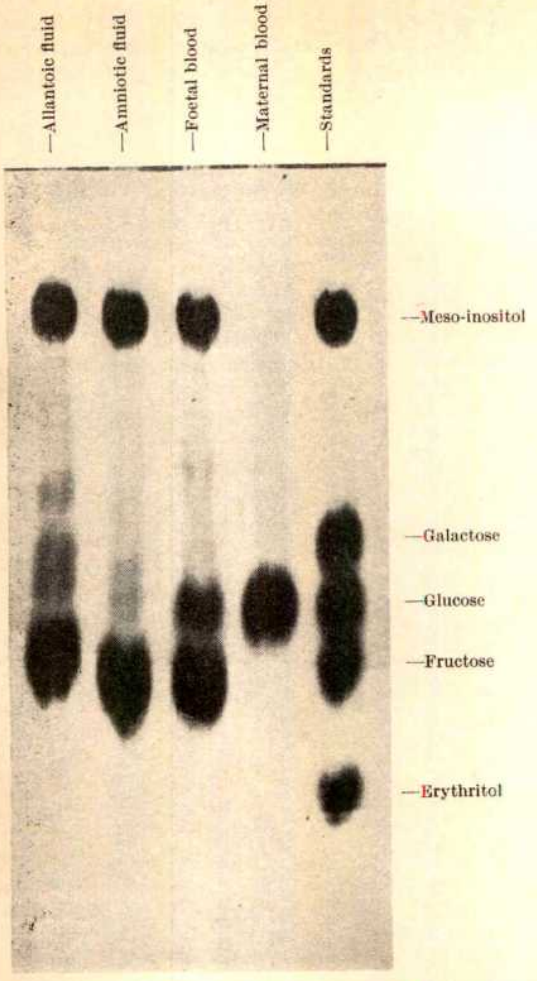


Fig. 3. Paper chromatogram of allantoic and amniotic fluids and foetal and maternal blood of the bush-baby (*Galago senegalensis senegalensis*) developed in amyl alcohol : pyridine : water for 18 h and visualized by silver nitrate

Galago senegalensis senegalensis, although an accurate determination may have been prevented by the presence of an interfering substance. Fahmy, Huggett and Pryce (unpublished) have obtained some evidence of its occurrence in the foetus of *Lemur mongoz*, which, like the galago, has an epitheliochorial placenta. These observations would thus appear to strengthen further the postulation that fructose in foetal life may, in some way, be related to placental histology.

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Liberation of Adenine from Deoxyribonucleic Acid by Hydrogen Peroxide

Recently[1], we have established a metabolic cycle producing virogens and mutagens, in which hydrogen peroxide performs a central function. It is assumed that these virogenic and mutagenic metabolites can act on nucleic acids in cells. We have now demonstrated that adenine is specifically liberated from deoxyribonucleic acid molecule by the action of hydrogen peroxide.

Deoxyribonucleic acid was prepared from calf thymus according to the method of Kay[2] and dissolved in 0·14 M sodium chloride + 0·014 M sodium citrate. A mixture consisting of 2 ml. of deoxyribonucleic acid (4 mg/ml.) + 1 ml. of 2 M hydrogen peroxide + 1 ml. of 0·2 M *tris* buffer (*p*H 7·5) was kept at 37° C for 48 h. The solution was spotted on a 'Toyo' No. 51 filter paper and developed with an isopropanol–12 N hydrochloric acid–water (170 : 41 : 39) solvent. Guide spots were also made with a standard solution containing adenine, guanine, cytosine and thymine. Exposure to ultra-violet light revealed that the solution treated with hydrogen peroxide produced a spot of R_F 0·37 corresponding to adenine. To confirm this finding, the spot was cut off, extracted with 0·1 N hydrochloric acid and the ultra-violet absorption of the extract was measured. The curve obtained corresponded to that of adenine, having a maximum at 260 mμ and a minimum at 239 mμ. The reaction mixture after treating with 0·5 M hydrogen peroxide was also subjected to chromatography with a butanol–acetic acid–water (5 : 2 : 3) solvent. Only one spot of R_F 0·75 appeared on the paper and the solution obtained by extracting this portion with 0·1 N hydrochloric acid gave an ultra-violet absorption curve corresponding to adenine.

The reaction mixture, on the other hand, was dialysed against water and hydrolysed with 6 N hydrochloric acid at 100° C. Chromatography was carried out using an isopropanol solvent as already mentioned. The results showed that, while most of the adenine and thymine remained in the deoxyribonucleic acid-digest, guanine and cytosine were almost completely decomposed by 0·5 M hydrogen peroxide.

The experiments, therefore, were then conducted with 0·1 M hydrogen peroxide. The reaction mixture was incubated at 37° C for 72 h and a portion of it subjected to chromatography with the isopropanol solvent. Only one spot corresponding to adenine was produced. The spot was extracted as already described and the absorption of the extract measured at 260 mμ. The phosphorus of organic form in the reaction mixture was also determined according to the method of Eto[3]. From these analyses it was calculated that 35 per cent of adenine in the deoxyribonucleic acid used was liberated in the free state.

Another portion of the reaction mixture was dialysed and hydrolysed with 6 N hydrochloric acid. Chromatography was performed with isopropanol solvent. Four spots with R_F values corresponding to adenine, guanine, cytosine and thymine were formed on the paper. Extracts were separately prepared from these spots with 0·1 N hydrochloric acid. Their absorption was then measured at 260, 250, 275 and 265 mμ respectively. The estimation of phosphorus was further made for the reaction mixture after dialysing. The degree of decomposition of four bases in deoxyribonucleic acid by 0·1 M hydrogen peroxide was calculated from the data obtained. The results are given in Table 1.

These experiments revealed that, although considerable portions of all bases remained in deoxyribonucleic acid treated with a diluted hydrogen peroxide, only part of the

Table 1. Decomposition of Bases in Deoxyribonucleic Acid by 0·1 M Hydrogen Peroxide

	Relative amounts of bases			
	Adenine	Guanine	Cytosine	Thymine
Before treatment	0·29	0·21	0·21	0·29
After treatment	0·19	0·07	0·10	0·16

adenine was liberated. Supplementary experiments disclosed, however, that the proportion of the remaining bases varied to a certain extent with the concentration of peroxide or time of reaction, and that a liberation of adenine deoxyribotide occurs sometimes under mild conditions.

In Yamafuji's cycle, it is shown that nitrite, hyponitrite, hydroxylamine and oxime inhibit cellular catalase, thus leading to an accumulation of hydrogen peroxide[4]. Omura, at our Institute (personal communication), found that adenine and guanine were liberated from deoxyribonucleic acid by the action of hydroxylamine. In addition, Watanabe (personal communication) proved that the mechanisms of the reaction of hyponitrite and glucosoxime with nucleic acid-components were different from those of nitrite and hydroxylamine. These results indicate that four kind of nitrogenous mutagens and peroxide specifically act on deoxyribonucleic acid. Our present investigations also suggest that in a very low concentration these metabolites may cause a sufficient denaturation of nucleic acid molecules in cells after a long period.

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Possible Structural Similarity between Insulin and Glucagon

Insulin and glucagon are pancreatic protein hormones which produce opposing physiological effects, the former lowering and the latter raising the concentration of sugar in the blood. With the elucidation of the complete amino-acid sequences of insulin[1] and glucagon[2] it became possible to compare the two structures in the hope of finding some correlation or complementary features in their sequences. Heretofore such comparisons have not revealed striking similarities or parallels between the two primary structures. Indeed, there are only four dipeptides which the two proteins have in common. This communication points out a similarity between the two primary sequences which are seen in Fig. 1.

The two interchain disulphide bonds in insulin form an 85 membered ring—27 amino-acid residues. The glucagon chain has 87 atoms in the backbone structure—29 amino-acid residues. If the NH_2 terminus of glucagon, histidine, is placed next to the histidine in the *B* chain of the large insulin ring, in the manner seen in Fig. 2, an interesting result follows. As the glucagon chain (indicated by the dotted line in Fig. 2) is laid along the insulin ring it is seen that the next residue, serine, coincides with a serine residue in insulin. The remaining two serines in the large insulin ring are exactly matched by serines in glucagon. The remaining serine in glucagon is matched by a glutamic acid residue in insulin. There are two other matching points in the sequences when compared in this way—threonine and tyrosine—as seen in Fig. 2.

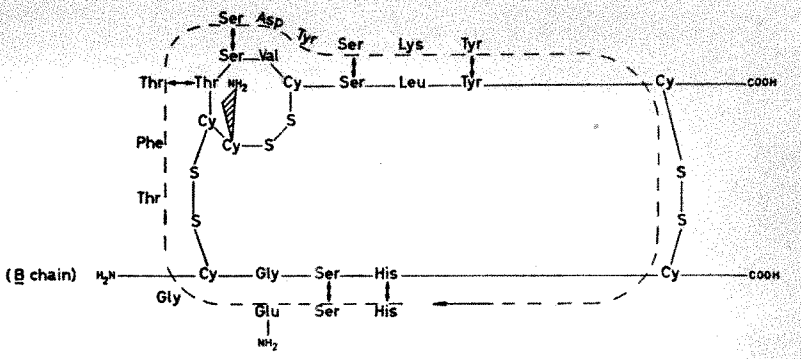


Fig. 2. Insulin is represented by the solid line and glucagon by the dotted line. The polypeptide chains are not drawn to scale. Distances between the amino-acids (with respect to histidine) are given in Table 1

In all, of the four hydroxl groups in the insulin ring, three are found to coincide with similar functional groups in glucagon. There are five amino side groups and two guanido side groups in glucagon, whereas the large insulin ring has one amino group.

A test of the closeness of fit of the large insulin ring and that proposed for glucagon shows good agreement. The difference in the total lengths of the two fully extended polypeptide chains is 3 Å, using the parameters of the fully extended trans-polypeptide chain[3]. The disulphide and sulphide bonds are relatively large, 2·04 Å and 1·82 Å respectively, and make up for the fewer number of peptide bonds in the insulin ring.

Table 1 summarizes the distances along the two fully extended chains. The correspondence is quite good in each case, showing that about one-fifth of all the amino-acids in glucagon have exact counterparts in insulin when the sequences are compared in this manner. There appears to be no sequential correlation according to hydrophobic or hydrophilic side-chains when the two rings are compared. However, it is true that the large insulin ring is richer than glucagon in the total number of hydrophobic side-chains, whereas the latter has more ionizable groups.

It is highly unlikely that either of these proteins exists in the fully extended conformation *in vivo* and yet there appears to be nothing in the structure of glucagon which would prevent it from assuming the conformation of the large insulin ring, whatever that may be. It is possible that the similarities between insulin and glucagon which are pointed out here are somehow related to the physiological action of these hormones and suggests that they may bind to a common site.

Two of the insulin residues involved in the correlation shown in Table 1 (threonine at 23·2 Å and serine at 26·9 Å) vary according to the animal source of the protein[5]. In beef insulin alanine is substituted for threonine, in horse insulin glycine is substituted for serine, and in sheep insulin both of these substitutions occur. Whether or not these differences

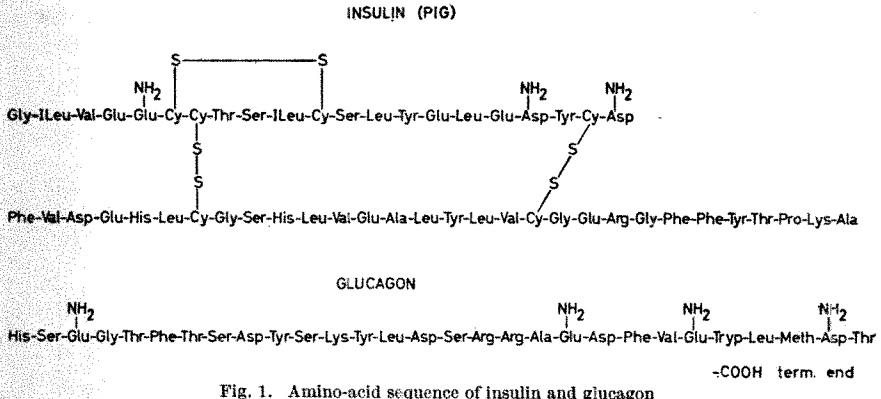


Fig. 1. Amino-acid sequence of insulin and glucagon

Table 1. Comparison of Distances between Amino-acid Residues when Insulin and Glucagon are aligned as in Fig. 2, in a Fully Extended Configuration

The distances are in angstroms and indicate the distances between α carbon atoms (refs. 3, 4)

Insulin (pig) Amino-acid	Distance (Å)	Glucagon Amino-acid	Distance (Å)
His	0	His	0
Ser	3·6	Ser	3·6
Thr	23·2	Thr	21·7
Ser	26·9	Ser	25·3
Ser	37·7	Ser	36·2
Tyr	45·0	Tyr	43·5

confer slightly different activities cannot be determined with current methods. Biological assays indicate that insulin from different animals has about the same activity[6].

One possible test of the significance of the correlations pointed out here would be to assay the activity of glucagon after it has been cyclized to form a new peptide bond across the ends. Such a reaction should be possible with current synthetic methods. If the foregoing similarity between insulin and glucagon is significant with respect to their mode of action then it is expected that the cyclized form of glucagon would be biologically active.

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Effect of Age on Serum-bound Sialic Acid in Rats

Changes in the level of glycoproteins have been reported for a large number of conditions, primarily in man[1–6]. Since glycoproteins contain a number of sugar components for which quantitative methods are available, many of these methods have been used to estimate the serum- or plasma-bound carbohydrate. With the refinements of the thiobarbituric method[7] for a semi-specific assay of *N*-acetyl neuraminic acid, we have made use of this method to investigate the changes with age in the concentration of plasma-bound sialic acid in the rat in an initial effort to use experimental animals in the investigation of the physiological significance of glycoproteins.

Male Long–Evans Hooded Wistar rats were obtained from Simonsen Laboratories, Gilroy, California. After weaning, all animals were maintained on 'Purina' laboratory chow and water *ad libitum*. About 0·5–1 ml. of blood was obtained from the tail vein while the rats were under light ether anaesthesia. The blood was clotted and centrifuged and the serum was removed and stored at −40° C. Serum was diluted 1 : 50 and 1 : 30 in duplicates and hydrolysed in 0·05 N H_2SO_4 at 90° C for 30 min. *N*-acetyl neuraminic acid was determined by the Warren thiobarbituric method utilizing a known *N*-acetyl neuraminic acid preparation (an *N*-acetyl neuraminic acid of 95 per cent purity was obtained from Sigma Chemical Co., St. Louis, Missouri).

Data on the effect of age on serum-bound *N*-acetyl neuraminic acid are shown in Table 1. The results are

Table 1. Effect of Age on the Level of Bound *N*-Acetyl Neuraminic Acid in Serum

Age	No. of animals	Percentage of the 6-month value
22 days	10	28·8 ± 7·60
31 days	10	33·5 ± 8·28
55 days	9	96·5 ± 18·57
6 months	8	100 ± 33·02
14 months	9	162·9 ± 28·70

expressed as the percentage of the 6-month value. The 6-month value was 125·1 ± 41·3 mg of *N*-acetyl neuraminic acid per 100 ml. of serum in reference to the commercial preparation. These data clearly demonstrate that there is an increase in the concentration of the acid-labile serum *N*-acetyl neuraminic acid with age. The function or physiological significance of this elevation in the level of serum sialic acid and the chemical identity of the augmenting fractions is now being investigated.

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Role of Lysosomes in Adrenal Necrosis caused by Dimethylbenzanthracene

It has long been recognized that carcinogenic chemicals, including polybenzenoid hydrocarbons, have injurious effects on cells[1,2]. Evidence has been presented that polybenzenoid hydrocarbons are concentrated in the lysosomes of living cells and the suggestion was made that release of lysosomal enzymes may play an important part in their cytotoxic action[3]. The necrosis of rat adrenal cortical cells produced by 7,12-dimethylbenz(*a*)anthracene (DMBA)[4] provided an opportunity to test this hypothesis in more detail, because it is specific in two ways. First, the degeneration is organ specific, adrenal cortical cells of the rat being much more sensitive than the cells of other organs. Secondly, it appears to be specifically produced by one compound, 7-hydroxymethyl-12-methylbenz(*a*)-anthracene (7-OHM-12-MBA), which is a metabolite of DMBA: an isomeric metabolite, 12-hydroxymethyl-7-methylbenz(*a*)anthracene (12-OHM-7-MBA), does not produce adrenal damage[5]. Rats can be protected against adrenal damage after administration of DMBA by previous treatment with a number of aromatic hydrocarbons and amines, which diminish the formation of 7-OHM-12-MBA from DMBA[5]. Hence it seems that the metabolite is more active than the parent compound in producing adrenal necrosis.

In order to ascertain whether there is any relationship between *in vivo* damage and release of lysosomal enzymes, particulate fractions of organelles from rat adrenals, kidneys and livers were prepared. In the case of the adrenal and kidney, the whole mitochondrial fraction, prepared by centrifugation of homogenates at 10,000*g* for 15 min, was used. The *F*3 fraction, a partially purified lysosomal preparation from rat liver, was used for comparison. This preparation, which was obtained by a sucrose layering technique, showed a twelve-fold increase in specific acid phosphatase activity as compared with the whole mitochondria preparation. The suspensions, adjusted by a standard turbidity (E_0 (500 mμ), 2·0) were incubated in 0·25 M sucrose buffered with 0·02 M *tris* pH 7·4. The carcinogen was dissolved in dimethyl sulphoxide (DMS) and added at the beginning of incubation. The

Table 1. Release of Acid Phosphatase from Particulate Fractions of Rat Adrenal, Kidney and Liver

	Conc. (μg/ml.)	Incubation time (min)	Percentage release of acid phosphatase Adrenal	Kidney	Liver
7-Hydroxymethyl-12-methyl-benzanthracene	100	15	23	—	5
7-Hydroxymethyl-12-methyl-benzanthracene	100	15	27	10	—
12-Hydroxymethyl-7-methyl-benzanthracene	100	15	7	—	—
DMBA	100	15	13	15	—
DMBA	100	30	22	—	6
DMBA	50	30	14	—	0
DMBA	10	15	9	7	—
Methylcholanthrene	100	15	5	0	—
Methylcholanthrene	10	15	2	0	—
Retinol	50	30	26	—	36
Control	—	15	2	3	—
Control	—	30	4	—	2

final DMS concentration was 2·5 per cent (v/v). At the end of the incubation period the preparation was centrifuged at 10,000*g* for 15 min and the free acid phosphatase estimated in the supernatant with *p*-nitrophenylphosphate as substrate. In some experiments retinol, a derivative of vitamin A known to increase permeability of lysosomal membranes[6], was used as a control. Other controls had the solvent alone. The percentage enzyme release is expressed as a fraction of that released by Triton X-100.

The results of several experiments, some of which are summarized in Table 1, show that retinol causes considerable release of enzyme from both adrenal and liver lysosomes, whereas 7-OH-12-MBA had a well-marked effect on adrenal lysosomes, but only a slight action on those of liver or kidney. 12-OH-7-MBA and 20-methylcholanthrene brought about no significant release of enzymes from either adrenal or kidney lysosomes. DMBA had some effect on adrenal lysosomes, especially after the longer incubation period shown in Table 1. The effect was, however, much less than that of 7-OH-12-MBA, and the possibility of metabolism of DMBA in this system has not been excluded.

It is clear that the membranes of lysosomes from different organs do not respond uniformly to the presence of chemicals. The membranes of rat adrenal lysosomes are highly sensitive to 7-OH-12-MBA, but less so to the isomer 12-OH-7-MBA, to DMBA, or to other hydrocarbons. Thus the *in vitro* results parallel the sensitivity *in vivo*, and it seems very likely that the adrenal necrosis observed in rats treated with DMBA is due to the concentration of 7-OH-12-MBA in adrenal cortical lysosomes with release of sufficient lytic enzymes to destroy the cells. There is other evidence that release of enzymes from lysosomes can irreparably damage cells[7].

The relationship of these results to carcinogenesis by DMBA and other hydrocarbons is not yet clear, but it has been suggested[3,7] that gradual release of enzymes from lysosomes over long periods may be implicated in malignant transformation. This process is likely to be different, quantitatively if not qualitatively, from the sudden and massive release of lysosomal enzymes which is probably involved in adrenal necrosis.

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PHYSIOLOGY

Behaviour of Carbonic Anhydrase in the Uterus of Hens during Moult

The carbonic anhydrase present in the uterus of hens is involved in the calcification of the egg-shell[1]. The activity of this enzyme in the uterus seems to be greater in 'good' than in 'poor' layers or in non-layers[2]. Recently we pointed out that this enzyme is located at the apical pole of the cells of the tubular glands[3] (Fig. 1). We succeeded in demonstrating, by a histochemical method[4], that the reactivity of carbonic anhydrase is reduced in the uterus gland cells in moulting hens, whereas moulting has no influence on the carbonic anhydrase activity in the erythrocytes (Fig. 2). These results suggest an enzyme activation or an induction of enzyme synthesis similar to the effect of progesterone on the carbonic anhydrase in the endometrium of mammals[5,6].

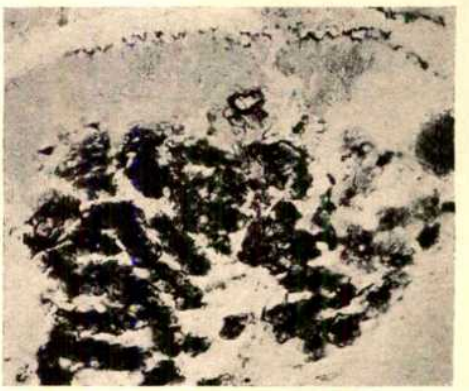


Fig. 1. Carbonic anhydrase activity in the tubular glands of the uterus of a laying hen. (× 270)

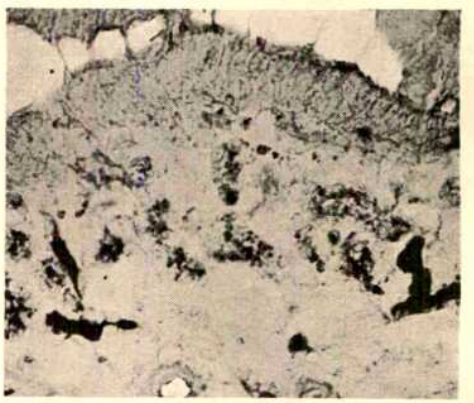


Fig. 2. Reduced carbonic anhydrase activity in the tubular glands of a moulting hen. Capillaries filled with enzyme-active erythrocytes. (× 270)

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In vitro Oxygen Consumption of Slices from Kidney, Brain, Cortex and Liver in Hypoxia

Most authors assert that the acute renal insufficiency observed in shock, or after haemorrhage, is due to the damage caused by hypoxia.

Munck's *in vivo* experiments showed that in haemorrhagic shock the oxygen saturation of renal cortex capillary and of renal venous blood was scarcely decreased. He therefore doubted the importance of hypoxia in his cases[1]. The degree of hypoxia is only one of the possible injurious factors; the other factor could be the sensitivity of different tissues to hypoxia.

In different mixtures of gases containing oxygen and nitrogen, we compared the *in vitro* oxygen consumption of renal cortex, brain cortex and liver slices.

White female rats (body-weight 130–150 g) were used. The animals were killed by decapitation. The rapidly removed organs were placed into Ringer phosphate buffer (*p*H 7·2; 0° C). The thickness of the slices was checked by Warburg's method. The respiration of 300 mg fresh wet tissue placed into 3 ml. buffer was measured in a Warburg apparatus. 0·1 ml. 20 per cent sodium hydroxide solution was used for absorbing carbon dioxide. Oxygen–nitrogen mixtures of different compositions were streamed through the flasks. Measurements were made at 30° and 37° C.

The oxygen consumption of renal cortex slices at 37° C was so strong that the deviation due to the frequent reading of the manometer was bigger than usual (see Tables 1 and 2).

Table 1. Oxygen Consumption of 300 mg Fresh Tissue/h, 30° C

	100% O_2	50% O_2	20% O_2	10% O_2
Kidney	476	416	254	164
$n = 12$	±37	±9	±43	±3
Liver	172	140	126	97
$n = 12$	±33	±4	±15	±10

Table 2. Oxygen Consumption of 300 mg Fresh Tissue/h, 30° C

	100% O_2	50% O_2	20% O_2	10% O_2
Kidney	442	323	247	148
$n = 16$	±45	±17	±13	±9
Brain	251	241	157	124
$n = 16$	±37	±11	±12	±9

Our experiments show that the oxygen consumption of the renal cortex is greatly influenced by hypoxia: (1) The oxygen consumption *in vitro* of renal cortex, brain cortex and liver slices is directly proportional to the logarithm of the oxygen content of the gas mixture (see Figs. 1–3). (2) The oxygen uptake of renal cortex slices decreased relatively more in gas mixtures with falling oxygen content than did that of the brain cortex and liver slices.

It can be assumed that the renal cortex is unable to take up the needed amount of oxygen even if the oxygen tension is relatively high. This means that a degree of hypoxia which is too small to damage other organs may produce severe alterations in the kidney.

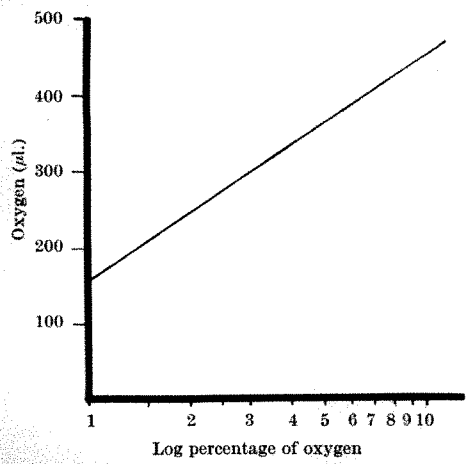


Fig. 1. Relationship between *in vitro* consumption of oxygen by the renal cortex slices and the log oxygen content of gas mixture. $y = 321{\cdot}9 = 293{\cdot}7\ (x - 1{\cdot}55)$

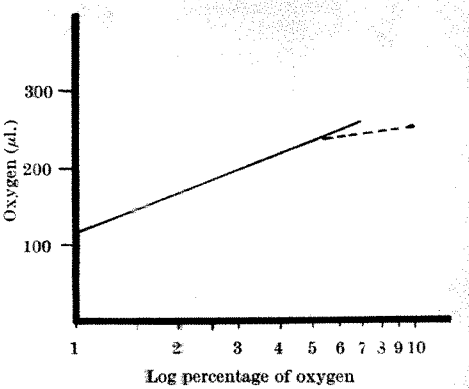


Fig. 2. Relationship between *in vitro* consumption of oxygen by brain cortex slices and the log oxygen content of gas mixture. $y = 175{\cdot}07 = 171{\cdot}4\ (x - 1{\cdot}34)$

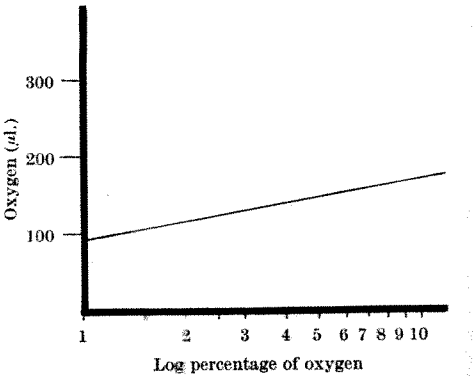


Fig. 3. Relationship between *in vitro* consumption of oxygen by liver slices and the log oxygen content of gas mixture. $y = 139{\cdot}2 = 79{\cdot}90\ (x - 1{\cdot}55)$

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Time Course of Failure of Neuromuscular Transmission after Motor Nerve Section

After transection of a motor nerve, the severed part of the axon undergoes progressive changes known as Wallerian degeneration. But before the degenerating axon loses its ability to conduct action potentials, function and structure of the motor nerve terminals are impaired and neuromuscular transmission fails[1]. Several workers have noted that this failure occurs at a time which depends on the length of the degenerating nerve segment[2,3]. This communication describes experiments which make it possible to relate quantitatively the length of the degenerating axon to the time at which neuromuscular transmission fails.

The experiments were made on the phrenic nerve-diaphragm preparation of female albino rats (140–170 g). Operating under anaesthesia, the left phrenic nerve was sectioned, either near its emergence from the spinal cord in the neck, or in the thorax a few mm from the entry of the nerve into the diaphragm. In the former case, 3·5–4·0 cm of extramuscular nerve remained connected to the

diaphragm. At various times after nerve section, muscles were isolated and examined at room temperature (about 22° C) with intracellular microelectrodes to determine the functional state of the motor endplates. The endplates in the diaphragm are distributed within a band about 2 mm wide, the centre of which is about 1·5 mm toward the costal margin from the main intramuscular nerve trunk. In a normally innervated diaphragm spontaneous miniature endplate potentials (m.e.p.p.s) resulting from transmitter leakage from the nerve terminals are observed in more than 95 per cent of electrode penetrations made within this band. Failure to record m.e.p.p.s from this region of a muscle fibre is therefore a satisfactory indication that the discharge has ceased at that endplate. Endplates were sampled at 0·5 mm intervals following a course parallel to the intramuscular nerve trunk and making a total of 70–80 penetrations in most muscles. The presence or absence of m.e.p.p.s was recorded after each electrode penetration. Muscle fibres from which no spontaneous activity was recorded were examined for at least 1 min and many for longer periods. In those experiments in which the long length of nerve remained attached to the muscle, the nerve was stimulated to test whether transmission had failed at each junction.

The percentage of fibres examined at which m.e.p.p.s were observed (*a*) when the nerve was left long and (*b*) when it was cut short is plotted against the time after nerve section in Fig. 1. Each point summarizes the results from 150–200 impalements. The time course of the failure of transmission when the nerve was long was almost identical to that for the failure of m.e.p.p.s. In only a few instances were m.e.p.p.s recorded from muscle fibres which did not respond to nerve stimulation. In no case was transmission observed when m.e.p.p.s were not present. It is clear from these results that when the nerve had been cut far from the muscle, the failure of m.e.p.p. activity occurred about 3 h earlier than when the nerve had been cut close. It is also apparent that once the junctional failure started, it developed with a similar time course in both cases.

The results of Fig. 1 were further analysed to determine whether a varying length of the intramuscular nerve also influenced the time at which failure of m.e.p.p.s occurred. For this purpose, the observations were grouped, according to the distance of the endplate from the nerve entry, into four divisions each 0·5 cm wide. The results from each region were plotted as in Fig. 1, and the time at which m.e.p.p.s had failed at 50 per cent of the endplates ($t_{50\%}$) was read off the curve. These times have been plotted in Fig. 2 against the length of the sectioned nerve up to the mid-point of each region.

With the exception of the one point corresponding to the shortest length of nerve, there is a nearly linear relation between nerve length and $t_{50\%}$. The discrepancy in the region nearest the site of nerve section may be due to local acceleration of degenerative processes resulting from injury of the nerve at the nearby point of section. The regression line calculated for the remaining seven distances is given by $t_{50\%} = 0{\cdot}67\ L + 10{\cdot}4$, where L is the nerve length (cm) and $t_{50\%}$ is in hours.

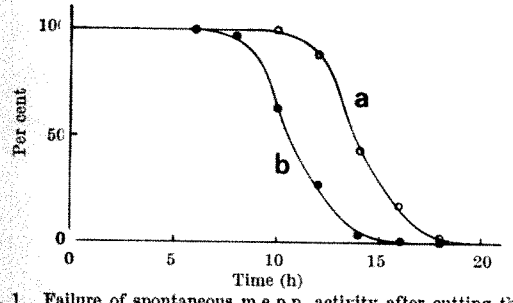


Fig. 1. Failure of spontaneous m.e.p.p. activity after cutting the rat phrenic nerve: (*a*) in the neck, 3·5–4·0 cm from the diaphragm (open circles); (*b*) in the thorax, 1–3 mm from the diaphragm (filled circles). Abscissa, time in hours after cutting the nerve; ordinate, percentage of impaled fibres at which spontaneous activity was observed. Pooled results from 1,500 penetrations in 23 muscles

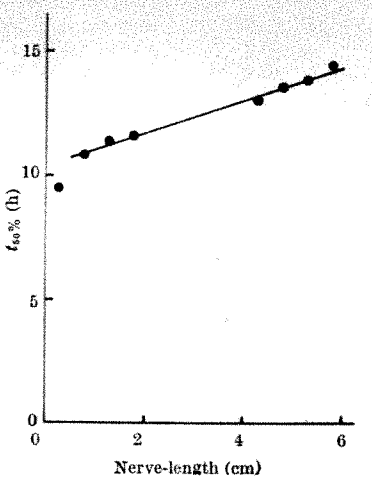


Fig. 2. Influence of the length of peripheral nerve stump on the time of m.e.p.p. failure. Abscissa, length of peripheral nerve stump in cm; ordinate, time at which m.e.p.p. activity has failed at 50 per cent of endplates

These results indicate that the presence of the peripheral nerve stump delays the failure of neuromuscular transmission after nerve section. For each cm of intra- or extra-muscular phrenic nerve remaining, the failure of m.e.p.p.s is delayed about 40 min. This is a smaller effect of the nerve than has been reported for peripheral nerve of the cat, where each cm adds about 5 h (ref. 3).

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PHARMACOLOGY

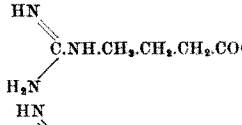
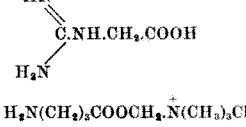
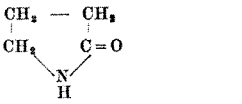
Inhibition of *Ascaris* Muscle by γ-Aminobutyric Acid: a Possible New Assay Method

Ascaris lumbricoides, a round worm commonly found in the intestine of pig, has been used as an isolated muscle preparation for the investigation of anthelminthic potency[1]. We are now making a wider assessment of *Ascaris* as a pharmacological tool. Responses were recorded from a length of dorsal muscle, cut from the anterior region of the worm, mounted in an organ bath containing modified Tyrode's solution at 37° C (refs. 2 and 3). In our experiments the most striking pharmacological effects have been seen with γ-aminobutyric acid (GABA), which produced a rapid reversible inhibition of *Ascaris* muscle, reflected as a relaxation of the preparation, in concentrations as low as 0·5 μg/ml. The time required for full development of relaxation at this concentration is 3–4 min. In contrast, the common anthelminthic agent piperazine produces a much slower relaxation (more than 10–15 min) at concentrations of the order of 50 μg/ml. Similar relative concentrations of GABA and piperazine have been shown to depress spike potentials in *Ascaris* muscle[4,5].

The high potency of GABA suggested that its action might be structurally specific and this has been sub-

stantiated by the behaviour of some available congeners (Table 1). The spatial relationship of the amino and carboxyl group in GABA, and the primary character of the amino group, appear to be critical. The lactam, 2-pyrrolidone, for example, is inactive. The importance of the spatial relationship is particularly well illustrated by the high potency of guanidinoacetic acid. Alteration of chain-length and substitution of the amino or methylene groups markedly lowers activity.

Table 1. INHIBITION OF *Ascaris* MUSCLE BY GABA AND RELATED COMPOUNDS

Compound	Structure	Activity (GABA = 100)
γ-Aminobutyric acid	$H_2N.CH_2.CH_2.CH_2.COOH$	100
β-Aminopropionic acid	$H_2N.(CH_2)_2.COOH$	1·5
δ-Aminovaleric acid	$H_2N.(CH_2)_4.COOH$	9
ε-Aminocaproic acid	$H_2N.(CH_2)_5.COOH$	0·1
β-Aminobutyric acid	$H_2N.CH(CH_3).CH_2.COOH$	< 0·001
α-Aminobutyric acid	$H_2N.CH.COOH$ (C_2H_5)	< 0·001
Sodium butyrate	$CH_3.CH_2.CH_2.COONa$	< 0·001
Butylamine hydrochloride	$H_2N.CH_2.CH_2.CH_2.CH_3$, HCl	< 0·001
γ-Amino-β-hydroxybutyric acid	$H_2N.CH_2.CH(OH).CH_2.COOH$	< 0·001
Methylaminobutyric acid	$CH_3.NH.CH_2.CH_2.CH_2.COOH$	< 0·001
Glutamic acid	$H_2N.CH(COOH).CH_2.CH_2.COOH$	< 0·001
γ-Guanidinobutyric acid		7
Guanidinoacetic acid		43
γ-Aminobutyrylcholine chloride hydrochloride	$H_2N(CH_2)_3COOCH_2.\overset{+}{N}(CH_3)_3Cl^-.HCl$	2·5
2-Pyrrolidone		< 0·001

Ascaris is a convenient preparation for the assay of GABA. Concentrations between 0·5 and 2·0 μg/ml. give graded responses which lie on a linear regression line when plotted against the logarithm of the dose (Fig. 1). Results with other smooth muscle preparations have been described as variable[6,7]. The most satisfactory alternative involves inhibition of 5-hydroxytryptamine-induced contractions of the guinea-pig ileum, but this preparation, unlike *Ascaris*, is affected by many common endogenous substances. The usual method for assaying GABA, in which impulses are recorded from the crayfish stretch receptor neurone[8], is apparently reliable but technically more demanding.

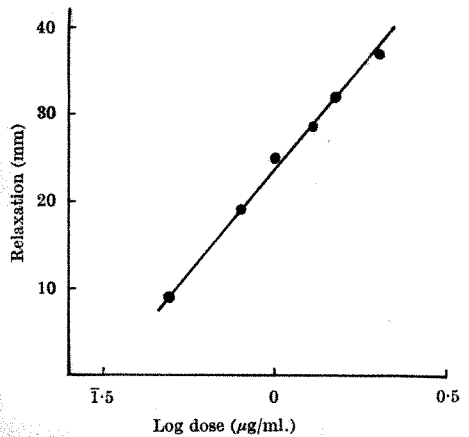


Fig. 1. Relaxation of isolated *Ascaris* muscle in modified Tyrode's solution by GABA. Doses were administered in random order at 7-min intervals

Ascaris is insensitive to many of the pharmacologically active substances present in brain, for example, noradrenaline, 3,4-dihydroxyphenylalanine, 3,4-dihydroxyphenylethylamine, 5-hydroxytryptamine and histamine in concentrations up to 2 mg/ml. Acetylcholine (0·2–2 μg/ml.), however, contracts the *Ascaris* preparation but the contractions may be blocked by *d*-tubocurarine[9] (20 μg/ml.); they are only slightly reduced by atropine (10–50 μg/ml.). We have not yet tested substance *P*, but on present evidence *Ascaris* appears to provide a simple and convenient means of studying the neuroanatomical distribution of GABA. The precision of the assay method is now under investigation.

GABA occurs in high concentrations not only in the central nervous system of vertebrates but also in the nervous tissue of some invertebrates[10]. Glutamic acid, the natural precursor of GABA, has been detected in *Ascaris*[11], but the possible occurrence or significance of GABA in *Ascaris* remains an open question.

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HAEMATOLOGY

Effect of Basic Proteins on the Adhesiveness of Red Cells

THE methods already described[1,2] for measuring the stickiness of red cells to surfaces of various composition cannot be applied to the stickiness of red cells to each other ('adhesiveness' in the sense used by Conan[3]), for counting becomes difficult or impossible when neighbouring red cells form even small aggregates. Adhesiveness, however, can be measured in the following way.

A substance, for example histone, which increases the adhesiveness of red cells to each other, is dissolved in 1 per cent sodium chloride in a concentration of 2 mg/ml. Two ml. of this is diluted in a series of small tubes by powers of 2, and 0·5 ml. of a human red cell suspension in saline (50 mm³/10 ml. saline) is added to each tube. The contents of the tubes are allowed to sediment at 4° C, for 18 h. If the cells stick to each other, even in small clumps, they cover the bottom of the tube diffusely; if they do not stick to each other, they form a sharply defined 'button' at the foot of the tube. This effect is well known to serologists, and provides a very sharp end-point differentiating clumping (adhesiveness or stickiness of red cells to each other) from its absence. The end-point can be verified microscopically.

The effect of a second substance, for example albumin, on the adhesiveness produced by the first substance can be demonstrated by making serial dilutions in powers of 2 of the second substance (1 ml. of a concentration of 2 mg/ml., 1 mg/ml. . . .) and adding 1 ml. of the first substance in constant concentration (for example, 2 mg/ml.) and 0·5 ml. of red cell suspension to each tube. Readings

are made after 18 h at 4° C, using the same criteria as before.

The histone referred to below was prepared from calf thymus by acid extraction[4]; the salmine and lysozyme were pure preparations supplied by I.B.F. and N.B.C., respectively. All were dissolved in 1 per cent sodium chloride in concentrations of 2 mg/ml.; in this concentration the *p*H was between 6·7 and 6·9. The proteins keep in the refrigerator, but solutions must be freshly prepared. The other proteins referred to were purified samples, dissolved similarly.

Adhesiveness. The basic proteins histone and salmine produce increased stickiness of human red cells to each other, that is, increased adhesiveness, in concentrations greater than 0·015 ± 0·01 mg/ml. This effect is virtually irreversible by washing with saline, is unaccompanied by haemolysis in concentrations less than 2 mg/ml., and is almost independent of the red cell concentration in the system; this is probably because, as the concentration increases, the chances of contact between cells increases. These effects on adhesiveness are related to the effect of histone and salmine on the electrophoretic velocity of the red cells, which is rapidly reduced to zero in concentrations of the order of 1 mg/ml.

Lysozyme, in concentrations of 2 mg/ml. or less, has no effect on the adhesiveness of human red cells; in higher concentration it is haemolytic, and in much higher concentration (20 mg/ml.) it reduces the surface charge of human red cells[5].

Effect of fibrinogen and gelatine. The only plasma protein which affects the agglutinating effect of histone and salmine is fibrinogen, which, while not producing an increase in stickiness itself, increases red cell adhesiveness about eight-fold. Serum albumin and serum globulin have no effect in concentrations of 2 mg/ml. or less, although in much higher concentrations they form complexes with histone[5].

Gelatine in concentrations of 2 mg/ml. (at which level it decreases the stickiness of red cells to glass probably because it coats the glass—it is well known that it is not adsorbed on red cells) decreases the adhesiveness of human red cells to each other about four-fold in systems containing histone or salmine.

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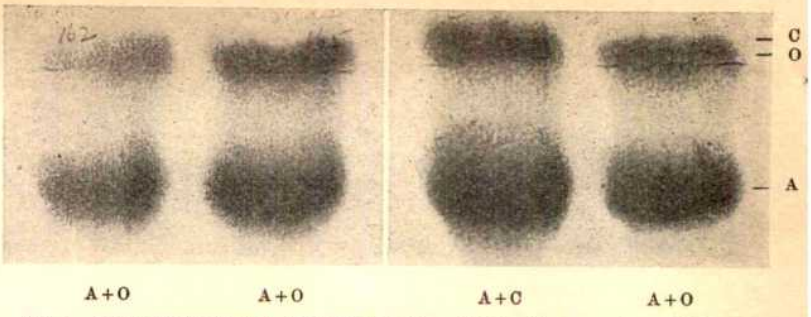


Fig. 1. Paper electrophoresis, barbiturate buffer *p*H 8·6. Three samples of HbA+O and one HbA+C control

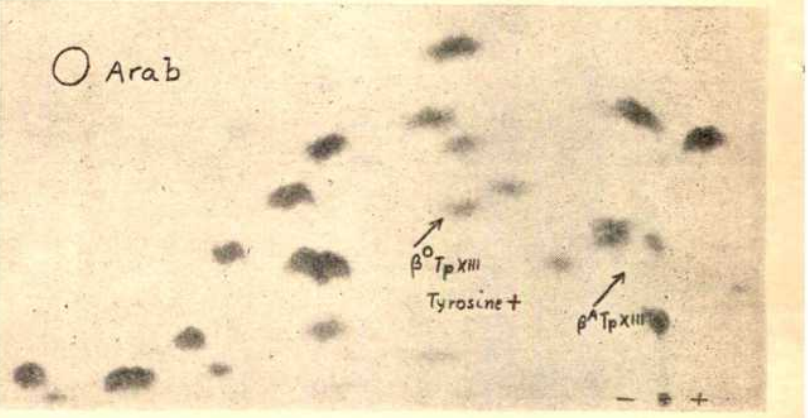


Fig. 2. Fingerprint of the soluble peptides from the tryptic digest of haemoglobin O from the Sudan. It shows the feature of HbO Arab[10]. β^{A}TpXIII is missing and a new tyrosine-containing peptide with additional positive charge can be demonstrated

Haemoglobin O Arab in Sudanese

THE Northern and Southern Sudanese differ from an anthropological point of view. The first are primarily Arab, but with varying degrees of African mixture, while the second are Nilotic–Hamitic Africans. The latter can be divided into Northern and Southern Nilotes. Among some groups of Southern Nilotes, haemoglobin S is common[1,2], but it is absent or low in the Northern Nilotes, for example, Dinka, Shilluk and Nuer[3]. Occasionally, also, haemoglobin Stanleyville II is found which relates these populations to their southern and western neighbours[4,5]. In the Northern Sudanese, sickling is found at a low, but constant, rate in Khartoum (182 of 9,100 examined, that is, 2 per cent) and at a more variable rate in Western Sudan[6]. It seems that the Northern Sudanese possess haemoglobin O which relates them to their Arab neighbours. Indeed, haemoglobin O Arab was first reported in an Arab family in Israel[7], but it has recently been found also in Bulgaria[8].

In 1962, we reported the finding of haemoglobin O in a Northern Sudanese woman in Khartoum[9]. Over a 4-year period we have examined 9,300 unselected Northern Sudanese in Khartoum and have found eleven instances of the carrier trait for haemoglobin O Arab. The haemoglobin O was identified in all instances in the Sudan and in England by its electrophoretic (Fig. 1) and other properties[7] and characterized as O Arab in three instances, by examination of the peptides arising from tryptic digestion of the isolated abnormal haemoglobin fraction by fingerprinting[10] (Fig. 2).

The incidence of haemoglobin O Arab in Northern Sudanese is admittedly low (about 0·1 per cent), but when viewed in the light of other surveys numbering many thousands of persons in Africa, it assumed some significance. It is tempting to consider the haemoglobin to be an Arab feature, particularly since it was first found in an Arab family. However, one of the large-scale surveys which failed to reveal the presence of haemoglobin O Arab was conducted in Saudi Arabia[11]. It is thus possible that the haemoglobin O Arab of the Northern Sudanese may be part of their non-Arab heritage related to pre-simitic Egypt. It is worth recalling that sickling was also present in the Arab family in which haemoglobin O Arab was first described, and that sickling is not a feature of pure Arab ('Bedouin') stock, and that haemoglobin O Arab has recently been reported in three generations of an American Negro family[12]. Kantchev *et al.*[8] have suggested that haemoglobin O Arab in Bulgaria may be the outcome of an independent mutation. Before accepting this unreservedly, one has to consider carefully

the links between Bulgaria and various parts of the middle-east and north-east Africa provided by the movement of armies during the prolonged Turkish occupation of these regions.

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Haemoglobin Typing of the Kerry Breed of Cattle

Two types of haemoglobin, designated Bov A and Bov B, have been identified in the blood of cattle by Bangham[1]. These are distinguished by their motility in paper electrophoresis; Bov A is the slow-moving and Bov B the fast-moving component. The type of haemoglobin which occurs in an animal is controlled by two simple allelic autosomal genes which give rise to three phenotypes A, AB and B.

In a survey of stud bulls in Britain, Bangham[2] found that with the exception of the Jersey, Guernsey and South Devon breeds all other British breeds exhibited A type haemoglobin exclusively, while in the case of Channel Island and South Devon bulls both B and AB types were encountered in addition to A type haemoglobin. In a subsequent survey of some cattle breeds of Europe and Africa, Bangham and Blumberg[2] found that B type haemoglobin occurred in breeds located geographically along a line running southwards through France into Africa, while cattle of north-east France, Holland and Denmark showed A type haemoglobin exclusively. This finding tended to support the suggestion of Boston[3] that one ancestral line of the Jersey breed originated in the Indus valley and permeated through Africa to Europe. The type of haemoglobin occurring in Kerry cattle—a breed native to Ireland—has not previously been reported and it was felt that haemoglobin typing might throw some light on the views of Moyles[4], who has suggested that the Kerry breed originated in mid-Asia.

The standards for the different types of haemoglobin were established according to the method of Bangham[1] using blood samples from different breeds, including Friesians, Shorthorns, Herefords, Guernseys and Jerseys, which exhibited the A, AB and B types. Blood samples were then collected from 25 Kerry cattle in a pure-bred Kerry herd and these were run on paper electrophoresis side by side with samples from other breeds. All twenty-five samples from the Kerry cattle contained the commonly occurring A type haemoglobin. This finding has more recently been confirmed by Dabczewski[5].

Haemoglobin typing, therefore, would suggest that the Kerry breed of cattle has no link with that ancestral line of Channel Island cattle but would appear to have an origin resembling that of other northern European breeds.

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IMMUNOLOGY

Active Immunization of Mice with Chagastoxin

Previously we reported on the immunology, immunochemistry and immunochemotherapy of experimental *Trypanosoma cruzi* infection in mice. *T. cruzi* has at least 9 antigens. Sonic lysates of the cultures contain antigens A, B, C, D, E, F, G and H, and the sediment contains B, E, D and X. Living trypanosomes have antigens B, E and G. Saline supernatant has antigens A, B and F, while distilled water supernatant has A, B and E, and sediment antigens B and X. The recently phenolized cultures have A, E, G and X, and old phenolized cultures have only B. 50 per cent extraction with ammonium sulphate yields A, B, E, G and F. 100 per cent saturation still extracts B, while precipitation with alcohol after full saturation with ammonium sulphate still extracts antigen B. The phenol extract and sediment (Westphal) contain no antigens, but phenol aqueous extract contains antigen B. The antigens B, E and G are major antigens. The agglutinin titre of hyperimmune rabbit serum was 1 : 10,240 and that of horse serum was 1 : 327,600. Both rabbit and horse hyperimmune sera had 1 : 4 titre of agar-gel precipitin. Immunotherapy with hyperimmune rabbit serum prolonged the survival time in fatal *T. cruzi* infection in mice two- to three-fold. Chemotherapy with 1-furaltadone, in doses of 50 mg/kg/day for two weeks, followed by 12·5 mg/kg/day for 2·5 months, protected 86·7 per cent of infected mice, while combined immunochemotherapy with hyperimmune rabbit serum plus 1-furaltadone protected all mice for at least 5 months[1–3].

The present report deals with the toxicity and immunizing properties of the lipopolysaccharide, chagastoxin.

Phenol extraction. Chagastoxin is prepared or extracted using the following modified Westphal method[4], used for the extraction of lipopolysaccharides from the cell walls of Gram-negative rods. *Trypanosoma cruzi* (Tulahuan) is grown for 10–12 days on brain heart blood agar (Difco), containing 5–8 per cent citrated human blood obtained from the blood bank, in Roux bottles. The cultures are pooled, should contain no bacteria and erythrocytes, and are sonically lysed for 90 sec in Raytheon sonic oscillator model *DF*01, 10 kc/s, 250 W, 115 V and 60 c/s. After spinning, the sediment and supernatant are treated with an equal volume of 88 per cent phenol for 30 min in the refrigerator. It is then centrifuged at 3,000–4,000 r.p.m., and the supernatant is then dialysed for 3 days in running tap water and for 24 h against distilled water. The sediment is precipitated with alcohol or is directly lyophilized. The phenol fraction is precipitated with 95 per cent alcohol (6–10 volumes); the sediment is washed in saline and then lyophilized. Gentle handling is

necessary, otherwise the phenol sediment may disperse in the aqueous or phenol phases.

Ammonium sulphate extraction. The lysed trypanosomes are centrifuged, and the supernatant is then treated with ammonium sulphate so that its final concentration is 50 per cent. When 54·2 g ammonium sulphate is added to 100 ml. distilled water, the resulting solution is fully saturated. For 100 per cent saturation, ammonium sulphate crystals are added until full saturation is reached. The precipitate, after full saturation, is then dialysed against tap water for three days, distilled water 24 h, and then lyophilized.

Sheep were immunized with formalin–phenol-killed *T. cruzi* culture, had an agglutinin titre of 1 : 20,280, but they had no agar-gel precipitin antibodies. The agglutinin titre of sheep immunized with ammonium sulphate extracts was 1 : 5,120, and they had no agar-gel precipitin antibodies.

Protective effect of chagastoxin. Formalin–phenol-killed cultures of *T. cruzi* have no protective effect. Freshly lysed virulent cultures have low or weak protective value. The quantity of antigenic material in lysed cultures is low, and a large volume of material cannot be injected into mice. 50 and 100 per cent ammonium sulphate extracts also have low or weak protective value. Old lysed cultures do not have protective value. The immunizing/protective factor is very unstable.

Forty-five mice weighing 8–10 g were injected intraperitoneally with 0·5 mg chagastoxin, twice a week, for seven doses. There were fifteen control mice, of the same age and weight. Two weeks after the last dose of toxin, all mice were challenged with 120 *M.L.D.* of *T. cruzi* from an infected mouse. *M.L.D.* of *T. cruzi* was about 1,000 trypanosomes from an infected mouse. The controls died in less than 7 days, while the immunized mice lived up to 40 days. Naturally, such heavy infection does not occur in persons who are exposed to triatomid bugs which are infected.

Toxicity of chagastoxin. According to the criteria of Milner *et al.*[5], the surface antigens or lipopolysaccharides of Gram-negative bacteria should fulfil the following requirements: lethal effect expressed in $L.D._{50}$, protective or immunizing properties, pyrogenicity in rabbits, Shwartzman reactions and skin tests. Freshly lysed virulent cultures, phenol–formalin-killed cultures, ammonium sulphate extracts, phenol extract and phenol sediment are all non-toxic to mice. Chagastoxin (lipopolysaccharide) as a phenol aqueous supernatant is toxic to mice. 2·5 mg injected intraperitoneally each day for three successive days, in mice weighing 10 g, or 5 mg daily for three days in mice weighing 25 g, killed 50 per cent of animals within less than a week. 1γ of chagastoxin injected intravenously in rabbits raised the temperature 2° in 2–3 h, and 10γ raised the temperature 6·8° F during 2 h, the temperature then falling gradually. 1 mg chagastoxin injected intradermally in clean rabbits failed to give rise to a dermal reaction, but the same amount injected in rabbits immunized with killed and live cultures gave rise to a positive dermal reaction.

At present we are actively investigating the problem of immunization, using various doses and durations, intervals between immunization and challenges with virulent trypanosomes, and varying the doses of the challenge. We are also investigating the effect of the booster dose of chagastoxin, the pharmacology and pathology of the toxin in animals, and the effect of chagastoxin in the therapy of chronic lesions in animals. It should be borne in mind that, in the chronic stages of the disease, there are few parasites in the cells or tissues. Probably most of the damage at cellular or tissue level could be the result of the Arthus phenomenon, through exposure of the host to the antigen to the parasite. Chagastoxin may play a part in desensitizing the tissues to further damage by the parasite, or it may increase the permeability of the cell membrane to 1-furaltadone, thus increasing the lethal action of the drug to the pathogen. At present immunochemotherapy in chronic Chagas disease in animals is being investigated, using chagastoxin plus 1-furaltadone.

Note added in proof. Since the submission of this paper to *Nature*, we would like to report the following important experimental observations: (1) Sixteen doses of 500 μg chagastoxin intraperitoneally in mice, and challenged four weeks later with one to two thousand virulent trypanosomes, completely protected the animals during 140 days of observation, whereas control mice died in 7–10 days. A booster dose of four doses of 250 μg chagastoxin prolonged the duration of protection in previously immunized animals. The height of protection was attained one month following immunization. (2) Smaller doses of 25–200 μg chagastoxin rendered partial protection. (3) Formalinized chagastoxin was less effective than freshly prepared suspension. (4) Chagastoxin had a cortisone-like effect. Three doses of 10–500 μg activated fatal trypanosomiasis in chronic latent trypanosome infection in mice, but smaller doses failed to activate acute infection in chronically infected animals. (5) Chagastoxin induced marked leucocytosis in rabbits.

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PATHOLOGY

Long-term Culture of Lymph Node Tissue from a Patient with Lymphocytic Lymphoma

Attempts to grow normal lymphoid tissue for extended periods of time in a state of morphological and immunological differentiation have been met by failure[1,2]. An alternative procedure involves short-term incubation of lymph node or spleen cells from previously immunized donors[3–7]. A short time ago Ioachim[8] reported successful long-term cultures of normal splenic tissue with continuous production of plasma cells from several mammalian species, including man. Simultaneous attempts with lymph node explants were unrewarding. On the other hand, continuous *in vitro* growth of mouse lymphomatous cells has been repeatedly accomplished[9–11]. Recently, Epstein *et al.*[12] reported long-term cultivation of Burkitt lymphoma cells.

This communication refers to a unique cell line obtained after *in vitro* culture of lymph node tissue from a patient with a well-differentiated lymphocytic lymphoma. The growing population is characterized by cell heterogeneity and evidence of morphological and immunological differentiation.

The patient is a fifty-eight-year-old male who noted an enlarged lymph node behind his ear in August 1962. He came to the University of Texas M.D. Anderson Hospital and Tumor Institute in October 1964 with generalized lymph node enlargement, the largest node being 3 cm in diameter. The liver and spleen were palpable. White blood cell count at that time was 41,500 with 3 per cent neutrophils, 91 per cent lymphocytes, and 6 per cent immature lymphocytes. A cervical lymph node biopsy was diagnosed as lymphocytic nodular lymphoma. He was

treated with 'Leukeran' for about 2 weeks. The patient has remained relatively well until the present time and his most recent count was 104,000, 85 per cent of which were mature lymphocytes.

A portion of his initial lymph node biopsy was immediately incubated with 'Colcemide' for chromosomal analysis, and the rest placed in a sterile Petri dish partially filled with Hanks's solution. The tissue was finely minced and the small pieces were distributed under perforated 'Cellophane' membrane in *T*-30 flasks. Approximately 4–5 ml. of culture medium was added to each container. The culture medium in use in our laboratory is *F*-10 (Colorado Serum Co., 4950 York Street, Denver, Colorado) supplemented with 20 per cent foetal calf serum (Hyland Laboratories, 4501 Colorado Blvd., Los Angeles, California) and 'Neomycin', 0·1 mg per ml. The vessels were then incubated at 37° C, and the medium changed every 3 days.

Outgrowth of spindle cells from the margins of the small explants were noted 2 weeks later. From the beginning phase microscope observation revealed two different types of cells: one larger, partially elongated and occasionally flattened containing a central nucleus and abundant granular cytoplasm, and the other, smaller, round or ovoid in shape with a lesser amount of cytoplasm and often condensed nucleus (Fig. 1). Since these latter cells were initially considered to be remaining lymphocytes it was assumed that with periodic change of medium they would disappear. However, this did not occur. With routine sub-culture every new flask contained the two cell types.

The reticular cells differed from the usual fibroblastic growth obtained from cultures of human skin, leukaemic bone marrow, and normal lymph nodes. They attached to the glass surface and proliferated, forming an interlacing network with nest-like spaces occupied by clusters of round cells. The latter elements predominated in the supernatant and often were seen floating freely in the medium (Fig. 1). Multiple cytoplasmic budding was also observed in both cell types. After centrifugation of the medium used, the resultant pellet was successfully utilized to start new cultures, which disclosed similar growth patterns.

It is of interest to mention that similar symbiotic cultures of reticular and lymphocyte-like cells have been reported in other species by a few investigators[13,14]. In a recent work Ioachim and Furth[15] described a long-term culture of virus-induced thymic lymphoma of rats which grew in a manner very similar to ours. At present, 12 months after the initial explants, our cell line still retains the heterogeneous population described. A permanent stock of these cells has been stored at minus 70° C in 5 per cent glycerine medium. A few ampoules of this material have been re-used with good recovery.



Fig. 1. Phase-contrast microphotograph of a 12-month-old culture from lymph node tissue of a patient with lymphocytic lymphoma. Two cell types are clearly seen. (× *c.* 400)

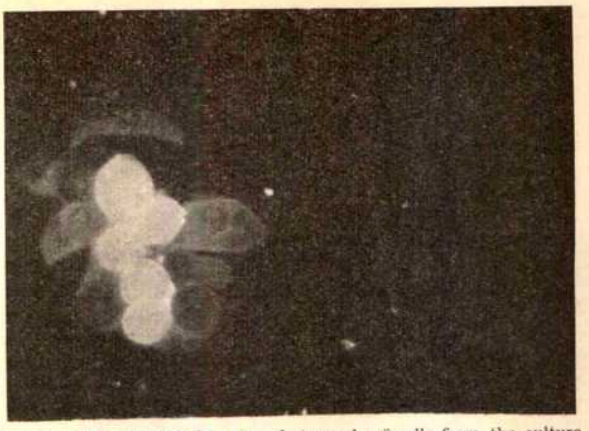


Fig. 2. Ultra-violet light microphotograph of cells from the culture shown in Fig. 1 after direct exposure to fluorescein-labelled rabbit anti-human gamma globulin serum. The round cells fluoresced strongly while the reticular elements remained unaffected. (× *c.* 400)

To investigate further the significance of this cell heterogeneity we used specific rabbit anti-human gamma globulin (Baltimore Biological Laboratories Division, 2201 Asquith Street, Baltimore, Maryland) labelled with fluorescein isothiocyanate (Calbiochem, 3625 Medford Street, Los Angeles, California). This was applied directly to the cultured cells and incubated for half an hour. Simultaneously, indirect techniques requiring previous exposure to unlabelled rabbit anti-serum were also carried out. Several normal and neoplastic human cell lines (fibroblasts, melanoma) were used as controls. With the direct technique the round cells revealed striking positive fluorescence (Fig. 2). The reticular cells were negative. Indirect techniques considerably reduced the fluorescence and the controls were consistently negative.

On the basis of these results it can be postulated that the two cell types observed under the phase microscope are functionally different: the spindle variety represents a reticular undifferentiated cell and the other, a more mature one, probably of lymphocytic origin. The fact that these round cells appear to be able to produce a protein molecule which can react specifically with the anti-human gamma globulin is most unique—especially considering the age of this cell line.

The initial chromosomal analysis showed euploidy, but subsequent periodical investigations indicated a change to aneuploidy around the sixth month. However, no evidence of a predominant mode has been found. More detailed cytogenetic data, as well as the results of histological, immunological, microcinematographic and other studies now in progress will be reported at a later date.

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Aflatoxin: a Site of Action in the Rat Liver Cell

Aflatoxin is the name given to a group of related toxic agents produced by the mould *Aspergillus flavus* and which have been characterized chemically[1,2]. Diets containing aflatoxin, such as toxic ground-nut meal, have been found to produce pathological changes[3–6], in particular, liver injury[7], in a wide variety of species of animals. The most toxic member of the aflatoxin group so far recognized has been designated aflatoxin B_1 (ref. 8). The rat is considerably less susceptible to aflatoxin than most other species investigated and for male rats the LD_{50} is 7 mg aflatoxin B_1/kg body-weight (administered by stomach tube, dissolved in dimethylformamide 5 mg/ml.)[9]. Such a dose produces an extensive hepatic periportal necrosis after 48 h. It seems likely that the toxic agent has a direct action on the liver as within 30 min of administration it is possible to detect aflatoxin B_1 in the liver[10] and within 60 min most of the administered dose has been concentrated there.

We have found that by 1 h after poisoning slices cut from the liver of the poisoned rat have a reduced ability *in vitro* to incorporate radioactive amino-acids into the liver cell protein. Using radioactive labelled DL-leucine-1-^{14}C, L-alanine-^{14}C (U) or mixed amino-acids-^{14}C(U) prepared from a *Chlorella vulgaris* hydrolysate, percentage inhibitions after 1 h of incubation of 48, 44 and 24, respectively, were demonstrated by comparison with slices from control animals. Similarly the addition of a final concentration of 0·032 mM aflatoxin B_1 to the incubation medium containing liver slices prepared from control animals inhibits the incorporation of DL-leucine-1-^{14}C into liver proteins by 46 per cent after 1 h. Inhibition of protein synthesis in both animals and plants following the administration of aflatoxin B_1 or diets containing toxic groundnut meal has been previously reported[11–14].

To study further the effect of aflatoxin B_1 on protein synthesis *in vivo* the induction of tryptophan pyrrolase by substrate or cortisone was measured in the livers of control and aflatoxin B_1-treated rats. Three hours after poisoning it was found that there was a 57 per cent inhibition of the cortisone-induced enzyme whereas there was no inhibition of the substrate-induced enzyme. These findings are similar to those reported for the effect of actinomycin *D* on tryptophan pyrrolase induction[15–17].

It has been shown that the inhibition of protein synthesis in the liver of the rat following administration of actinomycin *D* is secondary to an inhibition in ribonucleic acid (RNA) synthesis[18]. To determine whether a similar sequence of events occurs with aflatoxin B_1, using liver slices from control rats we examined the effect of the addition *in vitro* of the toxic agent on the incorporation of ^{14}C-orotic acid into RNA and ^{14}C-leucine into protein. Table 1 presents results of such an experiment showing that there was an immediate inhibition of orotic acid incorporation into RNA whereas the inhibition of leucine into protein took some 15 min to develop. It was shown that aflatoxin B_1 does not inhibit the entry of amino-acids into the liver slice. These results thus indicate a similarity in action of aflatoxin B_1 to that of actinomycin *D*.

It was also possible to demonstrate an inhibition of the incorporation *in vivo* of ^{14}C-orotic acid into the RNA of the liver cell nucleus in rats poisoned with aflatoxin B_1. In Table 2 are given the results of a typical experiment and it may be seen that there is no inhibition of incorporation of orotic acid into the nucleotide pool but there is a marked inhibition of incorporation of nucleotide precursors into the nuclear RNA. This would seem to indicate that there is an inhibition of RNA polymerase—the only enzyme known to be operative between these two stages[19].

Table 1. *In vitro* Effect of Aflatoxin B_1 on the Incorporation of ^{14}C-Orotic Acid into the RNA and on ^{14}C-Leucine into the Protein of Liver Slices

Duration of experiment (min)	Per cent inhibition of incorporation in the presence of aflatoxin B_1 into RNA	Protein
15	92	5
30	88	31
45	95	43
60	90	46

Liver slices were prepared from control rats and incubated in Krebs–Ringer phosphate, with oxygen as gas phase. 1 μc. of orotic acid-6-^{14}C or DL-leucine-1-^{14}C was added to the medium: aflatoxin B_1 when added was at a final concentration of 0·032 mM. The isolation and determination of radioactivity in the RNA and protein were as previously described[26].

Table 2. Effect of Aflatoxin B_1 on the *in vivo* Incorporation of Orotic Acid-6-^{14}C into the Nuclear RNA of the Liver Cell

	Incorporation into total nucleotides (c.p.m./density unit at 260 mμ)	Incorporation into nuclear RNA (c.p.m./μg RNA.P)	Per cent incorporation into RNA from nucleotide
Control	16,021	1,686	10·5
Control	14,313	2,825	19·7
Aflatoxin B_1	20,180	486	2·4
Aflatoxin B_1	21,477	327	1·5

Rats were poisoned with aflatoxin B_1 and at 3 h after poisoning received a caudal vein injection of ^{14}C-orotic acid in isotonic saline at a dose-level of 12·5 μc./150 g body-weight. They were killed 15 min later and the livers removed. From one portion of the liver nucleotides were extracted[27] and nuclei were isolated from the remainder of the liver from which the RNA was then extracted.

It is known that actinomycin *D* binds to deoxyribonucleic acid (DNA) and thus inhibits the DNA-dependent RNA synthesis[20]. The structure of actinomycin *D* is such that it wedges into a groove of the DNA helix with the quinoidal oxygen—the amino group and the lactone rings undergoing hydrogen bonding. A consideration of the structure of aflatoxin B_1 (refs. 1 and 2), with its three rings and groups capable of hydrogen bonding, suggested that it might bind to DNA in a similar manner. If such a reaction were to occur the DNA would be expected to alter the characteristic absorption spectrum of the aflatoxin B_1. We therefore examined the effects on the absorption spectrum of aflatoxin B_1 of adding varying concentrations of calf thymus DNA and found the difference spectrum showed a similar trace to that given by actinomycin *D* in the presence of calf thymus DNA[20]. The changes in optical density and shift of the absorption peak are given in Table 3.

Table 3. Effect of Calf Thymus DNA on the Spectral Properties of Aflatoxin B_1

Final concentration of DNA (μM DNA.P)	Decrease in *O.D.* at 355 mμ	Increase in *O.D.* at 385 mμ	Molar ratio aflatoxin B_1/DNA.P
397·5	0·085	0·063	0·059
662·5	0·187	0·088	0·035

The final concentration of aflatoxin B_1 was 23·4 μM and this and the DNA were dissolved in 0·01 M *tris*-HCl, *p*H 7·4, containing 0·01 M sodium chloride. Difference spectra were traced using a model 14 serial No. 273, Cary Recording Spectrophotometer[20]. Aflatoxin B_1 has a maximum absorption of 370 mμ in the *tris*-medium; on mixing with the DNA, maximum decrease in absorption is seen at 355 mμ and maximum increase in absorption at 385 mμ. The traces cross the base line from negative difference to positive difference at 377 mμ.

We have further studied the distribution of aflatoxin B_1 in the sub-cellular fractions of the liver cell some 30 min after poisoning and found that, although all fractions show the presence of the toxin, one-third of the total cell content was present in the nucleus.

We therefore propose the following sequence of events following poisoning with aflatoxin B_1: (1) The aflatoxin after entering the liver cell passes into the nucleus. (2) It binds with the DNA and thereby inhibits the RNA polymerase. (3) There is a reduced synthesis of RNA involving an inhibition of messenger RNA as exemplified by the inhibition of the cortisone-induced synthesis of tryptophan pyrrolase. (4) The inhibition of *m*RNA is reflected after some 15 min in a reduced protein synthesis. (5) The continued reduction of *m*RNA production is the cause of the degranulation of the endoplasmic reticulum which has been observed in electron micrographs prepared from the livers of rats poisoned with aflatoxin for 6 h (ref. 21). This would appear to be analogous to the endoplasmic reticulum changes occurring following administration of actinomycin *D* (ref. 22).

In view of the finding that aflatoxin B_1 binds with DNA it may be that the difference in toxicity shown by other varieties of aflatoxin is determined at least in part by the extent to which they are able to bind to DNA.

It is not yet possible to decide to what extent this biochemical lesion is responsible for the development of the periportal necrosis in the livers of poisoned rats. An inhibition of protein synthesis in the liver cell would not *per se* appear to result in hepatic necrosis. This follows from a consideration of the results with rats poisoned with ethionine[23], or *t*-butylmethylnitrosamine[24] or carbon-tetrachloride and treated with promethazine-HCl (ref. 25); in all these cases there is an inhibition of protein synthesis up to 24 h without the development of necrosis. Unlike these agents, however, a single dose of aflatoxin B_1 will depress protein synthesis for at least 48 h, and it may be that in these circumstances the inhibition of protein synthesis could play an important part in the development of the periportal necrosis.

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Influence of Thymic Mass on Murine Viral Leukaemogenesis

THE origin of lymphoma in the thymuses of mice is now well documented[1]. Of interest is the fact that regardless of the means used to induce the lymphoma (virus, chemicals, hormones, X-rays), the pattern of the thymic response is always similar. Recently, in pathogenesis investigations of a virus-induced lymphoma, attention was directed to the thymic anatomy. It was demonstrated that the two 'lobes' of 'the thymus' actually are two separate, paired organs[2–4]. This was of interest because it was noted that the lymphoma change occurred in only one of the two thymuses. Tumour cells frequently disseminated from a tumour-transformed thymus at a time when the opposite thymus was still histologically normal. The process of tumour formation studied histologically in serial sections suggested that the tumour cells evolved throughout a single thymus rather than at a single point[3]. Regarding the thymuses as organs which provide the site for the lymphoma evolution[5], it was of interest to investigate the anatomic unit of thymic tissue necessary for such a change.

To investigate the effect of reduced amounts of thymic tissue in viral leukaemogenesis, new-born random-bred Swiss mice were inoculated with Rich murine leukaemia virus[6], by protocols previously described[3]. At 4–5 days of age mice were either sham-thymectomized, unilaterally thymectomized, bilaterally thymectomized, or left intact. The sexes were equally represented in each group. Uninoculated intact and operated controls were included. The animals were palpated thrice weekly for enlargement of the spleen. When definite splenomegaly was detected, the animal was killed with ether and dissected. The mediastinal contents of mice in which both thymuses had been removed were fixed *en bloc*, and serially sectioned from the level of the thyroid to the bifurcation of the bronchi. In 49 of the 104 bilaterally thymectomized mice, residual microscopic fragments of thymus tissue could be identified histologically by the presence of Hassall's corpuscles, characteristic cortico-medullary differentiation, and the presence of 'PAS' cells. The residual amount of thymus tissue in the serial sections was measured with a calibrated ocular scale. The total volume was estimated by assuming the geometry of a double cone. Assuming a specific gravity of 1·0, the thymus fragments were estimated to weigh between 0·1 and 0·5 mg. By way of comparison, a single thymus of a control animal of comparable age weighed 20–30 mg. The larger fragments were easily visible on the stained slide, but were not discernible in the fresh tissue because they were embedded in mediastinal fatty connective tissue. These 49 animals were designated 'sub-totally' thymectomized. Only animals surviving past the first month were included in the investigation. An additional 51 virus-inoculated and 19 uninoculated, unilaterally thymectomized mice were killed between days 50–75 for pathogenesis examinations.

The results are summarized in Fig. 1. Disseminated thymic lymphoma (lymphoid leukaemia) occurred in

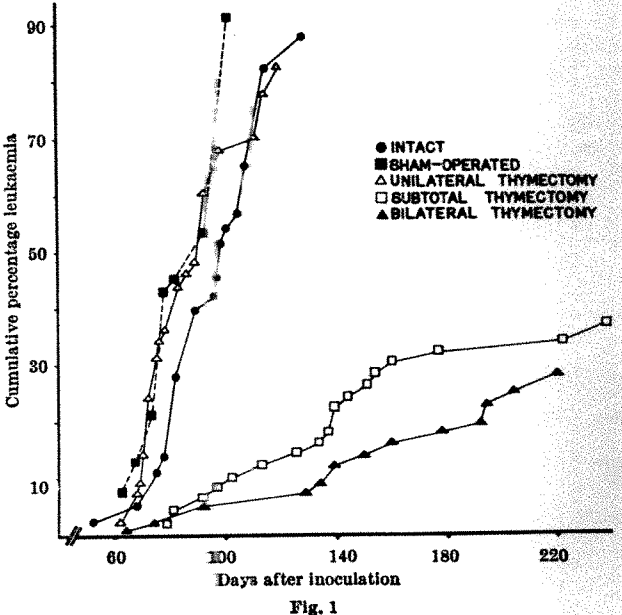


Fig. 1

31 of 35 intact, virus-inoculated mice; 35 of 41 unilaterally thymectomized, inoculated mice; and 14 of 15 sham-operated, inoculated mice. The differences between the mean latent periods in these three groups were not statistically significant. Thus, the incidence and latency of lymphoid leukaemia in mice with one thymus were the same as in mice with two thymuses. Mice possessing less than one complete thymus (sub-totally thymectomized) had a greatly decreased incidence of lymphoid leukaemia (18 of 49) with an increased latent period (137 days versus 91 days in controls).

Although not indicated in Fig. 1, two animals in which definite lymphoma could be seen in residual thymus fragments had concurrent myeloid leukaemia. This finding was also reported by Fiorre-Donati *et al.*[7].

The pathogenesis of the thymic lymphoma in unilaterally thymectomized mice was identical to that in mice with two thymuses, described previously[3]. Briefly, the remaining thymus in unilaterally thymectomized, virus-inoculated mice decreased in size and weight from a control value of 20–30 mg to 10–15 mg between 40 and 50 days. The decrease in weight was reflected histologically by a depletion of thymic lymphocytes and a drawing together of the epithelial reticulum which constitutes the tissue matrix or framework of the organ. Within a few days the contracted primitive cells had begun to proliferate, and formed continuous sheets of large, immature, lymphoblast-like cells. This proliferation was accompanied by a great increase in size and weight of that thymus, often weighing as much as 130 mg. Dissemination of tumour cells to other organs occurred after proliferation had begun. The degree of dissemination and the extent to which the tumour cells proliferated in distant organs was independent of the size of the primary thymic mass. In some instances specimens were obtained with large tumorous supracoecal (mesenteric) lymph nodes, and inconspicuous but completely tumour-replaced thymuses. Because of knowledge of the details of the pathogenesis of this tumour[3], the error of attributing to the largest tumour mass the site of orgin of the tumour was avoided.

No mouse histologically free by serial section of thymus tissue developed lymphoid leukaemia in this investigation during the 240 days of the experiment. Of 55 such mice, however, 16 (28 per cent) developed myeloid leukaemia by day 220 (solid triangles in Fig. 1). The development of myeloid leukaemia in thymectomized, virus-inoculated mice has been previously reported by Gross[8]. In many instances pigment changes in myeloid-infiltrated lymph nodes produced deep shades of green ('chloroleukaemia'). The pathogenesis of the myeloid leukaemia will be presented in a separate publication.

The data indicate that in murine viral leukaemia: (1) thymic tissue is necessary for the evolution of lymphoma, but not for the evolution of myeloid leukaemia; (2) animals with one thymus developed disseminated thymic lymphoma (lymphoid leukaemia) as quickly, as often, and in the same manner, as animals with two thymuses; (3) animals with less than one complete thymus have a markedly decreased incidence of lymphoma, which develops with a longer latency period.

These facts show that while small portions of thymic tissue (0·1–0·5 mg) are capable of evolving the lymphoma, they do so in greatly reduced incidence and after a longer period. While the longer latency may be due merely to the extra time required for a fragment to proliferate enough to make up for its initial small size, the decreased incidence (Fig. 1) suggests that a critical mass of thymic tissue may be a factor in lymphoma change. Study of this factor is now in progress in our laboratory.

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BIOLOGY

Inhibition of Fruit Drop and Colour Stimulation with *N*-Dimethylaminosuccinamic Acid

The retardation of vegetative growth of legumes and certain other species by foliar application of substituted maleomic and succinamic acids was reported by Riddell *et al.*[1]. The *N*-dimethylaminosuccinamic acid (designated *B*995) was found to be more stable in aqueous solution than the substituted maleomic[2] and was effective in retarding growth of the same species.

Similar retardation in vegetative growth of apple with foliar sprays of *B*995 has been reported[3]. Another effect of *B*995 application on apple, typical of a growth retardant, is the promotion of flowering of young trees[4]. Brooks[5] has also reported reduction of terminal growth of pear with sprays of *B*995. The spur development correlated with this reduced growth in pear is indicative of increased flowering. In contrast to these effects, tests in 1964 revealed that *B*995 may simulate the action of a growth promoter or auxin in controlling the abscission and pre-harvest drop of mature apple fruits. Auxins such as α-naphthaleneacetic acid (NAA) and 2,4,5-trichlorophenoxy α-propionic acid (2,4,5-TP) have been shown to delay the harvest drop of apples[6] and have been used extensively for this purpose. This report presents the results of several tests which characterize the effects of *B*995 in relation to fruit drop, anthocyanin formation and histological changes in the abscission zone of the fruit pedicel.

Mature apple trees (*Pyrus malus*, Linn. cv. McIntosh) were sprayed at various times and concentrations from full bloom (May 13) to a few days before harvest. From 4 to 8 trees were used per treatment in a randomized block design. Fruit growth measurements were made periodically during the season and fruit firmness, colour and drop data were obtained at the end of the season. Samples were collected from each tree for colour evaluation. Fruit firmness was determined with a Magness–Taylor pressure tester on a sample of 10 fruits from each tree at each harvest date. At normal harvest time for the control trees, fruit drop was occurring and had reached as high as 56 per cent of the total crop in some tests (Table 1).

Table 1. Harvest Drop and Fruit Firmness of McIntosh Apples treated with *B*995

Orchard	Treatment (p.p.m. *B*995)	Date applied (1964)	Fruit drop (%) Sept. 26	Oct. 10	Oct. 26	Fruit firmness (lb.) Sept. 26	Oct. 10	Oct. 26
48	500	June 5	5·5*	9·6	21·8	16·1*	14·6	9·4
	2,000	,,	1·3*	4·2	10·6	17·3*	16·7	10·5
	Control	—	56·6	—	—	14·5	—	—
K	500	Aug. 29	4·0*	—	—	14·7*	—	—
	1,000	,,	2·9*	—	—	15·5*	—	—
	2,000	,,	1·8*	—	—	15·6*	—	—
	Control	—	25·2	—	—	13·7	—	—

* Significantly different from control at 1 per cent probability level.

Trees sprayed with *B*995, even with concentrations as low as 500 p.p.m., had dropped less than 6 per cent of their crop by this date. Drop control was effected with all application dates used in these tests from full bloom until September. However, the late August application, approximately one month before harvest, was the most effective at the lower concentrations of *B*995.

The failure of *B*995 to control drop when applied just prior to harvest as the drop is initiated suggests that the mechanism of control is different from that achieved with auxin sprays such as NAA. With such compounds a delay in abscission and control of drop may occur within a few days after application.

A possible explanation for the control of fruit drop by *B*995 is that softening and maturity of the fruit are delayed and hence the fruits are not so mature as those on untreated trees at the time of conventional harvest. To elucidate this possibility some trees in the various plots were not harvested at the time of picking the control fruits. Pressure data and fruit drop records were obtained on these trees for an additional 3 weeks. The results in Table 1 show that drop was still inhibited on the treated trees even though the fruits had finally softened to the pressure of control fruits when they were picked on September 26 and had experienced heavy fruit drop.

Fruit pedicels and attached cluster bases of the spur were collected from tree units in the various plots on several dates. Freehand longitudinal sections were then prepared through this tissue to show the abscission zone. The distribution of fibres and schlerenchyma cells in the vicinity of the abscission zone was about the same in the pedicels collected from treated trees as from control trees when fruit pressures were comparable (Fig. 1). However, in no case was breakdown apparent in the pith cells of the abscission zone from treated trees. Separation of cells was apparent in the peripheral areas of the abscission zone in pedicels from control trees as well as through the pith in the central portion of this zone (Fig. 1). The absence of this separation and breakdown as the fruits were softening on treated trees would account for the tenacity of the fruit during the harvest period.

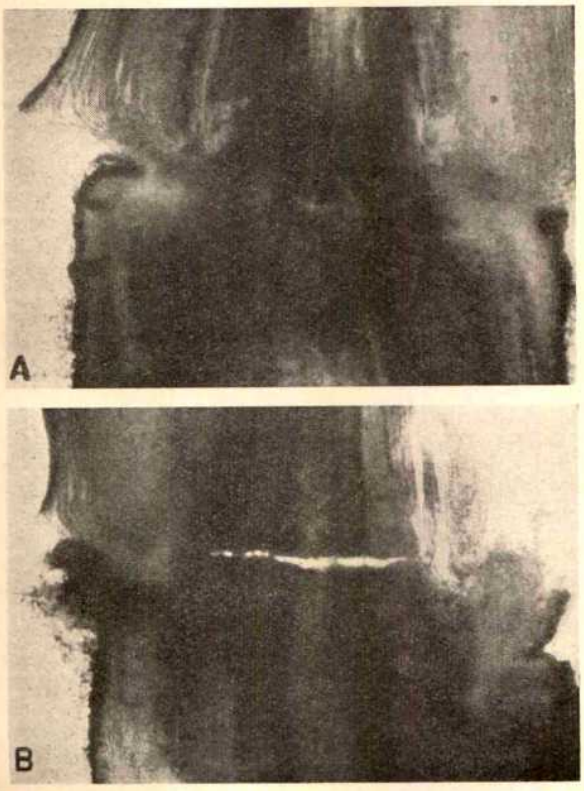


Fig. 1. Photomicrograph of longitudinal section through abscission zone of McIntosh fruit pedicels. (*A*) Treated with *B*995 1,000 p.p.m., August 29. (*B*) Control. Separation of cells and weakening of tissue are visible in control. Specimens collected October 1

It might be expected that anthocyanin formation would be enhanced by application of *B*995 if the fruits remain on the tree longer than the control fruits to reach a given stage of firmness and maturity. The exposure of the fruit to an additional period of light and cool night temperature which promote colour formation would contribute to this phenomenon. Fruits were examined from the replicate trees in the various treatments and the percentage of fruit surface with red colour was determined. The results are shown in Table 2. Intensity of anthocyanin (idaein) in the skin was also determined by optical density measurements according to the method described by Seigelman and Hendricks[7]. A direct colour stimulation by the treatment was indicated in two experiments where comparisons were made on the same harvest date (Table 2).

Table 2. Fruit Firmness and Anthocyanin Formation in McIntosh Apples

Treatment (p.p.m. *B*995)	Date applied	Pressure (lb.)	Surface colour (%)	Anthocyanin ($\mu g/cm^2$)*
		Orchard *Z*, harvested Sept. 14		
500	May 29	16·4†	67	7·9‡
1,000	"	17·1‡	65	7·4‡
5,000	"	19·0‡	74	8·3‡
Control	—	15·9	58	6·9
		Orchard 48, harvested Sept. 26		
500	June 5	16·1‡	80	6·8‡
2,000	"	17·3‡	85	8·0‡
Control	—	14·5	62	6·2

* Expressed as idaein.
† Significantly different from control at 5 per cent probability-level.
‡ Significantly different from control at 1 per cent probability-level.

These results thus show that delay in fruit abscission, an unexpected response of the application of a growth retardant, is not dependent solely on the delayed maturation of the fruit. Changes in the abscission zone occur which may inhibit separation of fruit pedicel from spur beyond the stage of maturity characteristic of control fruits at abscission. Firmer fruit at a given calendar date and enhanced formation of anthocyanin are likewise associated with this treatment.

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Germ Cell Migrations in a Cestode

Usually in cestodes the potential oocytes and associated cells of the female system leave the germinal primordium in the median line of the proglottid while the cells are still either undifferentiated or not easily distinguishable. In a member of the problematical order Tetrabothridea, oogonia, true vitelline cells, and what are considered to be shell-producing cells of two kinds, differentiate within the female primordium, although the whole process is delayed in this worm because of marked protandry. This delay causes the process of germ cell differentiation to coincide in time with the differentiation of the uterus, and the latter can thus be identified, for the first time, as the outer layer of the primordium enclosing the germ cells

except for a posterior aperture (Fig. 1). The shell-producing cells and oogonia migrate through this aperture in an organized sequence: first the compact gland cells, usually called 'vitelline', then the cells of a diffuse gland similar to those of the follicular glands of Pseudophyllidea, also usually called 'vitelline', and finally the oogonia. The true vitelline cells, arranged in the primordium in rosettes around the oogonia, degenerate within the primordium. The three sets of migrating cells, some of which were seen to be amoeboid, travel progressively less far into the medulla, which is itself differentiating at the same time (Fig. 1). The distance covered by the migrating cells would appear to be related to this medullary differentiation and thus indirectly to the degree of protandry. The migrating cells form two organs and not three as the diffuse gland contains both shell-producing cells and oogonia (Fig. 1).

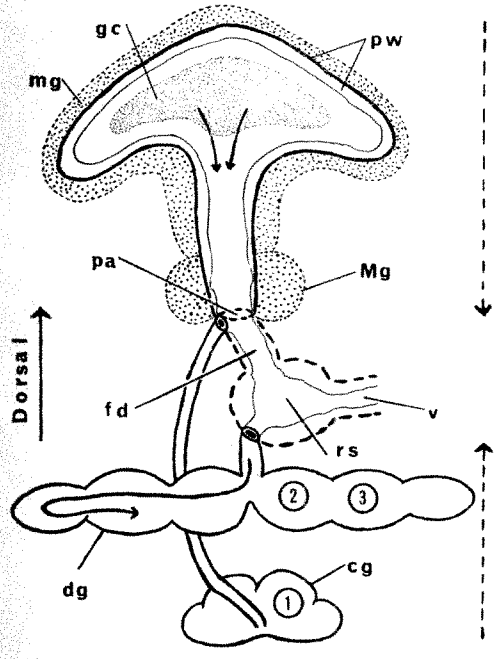


Fig. 1. Diagrammatic representation (not to scale) of morphogenesis of female primordium of *Tetrabothrius erostris* (Lönneberg). *c g*, Compact gland; *d g*, diffuse gland; *f d*, fertilization duct; *g c*, germ cells; *m g*, medullary gland cells; *M g*, Mehlis's gland; *p a*, posterior aperture; *p w*, fibroblast wall of ♀ primordium = wall of germarium = wall of uterus; *r s*, receptaculum seminis; *v*, vagina. Continuous arrows indicate movements of germ cells: (1) compact gland cells; (2) shell-precursor cells; (3) oocytes. (2) and (3) occur in both lobes of the diffuse gland. Dotted lines represent the latest proliferation of wall cells of primordium. Dotted arrows indicate direction of differentiation of medulla

During the development of the primordium into such a temporary germarium the whole is surrounded except for the posterior aperture by medullary gland cells secreting into it. When the germ cells are all expelled these gland cells cease to function except for a group, which becomes Mehlis's gland, around the posterior aperture. During the process the posterior aperture moves backwards so that the primordium becomes T-shaped (Fig. 1). The wall cells around the aperture proliferate to form the duct system along the route of the migrations and in addition forms a duct which has no precursors, the vagina. At this stage the posterior aperture of the primordium is recognizable as the so-called ootype which thus represents the posterior extremity of the uterus. The reproductive pattern is therefore determined by the timing of germ cell migrations, representing an epigenetic pathway of considerable biological significance.

All the medullary gland cells are periodic acid–Schiff-positive (with glycogen excluded) while they are functional and thus quite distinct from the rest of the medulla. Mehlis's gland is therefore thought to function in two phases in this worm; first, around the primordium while proliferation, differentiation and movement of germ cells occur; secondly, around the posterior aperture while this proliferates to form the duct system, persisting until embryonation occurs in the uterus. It is interesting that Rao[1,2], working on the chemistry of Mehlis's gland in *Fasciola hepatica* and in a pseudophyllidean cestode, considers the secretion to be a lecithin-like phospholipid involved in yolk formation in the uterus while embryos are being formed. This is the only place where the Mehlis secretion would have been expected to be found, as it is generally assumed that the germ cells pass through the region of Mehlis's gland only in one direction. I make the suggestion, based not on chemical evidence but on an embryological interpretation, that the secretion from the gland cells around the entire primordia, including the later restricted Mehlis's gland, may be the ultimate source of vitelline material for the proliferation and maintenance of the whole female reproductive complex. Such information about morphogenesis would be unavailable in forms where differentiation of the female system is extended in time by early germ-cell migration made possible by the evolution of a strongly polarized strobila of considerable length and the overlapping, in time, of the differentiation of male and female sex cells.

Early migration of germ cells, characteristic of most modern cestodes, would thus seem to be correlated with the more effective production of shell materials used as individual egg coverings and inversely related to egg protection by an elaborated uterus wall which, by definition, must have been primitively a closed structure with correlated apolysis. The Tetrabothridea, though considerably specialized in their modern form, combine significant characters of Cyclophyllidea, Pseudophyllidea and Tetraphyllidea, and these same characters are linked through *Tetrabothrius erostris* with many aberrant forms which collectively possess the following inter-related features which I believe to be basic to cestodes, namely: (1) no migration of female germ cells; (2) a closed uterus and permanent germarium; (3) apolysis and fragmentation associated with limited polarity; (4) marked protandry or the dioecious condition. On a basis of these characters, and on evidence leading to the conclusion that the primary organizer defines not three layers but two, namely, germinal and somatic, it is possible to construct a hypothesis for strobila organization which does not conflict with the reproductive metamerism theory of Hyman[3] and takes account of the elements of the zooid theory of earlier workers, while at the same time it supports the views expressed by Stunkard[4]. This hypothesis and the evidence supporting it will be presented in detail in a later publication.

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Proteolytic Enzymes of *Citellus citellus* and *Spalax leucodon*

With present knowledge and techniques, a more meaningful application of biochemical data towards classification schemes may be made at the family level and lower[1].

We have found it interesting to compare, from the biochemical aspect, two species of the order Rodentia which belong to different sub-orders[2], namely, families sub-order Sciuromorpha—fam. Sciuridae (*Citellus citellus*), and sub-order Myomorpha—fam. Spalacidae (*Spalax leucodon*).

According to recent taxonomy, these two families are phylogenetically very distant. *Spalax leucodon* is especially interesting, because of its origin (a very old species in this order—first data are from Oligocene) and specialized way of life.

All animals killed before investigation spent about one month under laboratory conditions. Food of *Spalax leucodon* was similar to that found in its natural environment (potato, onion, carrot); food of *Citellus citellus* consisted of grains of wheat, carrot and cabbage.

The tissue homogenate of the duodenum with a part of pancreas of both animals examined contained 8 mg duodenum and 1·87 mg of pancreas per 1 ml. of distilled water.

Proteolytic activity was determined by the casein digestion method[3]. The reaction was initiated after 5 min of equilibration at 37° C by addition of soluble casein to a concentration of 0·5 per cent in the following buffers: 0·075 M phosphate *p*H 6·0, 6·5, 7·0, 7·5 and 8·0. Enzymatic activity was terminated after 20 min incubation at 37° C by addition of 5 per cent trichloroacetic acid. Optical density was determined in filtered solutions at 280 mμ.

The relative protein content in each case was determined with the phenol reagent described by Lowry *et al.*[4]. All results were calculated on the same protein basis.

Results are shown in Fig. 1. From the graph, it is evident that protease activity of the duodenum with a part of pancreas differs in *Citellus citellus* from that in *Spalax leucodon* in relation to changes in *p*H. Protease activity of *Citellus citellus* at all points is much higher than that of *Spalax leucodon*: for example, maximal protease activity is about 3 times higher in *Citellus citellus* than in *Spalax leucodon*.

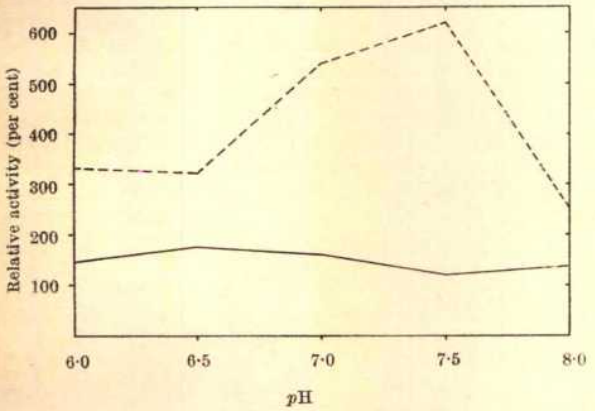


Fig. 1. Protease activity of the duodenum and part of pancreas of *Citellus citellus* and *Spalax leucodon* in relation to *p*H of the reaction mixture. - - -, *Citellus citellus*; —, *Spalax leucodon*

It is well known that the effect of *p*H on enzyme activity is highly important in studying enzymes, even though a satisfactory explanation of the effect is not available[5].

Protease activity of *Citellus citellus* under the conditions described is dependent on the *p*H of reaction mixture (optimum *p*H 7·0–7·5). On the contrary, protease activity of *Spalax leucodon* is not, in general, dependent on the *p*H of reaction mixture; under the same conditions it is more or less on the same level.

This fact may suggest differences between the nature of protease of these two species of Rodentia.

The cause of the aforementioned differences in protease activity between these two species must still be considered as unexplained. We have tried to consider it from two aspects.

It is known that high protein diet results in pancreatic juice, high in trypsin and chymotrypsin[6–8]. Maybe the difference in protease activity could be partially explained by differences in their food (*Citellus citellus* takes food which is richer in proteins than *Spalax leucodon*).

On the other hand, the very unusual form of curve of protease activity of *Spalax leucodon* suggests a connexion with the specialized way of life of this animal, which belongs to the family Spalacidae. In the evolution of order Rodentia this family appeared very early in the Oligocene, which is undoubtedly also reflected in its biochemical system.

Our unpublished results on alkaline phosphatases of the species examined also prove the same.

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Membrane in the Human Tooth

The inner structure of human teeth, the dentine, is occupied by the dental pulp. This is generally described as a syncytium, cell tissue in which the cell walls are not clear, though the outermost layer of the pulp consists of a well-defined layer of cells, the odontoblasts, firmly attached to the dentine.

An early investigation of cells and membranes in teeth[1] was followed by similar studies[2], almost all being of histological nature; interest in biological activity of membranes in general has increased. It was noted[3] that "the pulp tissue appears to be bounded by a membrane"; demonstration was incomplete. Further evidence has now been obtained from freshly opened human teeth, when the pulps were removed and examined by electron micrography.

A membrane composed of two layers about 100 Å apart is seen to surround the pulp (Figs. 1 and 2), lying between the body of the pulp and the layer of odontoblasts. When a pulp is removed from a tooth, the neighbouring cells —the odontoblasts—remain *in situ*[4]; this is not shown in the specimens illustrated.

Labelled ions (^{32}P as PO_4) pass through human teeth[5]. passage now being found to be part active, part passive, Ion transfer and the membrane are subjects of further inquiry; a fuller account is in preparation.



Fig. 1. ×, 6,000



Fig. 2. × 18,000

The E.M.F. of the membrane surrounding the human dental pulp was determined by the use of micropipettes; it has a value of about 45 mV.

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Selective Inhibition of Cation Absorption by Uranyl

Investigations into the interactions between cations in the ion absorption process of plant roots resulted in the observation that uranyl (UO_2^{++}) appeared to produce a differential inhibition of the uptake of certain cations. The only information found in the literature concerning such a differential effect by uranium was that reported by Rothstein[1]. He showed that uranium, in the form of uranyl chloride, inhibited the uptake of hexoses by yeast cells while having essentially no effect on the uptake and metabolism of other substrates such as alcohol and pyruvate. In the light of these observations detailed experiments were conducted to evaluate the effect of UO_2^{++} on the metabolic absorption of Li^+, Na^+, K^+, Mg^{++} and Mn^{++} by excised barley roots.

Barley seedlings (*Hordeum vulgare*) were grown and the experiments were conducted according to the methods outlined by Jacobson *et al.*[2]. The roots were placed into continuously aerated test solutions maintained at *p*H 5·0 and at a temperature of 25° C. At the end of the absorption period the roots were removed from the solution, washed with distilled water, dried overnight, and digested in nitric and perchloric acids. Li^+, Na^+ and K^+ were determined by flame photometry and Mg^{++} and Mn^{++} by atomic absorption spectrophotometry. (Due to the toxicity of uranium these instruments should be placed in a well-ventilated fume hood.) Standard solutions with and without UO_2^{++} were compared and interferences in the analyses were found to be negligible.

Previous work has shown that ion uptake by excised barley roots occurs in two phases. The first phase is a non-metabolic uptake into the apparent free space, whereas the second phase is associated with steady-state metabolic uptake. Only the metabolic uptake was evaluated in this study. Metabolic uptake of ions by barley roots reaches a steady state prior to 1 h and maintains this rate for periods in excess of 6 h. The rate of uptake was measured as the difference in uptake between a 1-h and a 6-h absorption period. All results are expressed in milli-equivalents uptake per kilogram of fresh root tissue per 5 h (m.equiv./kg/5 h). Differences between treatments of ± 1 m.equiv./kg/5 h are significant.

Table 1 presents the results of these experiments. The effects of adding 1·0 m.equiv. UO_2^{++}/l. to the test solution can be seen by comparing the first two columns in Table 1. From these results it is apparent that UO_2^{++} had considerably different effects on the various cations. Uranyl had essentially no effect on the uptake of Li^+, reduced Na^+ by 36 per cent, K^+ by 74 per cent, Mg^{++} by 83 per cent and Mn^{++} by 69 per cent.

The effect of pretreating the roots with UO_2^{++} prior to the absorption period was also evaluated. Roots were pre-treated by placing them into a solution of 1·0 m.equiv. UO_2^{++}/l. for 30 min. After the pre-treatment the roots were thoroughly washed in distilled water and then placed into the test solution. Except for the pre-treatment, these roots were handled in an identical fashion to the control roots. During the pre-treatment, approximately 5 m.equiv. UO_2^{++}/kg roots was bound by the tissue and was not removed by the distilled water wash. This UO_2^{++} was apparently bound very tightly since the bulk of it was still associated with the tissue at the end of the subsequent absorption periods. The data showing the effect of UO_2^{++} pre-treatment are given in the last column of Table 1.

Although the inhibitory effect of the UO_2^{++} pre-treatment was not as pronounced as the effect of including the UO_2^{++} in the bottle with the test solution, there was still considerable difference between the reaction of the various cations. Mn^{++} was unaffected by the pre-treatment; Li^+ and Na^+ were either unaffected or slightly stimulated; whereas K^+ and Mg^{++} still showed the inhibitory effects of UO_2^{++}.

Table 1. The Effect of Uranyl (UO_2^{++}) on the Uptake of Cations by Excised Barley Roots. (All Ions were present at 5 m.equiv./l. except for UO_2^{++} which was present at 1 m.equiv./l.; Pre-treatment was for 30 min in a 1 m.equiv./l. UO_2^{++} Solution; all Ions were provided as Chloride Salts)

Ion	Net uptake (m.equiv./kg/5 h) Control	+ UO_2^{++}	Pre-treatment
Li	9·0	8·1	11·7
Na	24·4	15·7	26·6
K	27·8	7·3	18·5
Mg	12·6	2·2	6·0
Mn	7·4	2·3	7·2

Dinitrophenol (DNP) and low temperature were used to determine if the portion of the ion uptake which was resistant to UO_2^{++} was metabolically mediated. 5×10^{-5} M DNP was found to completely inhibit the absorption of Li^+, Na^+ and K^+ in the UO_2^{++} treatments as well as in the control. Low temperature (0·5° C) was used in an independent verification of the metabolic nature of K^+ and Na^+ uptake. In this experiment low temperature almost completely inhibited the uptake of these two ions by both pre-treated and control tissues.

From the results obtained in the work recorded here, UO_2^{++} appears to hold promise as a means of separating and characterizing various cation transport sites. Unlike the common inhibitors of ion absorption (metabolic poisons, low temperature and anaerobiosis), UO_2^{++} shows a considerable degree of selectivity. This selectivity strongly suggests that UO_2^{++} reacted with specific sites intimately involved in ion absorption rather than interrupting some basic aspects of respiration which supplies energy for the general ion transport mechanism. This hypothesis is supported by the observation that the UO_2^{++}-resistant

absorption was inhibited by dinitrophenol and low temperature. Furthermore, the strong retention of UO_2^{++} by the tissue and the persistence of the effect of UO_2^{++} on certain ions following pre-treatment indicates that UO_2^{++} was probably bound tightly to some of these active sites.

Of particular interest is the differential effect that UO_2^{++} had on Na^+ and K^+ absorption, especially the effect of pre-treatment. Na^+ and K^+ are chemically similar and exhibit similar absorption characteristics in barley roots[3]. In contrast to animal systems, both cations may be accumulated inside plant cells, and are known to compete readily during absorption by this kind of root tissue[4]. In this work, K^+ was inhibited to a much greater extent than was Na^+; therefore, it seems reasonable to conclude that at least a portion of the Na^+ transport was handled differently than K^+ transport by this tissue. Although these roots initially contained more K^+ than Na^+ the loss of K^+ due to UO_2^{++} treatment was slight and cannot account for the observed differences.

The results with cation absorption are similar in many respects to the UO_2^{++} induced differential inhibition of the uptake of organic substrates by yeast cells as reported by Rothstein[1]. He concluded that the UO_2^{++} was bound tightly at the yeast cell surface and did not penetrate into the interior of the cell. Numerous substances in the cell are capable of binding UO_2^{++} (ref. 5). Phosphate, a constituent of many compounds present in the cell membrane, has a high affinity for uranyl. This high affinity of phosphate for uranyl may be significant in the light of recent speculation concerning the role of phosphorus compounds in the ion absorption process[6].

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Depressant Effect of Moonlight on Activity of Aquatic Insects

Investigations by Waters[1], Müller[2-5], and Wickett[6] have conclusively demonstrated the diurnal periodicity of downstream drift of aquatic insects and other invertebrates. Prior to these investigations, Moon[7] had shown that the nocturnal activity of aquatic insects is much greater than that observed during the day. Very little data are available to demonstrate whether these activity-levels are circadian rhythms or whether they are a direct response to the level of light intensity. Beeton[8] observed a depression of the vertical migration of the freshwater shrimp, *Mysis relicta*, due to increased moonlight. Waters[1] suggested that the magnitude of the nocturnal drift rate may be, in part, a function of moonlight. The following data appear to support Waters's contention.

Collections of aquatic insects drifting downstream were taken at intervals of 3 weeks during 1963 and 1964 in Berry Creek, near Corvallis, Oregon (lat. N. 44° 43′, long. W. 123° 16′). These collections were made as part of a project concerned with measuring the production rate and energy exchange in a woodland stream community.

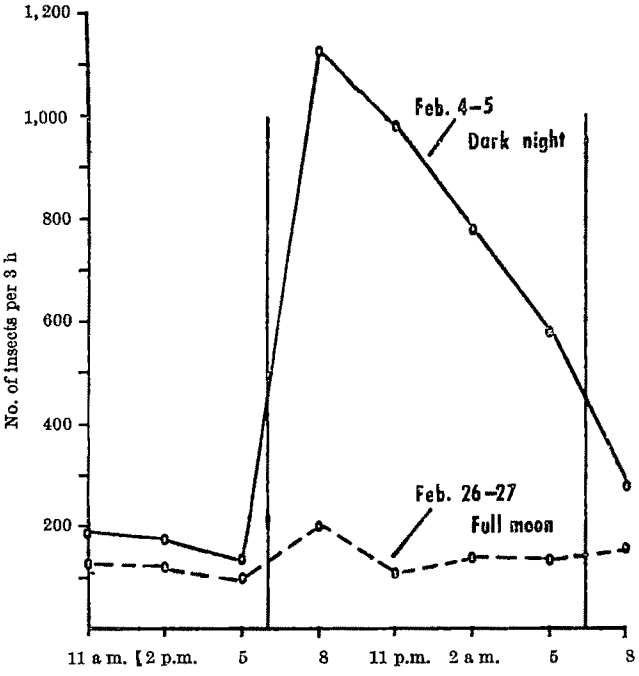


Fig. 1. Number of insects (Ephemeroptera, Plecoptera, and Simuliidae) collected per 3-h sampling interval on a dark night and a moonlit night. The vertical lines indicate approximate times of sunset and sunrise

The data reported here compare results from two consecutive collecting dates, February 4–5 and February 26–27, 1964. It is felt that differences in drift rate can be attributed to differences in intensity of moonlight on the two nights. The night of February 4–5 was clear, but dark, with the Moon in the last quarter, rising at 0012 Pacific Standard Time, and setting at 1118; on February 26–27, the night was again clear, but bright, as the full moon rose at 1659 and set at 0728. Weather and stream conditions for the two days were similar. Mean water temperature for the former was 8·3° C, and for the latter, 6·7° C.

The total numbers of insects in the three dominant taxa, Ephemeroptera, Plecoptera, and Simuliidae, collected during each 3-h sampling interval on the two dates are compared in Fig. 1. Chironomidae are abundant in Berry Creek and are an important component of the drift, but are not included because there was no evident increase in their drift rate at night. The drift rate of the dominant taxa for February 4–5 exhibited the typical pattern (compare Waters[1]), with a sharp increase occurring shortly after sunset, followed by a gradual decrease during the night. The drift rate during both day and night was nearly constant on February 26–27, with little or no evidence of the expected sharp post-sunset increase.

Table 1. Percentage of Larvae (Ephemeroptera, Plecoptera, and Simuliidae) 3 mm or Larger in Drift-trap Collections

	February 4–5 (dark night)	February 26–27 (moonlit night)
8–11 a.m.	17·9	18 6
11–2 p.m.	12·4	17·4
2–5 p m.	16 2	11·5
5–8 p m.	42 3	18 3
8–11 p.m.	41·5	28 1
11–2 a.m.	30 3	15 7
2–5 a m.	23 0	19 3
5–8 a.m.	11·5	12·4

The range of size of organisms collected was from 0·5 to 7·0 mm in length. Table 1 gives the percentage of insects over 3 mm in length collected during each sampling interval; these are arbitrarily considered 'large' insects. There is a high correlation between the increase in the percentage of these large insects and the post-sunset increase in drift rate on February 4, whereas on the moonlit night a much lower percentage of larger forms occurred

in the drift. Comparing the total night period (5 p.m. to 5 a.m.) with the daylight period (5 a.m. to 5 p.m.), the percentage of large insects in the drift was: February 4–5, day—14·1, night—36·1; February 26–27, day—16·0, night—19·0. There was no major emergence of adult insects between the two sampling dates that could have accounted for a decrease in mature insects; it is expected that there should be some slight increase in mean size due to growth at that time of year.

On the basis of the present set of observations, the diel periodicity of drift rate appears to be a direct response to lowered light intensity at sunset. The depressant effect of moonlight on drift rate results from the suppression of activity of the larger size classes of larval insects. Feeding behaviour and other activity patterns associated with periods of low light intensity may have evolved as a protective or avoidance mechanism to escape attack by predators that seek their prey visually.

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Differentiation of Nucleated Red Blood Cells during *in vivo* Culture of Mouse Spleen and Thymus Cell Suspensions

Differentiation of nucleated red blood cells has been observed when mouse bone marrow was cultivated *in vivo* in diffusion chambers[1]. Recently, during investigations of the survival of thymus and spleen cells similarly cultured, we found that differentiation of nucleated red blood cells also occurred. This was indicated by an increase in the number of erythrocytes after cultivation. The present report describes these findings.

Healthy male mice of the *BALB/cfSp* strains, 7–9 weeks of age, were used throughout. In experiment 1, six donor mice were used for each time period; in experiment 2, five donor mice were used. One chamber was inserted into each age-matched recipient mouse. All mice were killed by cervical dislocation.

The diffusion chambers were modified from those used by other workers[2,3]. They were constructed of 'Plexiglas' rings (16 mm outside diam., 9·5 mm inside diam., 2 mm thick, access hole 1 mm diam.) sandwiched between two filters. Filters were Schleicher and Schuell *B*-14 'BAC-T-Flex', having an average pore diameter of 0·1μ. The membranes were sealed to the rings with Rohm and Haas 'Acryloid *B*-7' cement. Chambers were allowed to dry for at least 18 h and then sterilized for 72 h at 78°–80° C dry heat. Each chamber, when completed, had an average volume of 0·14 ml. Empty chambers prepared in this manner and with the access hole sealed with a chip of heat-softened 'Plexiglas' were found to be completely unpenetrated by cells 5 days after they were inserted into the peritoneal cavity of recipient mice.

Donor mice were killed, washed in a 0·94 per cent Wescodyne solution and the thymus and spleen (experiment 1), or only the thymus (experiment 2), were removed. Cells from each organ were obtained by gentle dispersion in 0·4 ml. of Krebs–Ringer phosphate buffer solution fortified with Eagle's nutrients and adjusted to *p*H 7·3–7·4 (KRP) in a 1-ml. centrifuge tube by means of a 5-mm glass rod, tapered to fit snugly in the tube. The KRP had been sterilized by passage through a Millipore *HA* filter. The resulting suspensions were transferred aseptically to 12-ml. centrifuge tubes and diluted to 1 ml. Following thorough dispersion, a portion was removed and the cellular content of each suspension was determined. KRP was then added to adjust the cell density to 50–100 million cells per ml. Few broken or clumped cells were found on microscopic examination of suspensions prepared in this manner.

Recipient mice were anaesthetized with ether and the peritoneal cavity opened with a small incision of the midventral surface of the animal. The chambers were filled with 0·1 ml. of the organ suspensions, the access hole was sealed with a chip of softened 'Plexiglas' and the chamber was inserted into the abdominal cavity of the recipient. At the same time an aliquot of each suspension was taken for a repeat count and another portion was used for making a smear for the differential count. The recount was made to correct for possible changes in the cell content of the suspensions during the interval between the first count and the time the cells were inserted into the chambers.

The recipient mice were killed at various times after chamber insertion. The chambers were removed, their outer surfaces cleared of adherent host cells, then washey in KRP and wiped dry. One of the filters was carefulld removed and the entire content was transferred to a 5-ml. centrifuge tube and dispersed gently with a glass rod. A portion of the fluid removed was thoroughly mixed with an equal amount of KRP and a total cell count was made. The remaining portion was used for a differential count.

All smears made for differential counts before and after culture were air dried. In experiment 1, they were stained with haematoxylin and eosin; in experiment 2, they were stained with freshly prepared buffered Leishman's stain. A minimum of 100 consecutive cells were examined and the proportion of erythrocytes was determined. The total number of erythrocytes in the chambers before and after culture was then calculated from the total cell counts.

Marked variation was found in the number of erythrocytes in the donor organs and in the number of cells converted to erythrocytes (Tables 1 and 2). At all the times studied, the average number of erythrocytes in cultured spleen (Table 1) and thymus cell (Table 2) suspensions was increased. Such an increase was found in 18 of 24 (75 per cent) spleen and 23 of 29 (79 per cent) thymus cultures. Considerably more spleen than thymus cells

Table 1. Differentiation of Red Blood Cells during *in vivo* Culture of Spleens in Diffusion Chambers

Days after insertion	Total No. of cells inserted into chambers	No. of erythrocytes when implanted	No. of erythrocytes when removed	Cells converted to erythrocytes (per cent)
		Experiment 1		
1	572·9* (358–999)	128·5 (22–499)	278·1 (218–337)	36·7 (0–62)
2	593·1 (333–730)	115 0 (38–164)	227 9 (36–418)	23·2 (0–47)
3	503·1 (283–631)	105·5 (22–257)	161 5 (43–352)	15·1 (0–49)
5	680·7 (510–1,102)	103·6 (5–254)	133·2 (31–381)	10·1 (0–30)

* Figures are averages and ranges × 10^4.

Table 2. Differentiation of Red Blood Cells during *in vivo* Culture of Thymuses in Diffusion Chambers

Days after insertion	Total No. of cells inserted into chambers	No. of erythrocytes when implanted	No. of erythrocytes when removed	Cells converted to erythrocytes (per cent)
		Experiment 1		
1	468·1* (357–544)	1·5 (0–9)	2·7 (0–11)	0·3 (0–1)
2	601·5 (478–737)	3·8 (0–11)	7·8 (1–14)	1·0 (0–2)
3	533·4 (275–884)	3·5 (0–10)	30·8 (0–157)	3·5 (0–18)
5	525·8 (472–655)	2·3 (0–10)	21·0 (1–89)	3·8 (0–17)
		Experiment 2		
3	510·8 (160–678)	2 8 (0–7)	29·1 (0–94)	4·1 (0–13)

* Figures are averages and ranges × 10^4.

were converted to erythrocytes (Tables 1 and 2). Of the former, the number of cells converted was greatest after one day of cultivation; of the latter, the greatest number of cells converted occurred after 3 and 5 days of culture. These findings indicate that the process of erythroid maturation was longer in the thymus than in the spleen.

The reasons for the marked variations are not apparent at the present time. They may merely indicate that, although the mice belonged to a highly inbred strain, environmental conditions resulted in a different requirement for erythropoiesis in each mouse in reticuloendothelial sites other than the bone marrow. Alternatively, red blood cell formation may be a rhythmic process and the variations may result from the fact that erythropoiesis in the spleen and thymus of each mouse was at different stages of such a cycle at the time they were killed. These possibilities are at present under investigation.

The data suggest several conclusions. First, it is apparent that differentiation of nucleated red blood cells from spleen and thymus can occur during *in vivo* culture in diffusion chambers. Secondly, erythropoiesis in reticuloendothelial sites other than bone marrow normally is a common occurrence in healthy young adult male mice and may be quite extensive since as many as 62 per cent of the spleen cells were found to have an erythropoietic function. Inasmuch as bone marrows from comparable mice were normal, the possibility that the presence of nucleated red blood cells in the spleen and thymus is due to extramedullary hematopoiesis can be excluded. Thirdly, the thymus is an important site of erythropoiesis since as many as 18 per cent of its cells may have this function. This finding is in agreement with previous observations[4]. Fourthly, maturation of erythrocytes appears to differ in the thymus and the spleen. Fifthly, many of the cells in these organs, especially in the thymus, have been erroneously identified, in the past, as lymphocytes.

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ENTOMOLOGY

Intersterility as a Consequence of Insecticide Selections in *Tetranychus urticae* Koch (Acari : Tetranychidae)

In July 1961 specimens from a wild colony of *Tetranychus urticae* were gathered from *Sambucus nigra* L. in dunes in the Netherlands and cultivated on beans (*Phaseolus vulgaris* L.) in the laboratory. This colony (*S*) proved to be very susceptible to the ovo-larvicide 'Tedion' (2,4,5,4′-tetrachloro-diphenylsulphone—LD_{50}, 1·410 p.p.m.).

In 1961, a series of selections on a representative sample of this colony with the compound 'Tedion' resulted in a strain R_1, highly resistant to 'Tedion'. Three years later a second series of selections was performed, also obtained from the original colony, and a resistant strain R_2 was acquired.

A genetic analysis of resistance to 'Tedion' could not be executed, since an unexpected intersterility existed between the *S*- and *R*-strains[1]. In Table 1 the percentages of sterile eggs in the F_1 and F_2 progeny are given for all combinations of crosses.

As *T. urticae* has a generative parthenogenesis[2], I was able to establish the sterility of the haploid eggs in the F_2 separately by starting from unmated F_1-females. The opposite, a purely diploid progeny, cannot be obtained, as the mated females always gave a mixture of diploid and haploid eggs.

Table 1. Percentage of Sterile Eggs of Several Combinations of Crosses between Resistant Strains (R_1 and R_2) and the Wild Colony (*S*)

Mass crossing ♀×♂	F_1 diploid and haploid		F_2 diploid and haploid		F_2 haploid	
	Eggs	Sterility (%)	Eggs	Sterility (%)	Eggs	Sterility (%)
$S \times S$	559	6	453	7	311	6
$R_1 \times S$	390	7	566	26	516	31
$S \times R_1$	772	12	309	40	559	61
$R_1 \times R_1$	493	2	412	8	547	8
$R_2 \times S$	716	2	431	19	657	33
$S \times R_2$	685	3	324	28	648	62
$R_2 \times R_2$	282	3	706	4	656	5
$R_1 \times R_2$	267	3	—	—	162	6
$R_2 \times R_1$	282	3	—	—	187	1

The outstanding fact that arises from these observations is that selection with an insecticide has resulted in an appreciable genetic blockade. The intersterility factors accompany on both occasions the build-up of resistance in strains R_1 and R_2. The specificity of the intersterility is underlined by the lack of sterile eggs in the mutual crosses of R_1 with R_2.

The phenomenon of a decreased genetic affinity following the application of an insecticide has not, so far as I know, been reported before. It is interesting from the point of view of theories of evolution and also because it has consequences in the practical control of spider-mites.

A further analysis of 'Tedion' resistance and intersterility is being undertaken.

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MICROBIOLOGY

Stabilization of Autolysis in *Bacillus cereus* T

During investigations of the tricarboxylic acid cycle in intact, resting vegetative cells of *Bacillus cereus* T suspended in phosphate buffer, spontaneous autolysis was observed similar to that reported by Kronish *et al.*[1] in 0·05 M *tris* (hydroxymethyl)-aminomethane (*tris*) buffer, *p*H 7·5. Further examination of intact, resting cells was precluded unless the cells could be stabilized, remaining metabolically active for the required duration, usually 2–4 h, and preferably in the absence of agents such as sucrose or polyethylene glycol 4000 (ref. 1).

Since analysis in a marine pseudomonad can be prevented or reduced by several cations, principally Mg^{++} (refs. 2 and 3), a similar situation might prevail in *B. cereus* T, and consequently the effect of Mg^{++} on autolysis in this organism was investigated.

B. cereus T was grown in 'Trypticase Soy Broth' (Baltimore Biological Laboratory) from a suspension of germinated spores[4] for 4 h at 30° C. Cells at the filamentous stage of development[5] were collected by centri-

fugation, then washed twice with and suspended in 0·05 M *tris* buffer, *p*H 7·5, containing the appropriate concentration of $MgSO_4$, and shaken at 30° C. The cell suspensions were examined turbidimetrically at 600 mμ in a Bausch and Lomb Spectronic 20 colorimeter and by phase-contrast microscopy. Viability determinations were made on 'Trypticase Soy Agar' with the same concentrations of $MgSO_4$ in the dilution tubes as in the test suspending medium.

Above 0·05 M $MgSO_4$ the absorbency of the cell suspension increased over a 4-h period as compared to the initial reading (Fig. 1), suggesting a possible increase in the number or size of the cells or a change in the optical properties of the cells with increasing osmolarity[6,7]. Phase-contrast microscopy of the suspensions after 4 h indicated a large but variable number of spheroplasts at 0·05 M and 0·1 M. At higher concentrations there was an increasing proportion of shortened, partially refractile cells, relatively few spheroplasts identifiable as such, and numerous bright, round bodies that may have been spheroplasts modified optically by the relatively high solute concentration[6,7].

Relatively high degrees of viability were maintained for 2 h at all concentrations, 0·05 M and 0·1 M being the best in this regard (Fig. 2). After 4 h, however, the reduced viability at these concentrations may have resulted from spheroplast sensitivity to plating since respiratory activity at these lower concentrations decreased only moderately during the latter hours of the experiment, when most of the cells were spheroplasts.

Q_{O_2}, highest at 0·05 M and 0·1 M, decreased with increasing hypertonicity (Table 1), which is consistent with the decreased viability at these concentrations.

The increase in absorbency of the resting-cell suspensions at higher $MgSO_4$ concentrations would seem to be related to a combination of spheroplast formation[8] and a change in the optical properties of the cells in hypertonic solutions rather than to any substantial increase in cell numbers. The lower concentrations of $MgSO_4$, 0·05 M and 0·1 M, can then be used to maintain resting vegetative cells of *B. cereus* T in a sufficiently active metabolic state for several hours. Whereas Mg^{++} apparently is involved in maintaining the integrity of the spheroplast, it does not prevent enzymatic autolysis[9] from occurring. But it may be involved in delaying autolysis since the rate of spheroplast formation appeared to be lower than the corresponding decrease in turbidity in the *tris* control. The system described could be quite useful for studying cell-wall degradation at a depressed rate as well as for gaining insight to the problem of spheroplast integrity.

Stabilization is not necessarily restricted to hypertonicity alone; other factors are being examined and will be reported on later.

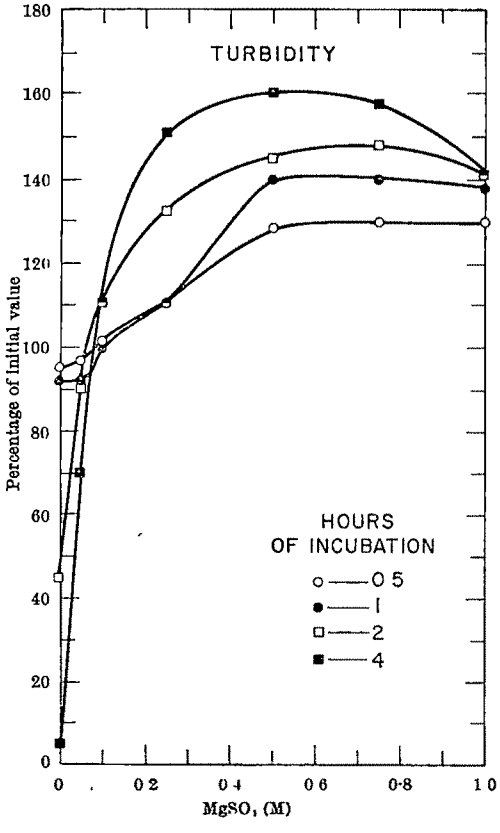


Fig. 1 Effect of $MgSO_4$ molarity on turbidity of resting-cell suspensions of filamentous stage *Bacillus cereus* T. Suspending medium consists of 0 05 M *tris* buffer, *p*H 7·5, and the appropriate concentration of $MgSO_4$

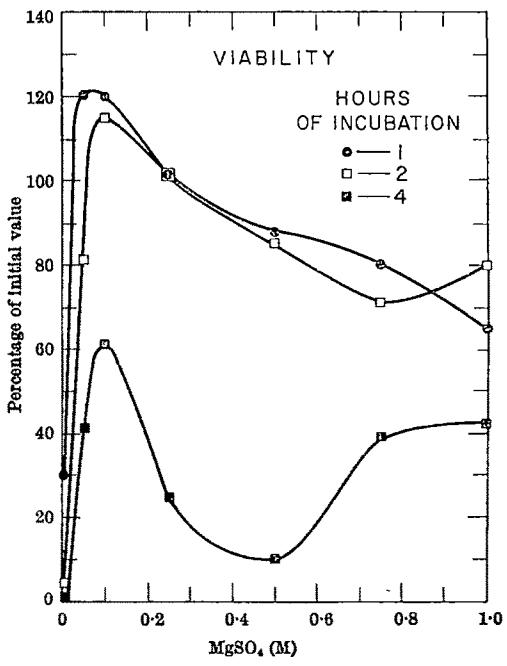


Fig. 2. Effect of $MgSO_4$ molarity on viability of resting-cell suspensions of filamentous stage *Bacillus cereus* T. Suspending medium consists of 0 05 M *tris* buffer, *p*H 7·5, and the appropriate concentration of $MgSO_4$

Table 1. Effect of Magnesium Sulphate Molarity on Q_{O_2} of Resting-cell Suspensions of Filamentous Stage *Bacillus cereus* T

$MgSO_4$ (M)	Q_{O_2} (μl./O_2/h/mg dry wt.)
0·05	108·2
0·1	97·2
0·25	81·7
0·5	48·5
0·75	25·1
1 0	8·3

Tris control omitted because of rapid lysis and concomitantly low respiration.

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VIROLOGY

Inhibition of Haemagglutinating Particles of Coxsackie *B5* Virus by HeLa Cell Cultures

It has been observed in recent years that many enterovirus strains (belonging to 18 types[1,2]) have the ability to agglutinate human red cells.

The receptor of human erythrocytes for some ECHO viruses is destroyed by chymotrypsin, but not by RDE or trypsin: it appears to be different from the erythrocyte receptor for myxoviruses and is probably a protein[3].

In 1962 we isolated from a rectal swab of a child in Ossi (Sardinia) a strain of Coxsackie *B* 5 virus (*SS* 31 strain) with an ability to haemagglutinate human erythrocytes[4]. In this it was similar to that of other strains of Coxsackie *B* 5 virus previously isolated by other authors[5,6].

Propagation in HeLa cell cultures of this strain of Coxsackie *B* 5 virus makes its haemagglutinating capacity disappear after the first or second passage, as was observed for haemagglutinating strains of ECHO viruses, types 3, 7, 11, 12, 19 (refs. 7, 8), and, to a lesser degree, for Coxsackie *A* 21 virus (Coe)[9].

A suspension of Coxsackie *B* 5 virus, *SS* 31 strain, in veronal buffer at *p*H 7·2, with a haemagglutinating titre of 1 : 64, was incubated 1 h at 37° C with: (*a*) HeLa cell medium (Eagle's, Hanks's and 5 per cent calf serum solutions), kept in contact with HeLa cells for 5 days; (*b*) control medium (Eagle's, Hanks's, and 5 per cent calf serum solutions), not incubated with HeLa cells; (*c*) HeLa cell monolayers washed twice with Hanks's solution.

Haemagglutinins (HA) were not found (titre < 1 : 2) except in the virus sample which was incubated with HeLa cell medium. HA titre was unchanged after incubation with the control medium and decreased only slightly after contact for 1 h with HeLa cells. In the HeLa cell medium, therefore, there is a factor which inhibits the haemagglutinins of Coxsackie *B* 5/*SS* 31 virus: this factor will here be referred to as 'AHAF' (anti-haemagglutinating factor).

AHAF withstood rapid freezing and thawing five times, heating at 56° C for 30 min, incubation at *p*H 2 and *p*H 12 for 6 h at 37° C. Heating at 100° C for 30 min, on the other hand, rendered it completely inactive.

The effect on AHAF of some chemical compounds and enzymes was investigated: neither ethyl ether nor merthiolate (1 : 10,000) altered its titre, but trifluorotrichlorethane and potassium periodate (5 mg/ml.) clearly decreased its titre; trypsin (1 mg trypsin 1 : 250 Difco/ml., for 6 h at 37° C and at *p*H 7·4) and pepsin (1 mg crystalline pepsin 1 : 60,000 Sigma/ml. for 6 h at 37° C and *p*H 2) brought about the complete disappearance of AHAF.

AHAF is probably a proteic substance perhaps related to human erythrocyte receptors for ECHO viruses. It behaves in a similar fashion to the haemagglutinin inhibitors for Coxsackie *A* 21 (Coe)[9].

Changes in the AHAF-level in the HeLa cell medium (Eagle and Hanks's solutions) were investigated, correlating them to incubation times at 37° C and to the percentage of calf serum in the medium (Table 1). For this purpose, the reagents (which had been diluted in a veronal buffer at *p*H 7·2) were used in the following ratios: (*a*) haemagglutinating virus (Coxsackie *B* 5/*SS* 31), 0·25 ml. 25 HA units; (*b*) HA inhibitor (AHAF), 0·25 ml.; (*c*) 1 per cent suspension of human erythrocytes, 0·12 ml.

HA and AHAF were incubated 1 h at + 37° C and then the suspension of erythrocytes was added: the final reading was made after 2 h incubation at room temperature. AHAF titres were expressed as an inverse ratio of the highest dilution of the medium which inhibits 25 HA units of virus.

Small quantities of an inhibitor were also found to be present in the medium, which contained 10 per cent calf serum before its use with HeLa cells, but they did not vary during incubation at 37° C; on the other hand, it would seem that (within the limits of the sensitivity of the method used) in neither the media with 5 per cent serum nor in the media devoid of calf serum was inhibitor present before their use with HeLa cells.

AHAF clearly increased in HeLa cell cultures from the second to the fifth day of incubation; it also reached moderate levels in HeLa cell cultures devoid of serum, before cell destruction.

This experiment shows that AHAF originates from prolonged contact between the medium and HeLa cells. The presence of proteins in HeLa cell medium does not appear necessary for AHAF production. This could be explained either by the fact that AHAF is a substance produced by HeLa cells and released in the medium, or by the fact that it originates from a modification of the medium components through the action of HeLa cells.

Repeated sub-cultures of Coxsackie *B* 5 virus, *SS* 31 strain, in primary monkey kidney (MK) cells and in newborn mice did not affect its haemagglutinating ability. On the contrary, ten passages of this virus in HeLa cell cultures brought about the irreversible loss of the haemagglutinating capacity, as has already been observed with ECHO virus type 19 (ref. 10): the haemagglutinating capacity of Coxsackie *B* 5 virus/*SS* 31 was not restored even after twenty subsequent passages in primary MK cells.

Acting on the hypothesis that during the propagation in HeLa cell cultures a selection of viral non-haemagglutinating particles (HA−), in comparison with haemagglutinating ones (HA+), might occur, the ratio of HA+ to HA− particles was checked in the original virus sample (sixth MK passage) and in the first four passages in HeLa cells.

For this purpose the material from individual viral plaques, obtained in MK cell cultures with agar overlay, was inoculated in MK cell tubes and the presence or absence of haemagglutinins was determined in the sample of virus resulting from each individual plaque. The results are set forth in Table 2.

In the serial sub-cultures of the virus in HeLa cells, first the HA− particles began to appear and then their titre quickly increased, whereas the HA+ particles were less than a twentieth of all the infectious viral particles present in the sample by the fourth passage.

Table 1. VARIATION OF AHAF TITRES* IN HELA CELL MEDIUM CORRELATED TO INCUBATION TIME AT + 37° C AND TO THE PERCENTAGE OF CALF SERUM IN THE MEDIUM

Incubation time (days)	Kolle 1 5% calf serum	Kolle 2 10% calf serum	Kolle 3 no calf serum	Kolle 4 control without HeLa cells 10% calf serum
0	0	1	0	1
2	2	2	1	1
5	8	8	4	1
7	8	8	4†	0
10	8	8	—	1
12	8	16	—	1
15	8	16	—	1
17	8	16	—	1
20	8	16	—	1
22	16	32	—	0

* Titre expressed as the reciprocal of the highest dilution showing haemagglutination inhibition of 25 HA units of virus.
† After 7 days' incubation the HeLa cell monolayer was destroyed.

Table 2. BEHAVIOUR OF HAEMAGGLUTINATING CAPACITY OF COXSACKIE *B* 5 VIRUS AND OF RATIO OF HA+ TO HA− PARTICLES AFTER ADAPTATION OF THE VIRUS TO HELA CELLS

Passage history of Cox. *B5/SS* 31	Age of cell cultures at the inoculation point (days)	Haemagglutinating titre: In the material of each HeLa passage	Haemagglutinating titre: After a further passage in MK cells	Studies of individual plaques: No. of plaques examined	Studies of individual plaques: Ratio of HA+ to HA− particles
MK 6		64	64	30	30 : 0
MK 6/HeLa 1	2	<2	128	36	35 : 1
MK 6/HeLa 2	4	<2	32	30	5 : 25
MK 6/HeLa 3	1	<2	16	30	5 : 25
MK 6/HeLa 4	7	<2	<2	20	0 : 20
MK 6/HeLa 5–9	1–5	<2	<2	—	—
MK 6/HeLa 10	3	<2	<2	30	0 : 30
MK 6/HeLa 10/ MK 1–20	—	<2	<2	—	—

From the data set out in Table 2 it can be deduced that the selective pressure which yielded the numerical reduction of HA+ particles in HeLa cell cultures ranged from 1 to 175 times in the individual viral sub-cultures, and showed a positive correlation with the age of cell cultures, expressed as days from the last change of medium, at the time of inoculation. For example, in the second passage in HeLa cells, at the time of inoculation, the medium was 4 days old and an increase of 175 times was demonstrated in the ratio of HA− to HA+ particles; in the third passage in HeLa cells, at the time of inoculation, the medium had just been replaced with a fresh one and on this occasion the relative increase in number of the HA− particles was absent or irrelevant.

Since all thirty plaques of the original sample of virus (MK 6) examined contained HA+ particles, this sample was subjected three times to incubation with human erythrocytes group O at +4° C, in the attempt to adsorb the HA+ virus particles and to enrich the HA− particles which might be present. Packed red cells were added to the virus suspension at a volumetric ratio of 0·2 ml. to 1·5 ml.; the incubation period each time was 1 h, and afterwards the decrease of the virus titre and the ratio of the HA+ to HA− particles was determined in the supernatant. As a control the non-haemagglutinating variant of the same viral strain (MK 6/HeLa 10/MK 6 passage of Coxsackie *B* 5 virus, *SS* 31 strain) was subjected to the same process; this variant had been purified by plaque method in agar-overlay cultures and contained only HA− particles.

Table 3. Adsorption on Human Erythrocytes of the following Samples of Coxsackie *B*5:
(*a*) MK 6 passage; (*b*) MK 6/HeLa 10/MK 6 passage. The former contains haemagglutinins, the latter is devoid of them

Passage history of the virus	Presence of haemagglutinins	Titre in P F U /ml. before adsorption (x)	Titre in P.F.U./ml. after adsorption (y)	Ratio of the titres $\left(\frac{x}{y}\right)$	Ratio of HA+ to HA− particles after adsorption
MK 6	+	$3{\cdot}25 \times 10^7$	$2{\cdot}9 \times 10^4$	$1{\cdot}12 \times 10^3$	20·11
MK 6/He La 10/MK6	−	$2{\cdot}15 \times 10^7$	$3{\cdot}8 \times 10^7$	0·57	0 : 30

From Table 3 it can be deduced that the HA+ particles were adsorbed to erythrocytes, whereas the HA− ones were not adsorbed at all. The ratio of virus HA+ to HA− particles in the MK 6 virus sample can be estimated as 3,155 : 1.

These experiments indicate that HeLa cell cultures affect a population of Coxsackie *B* 5 virus particles, in that they promote the rapid selection of the non-haemagglutinating particles, which are very rare in the original samples as compared with the haemagglutinating ones.

The mechanism of action of the factor which inhibits the infectiousness of HA+ particles of Coxsackie *B* 5 virus, *SS* 31 strain, remained unknown; other questions which remain to be answered are whether this factor is the same as the anti-haemagglutinating factor (AHAF) demonstrated in HeLa cell medium or whether it must be differentiated from AHAF.

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Isolation and Identification of Poliovirus from a Clinical Specimen

At present, the isolation and identification of viruses from clinical specimens is a slow, expensive and tedious process[1,2]. Modifying a technique previously described[3], we were able to isolate and identify in three rapid and relatively simple steps a prototype virus from a sample of faeces seeded with a known quantity of polio virus.

DEAE 'Sephadex' *A*-25 medium-grade columns were prepared and used as previously described[3]. All columns were 14 × 1·2 cm. The elution of virus from all columns was accomplished with M/15 phosphate buffer (*p*H 7·5) at a rate of 1 ml. per min. The first eluate in all experiments was 3 ml.; all others were 1 ml. quantities. Protein content of the eluates was determined by the method of Lowry[4]. The prototype virus used was the avirulent Sabin type I polio-virus. Virus assay was accomplished by plaque titration on HeLa cell monolayers propagated in 2*X* Eagle's basal medium supplemented with 10 per cent calf serum and 15 per cent tryptose phosphate. Antibody against this virus, prepared in rabbits[5], was tagged with fluorescein isothiocyanate[6]. The virus-antibody complex was dissociated by lowering the *p*H with an acid buffer[7].

1 ml. of a 1 : 10 dilution of fluorescein-tagged type I poliovirus antiserum was added to three columns. A fourth column served as the control and received no antiserum. 0·5 ml. of Sabin type I poliovirus having a titre of 5×10^7 P.F.U./ml. was added to these columns. The virus was in contact with the antibody for various time intervals prior to elution with buffer. The 16-h antigen–antibody association was performed at 4° C, whereas the 1 and 2 h reactions were carried out at room temperature. The above time intervals were followed, elution was accomplished with phosphate buffer, and the viral content of the eluate was determined.

Neutralization of poliovirus by tagged antibody suspended in a column. Fluorescein-tagged, immune serum (of any type), when added to a column, is held in that position. This is in contrast to untagged, immune serum which is rapidly eluted with phosphate buffer.

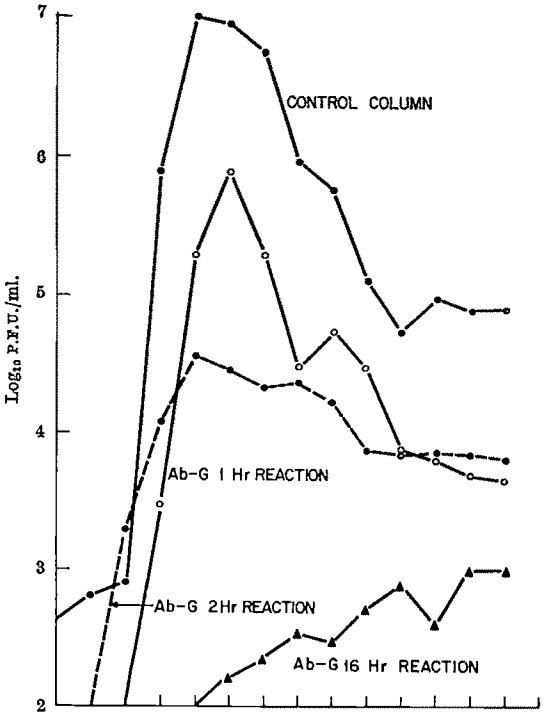


Fig. 1. Type I poliovirus retention by fluorescein-labelled type I antiserum in relation to the time interval of antigen-antibody association. Ab, antibody; G, antigen

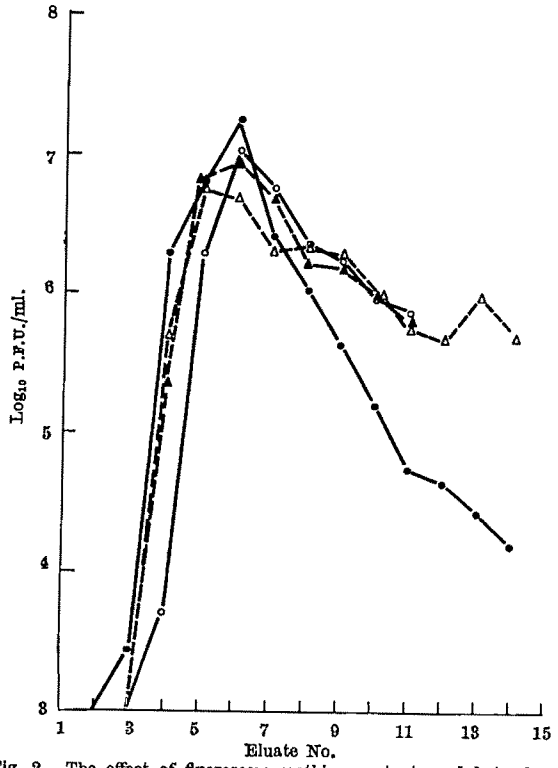


Fig. 2. The effect of fluorescein isothiocyanate tagged heterologous antiserum on the movement of Type I poliovirus in an *A*-25 column. ●—●, Control column; ○—○, Ab-G 1 h reaction; ▲—▲, Ab-G 2 h reaction; △—△, Ab-G 16 h reaction. Ab, anti-guinea-pig antibody; G, antigen

Results, as seen in Fig. 1, indicate that approximately 90 per cent of the virus failed to be eluted after 1 h of fluorescein-tagged type I poliovirus antiserum : type I poliovirus association; by 2 h, more than 99 per cent of the virus was retained; by 16 h, essentially all the virus was bound to the column.

Specificity of reaction. There was the possibility, of course, that the virus was not neutralized by specific antibody, but was physically retained, indicating a non-specific reaction.

The same procedure as in the foregoing experiment was followed except that fluorescein-tagged rabbit anti-guinea-pig antiserum was used instead of fluorescein-tagged type I poliovirus antiserum. Results, as seen in Fig. 2, demonstrated that almost all the type I poliovirus was recovered from each column. It can thus be concluded that the virus was not physically retained.

To test the specificity of the reaction, a column was loaded with 1 ml. of a 1 : 10 dilution of fluorescein-tagged polio type III antibody (kindly supplied by Dr. M. H. Hatch); 0·5 ml. type I poliovirus having a titre of 8×10^7 P.F.U./ml. was added and allowed to react for 2 h before eluting. The results, as seen in Table 1, are representative of several experiments and demonstrate that approximately 85 per cent of the virus was recovered. It can be concluded from these results that the column neutralization reaction is highly specific.

Table 1. Specificity of the Neutralization Reaction Sabin Type I Poliovirus reacted against Fluorescein-tagged Type III Poliovirus Antiserum

Eluate No.	Virus (P.F.U./ml.)
1	0
2	0
3	0
4	4×10^6
5	$9\,8 \times 10^6$
6	$1\,0 \times 10^7$
7	$6{\cdot}0 \times 10^6$
8	2×10^5
9	$1{\cdot}5 \times 10^5$
10	$8{\cdot}7 \times 10^5$
11	$8{\cdot}0 \times 10^4$
12	$9{\cdot}1 \times 10^4$
13	$4{\cdot}0 \times 10^4$
14	$2\,0 \times 10^4$

Table 2. Type I Antiserum Neutralization of Poliovirus in a Monkey Faeces Sample

Eluate No.	Column 1 (with antiserum) Protein (μg/ml.)	Column 1 (with antiserum) Virus (P.F.U./ml.)	Column 2 (without antiserum) Protein (μg/ml.)	Column 2 (without antiserum) Virus (P.F.U./ml.)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	9×10^2	0	$3\,0 \times 10^5$
5	0	$3{\cdot}7 \times 10^3$	0	$8{\cdot}1 \times 10^5$
6	0	$4\,5 \times 10^3$	0	$1{\cdot}0 \times 10^6$
7	0	$2{\cdot}0 \times 10^3$	20	$7{\cdot}5 \times 10^5$
8	20	$1{\cdot}4 \times 10^4$	20	$1{\cdot}7 \times 10^5$
9	20	$1{\cdot}4 \times 10^4$	20	$9{\cdot}4 \times 10^4$
10	20	$6\,2 \times 10^3$	40	$9\,0 \times 10^4$
11	20	$6\,0 \times 10^3$	20	$6\,0 \times 10^3$
12	20	$3{\cdot}0 \times 10^3$	60	$6{\cdot}0 \times 10^3$
13	20	$1{\cdot}0 \times 10^3$	40	$6{\cdot}1 \times 10^3$
14	20	$1{\cdot}2 \times 10^3$	40	$5{\cdot}8 \times 10^3$

Table 3. Recovery of Antiserum Neutralized Poliovirus from Column by Acid Buffer Dissociation

Portion of column dissociated	Virus P.F.U./ml.
Fluorescein labelled Type I antiserum Top (2 cm)	$2{\cdot}0 \times 10^4$
Middle (6·7 cm)	0

Isolation of poliovirus from faecal material. Type I poliovirus (8×10^6 P.F.U.) was added to 2 ml. of a heavy suspension of monkey faeces. After vigorous mixing, the suspension was centrifuged at 1,200 r.p.m. for 15 min. 1 ml. of the supernate was then added to a column which had previously been loaded with fluorescein-tagged type I poliovirus antiserum as above; an equal volume was added to a control column not containing the poliovirus antiserum. After 2 h, the columns were eluted. Eluates were analysed for protein and virus content.

The results in Table 2 show that less than 2 per cent of the virus was recovered from the column containing the fluorescein-tagged antiserum while about 80 per cent of the virus was recovered from the control column. The protein analysis similarly demonstrated the capacity of the column to provide a 'clean' preparation. One may conclude from these data that the poliovirus from the faeces sample had been specifically neutralized.

The fluorescein-tagged antiserum was removed from the column and the virus was dissociated with acid buffer. As a control, samples from the same column not associated with the antibody band were treated in a similar manner and assayed for virus.

The results, in Table 3, indicate that virus was present only at the site of antiserum localization.

It was apparent that this technique has application for the isolation and identification of certain viral agents. Subject to refinement of antigen–antibody dissociation techniques, viruses present in quantities commensurate with those found in a viral disease should be detectable. It is conceivable that by proper positioning of a variety of fluorescein-labelled antisera on a DEAE 'Sephadex' *A*-25 column, differential diagnosis could be achieved. The indirect fluorescent antibody technique can be utilized for this procedure[8], thereby eliminating the requirement for specific fluorescein-tagged antiserum. This method has further application in removing extraneous material from clinical specimens which might otherwise result in non-specific cytopathogenic responses in tissue cultures.

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CYTOLOGY

Chromosomal Constitution of Human Endometrium

It is generally assumed that there is a diploid number of chromosomes in nearly all human somatic cells. That this is not true for the endometrium was first reported by Timonen[1] in 1950. He found chromosome numbers ranging from 4 to 104 in 1,000 cells of human endometrium. Manna[2] observed similar aneuploidy in this tissue. Sachs[3], on the other hand, found no variation from the normal diploid number in either human or rat endometrium. Walker and Boothroyd[4], although they found chromosome counts ranging from 42 to 56, believed that the apparent aneuploidy was caused by the methods used, and suggested that Timonen's results were similar artefacts produced by the technique. Since all these investigators used squash preparations without previous hypotonic treatment, it is quite possible that in some cases accurate counts were not possible or that fragmentation occurred. Furthermore, the human diploid chromosome number was, at that time, believed to be 48, and this erroneous belief may also have biased the results obtained. Although new methods were introduced into human cytogenetics and the quality of the preparations was greatly improved, the question of aneuploidy in the endometrium has remained unanswered. In 1958, Tjio and Puck[5] reported that they had found only diploid and some tetraploid cells in endometrium grown in tissue culture. Takemura[6], however, found that of 109 cells only 51·4 per cent had 46 chromosomes, the chromosome counts in the others ranging from 33 to 95.

The purpose of our investigation was to establish the chromosomal pattern of the human endometrium as a part of a study concerned with reproductive disorders. The endometrium was obtained by biopsy from 28 women of child-bearing age, including seven normals, 14 with a history of sterility and seven with a history of repeated abortions. Of these, seven represented the proliferative and 21 the secretory phase of the menstrual cycle. The tissue was cultured *in vitro* after a modification of the method described by Chu and Giles[7]. In most cases primary cultures were used for chromosome preparations. Colchicine in a final concentration of 2–3 μg/ml. was added to the cultures 16 h before harvesting. The cultures were then trypsinized, and the cells were treated with 20 per cent Hanks's balanced salt solution in distilled water for 20 min, fixed, allowed to air-dry on slides, and stained with dilute Giemsa stain. All metaphase spreads of adequate technical quality were counted, and every attempt was made to exclude broken cells.

Chromosomes were counted in a total of 449 mitoses, with the counts ranging from 17 to 103 chromosomes per cell. 119 of the cells counted had the normal number of 46 chromosomes. The distribution of the counts is given in Table 1. Aneuploidy was found in every culture. No chromosomes with abnormal morphology were observed.

Table 1. Distribution of Chromosome Counts in 449 Cells of Human Endometrium

Chromosomes/Cell	No. of cells
14–18	2
19–23	2
24–28	10
29–33	14
34–38	48
39–43	117
44–48	208
49–53	20
54–58	4
59–63	5
64–68	2
69–73	3
74–78	4
79–83	4
84–88	4
89–93	—
94–98	1
99–103	1

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These results support the findings of high degrees of aneuploidy in the human endometrium. The chromosome counts are similar to those obtained by the earlier workers, all of whom used the direct squash method without tissue culture in their investigations. This indicates that the conditions of *in vitro* growth, as used in this work, do not significantly alter the chromosome complement or result in selective growth of cells with a given karyotype. Similarly, the only difference between our preparations from primary cultures and sub-cultures has been a higher mitotic rate in the latter.

It seems clear that there is aneuploidy in the human endometrium, and that aneuploidy is, indeed, normal for this particular tissue. Since aneuploidy is believed to be the result of certain abnormalities of either meiosis or mitosis, and since endometrium is a very rapidly growing tissue, it is likely that the high degree of aneuploidy observed in the endometrium reflects in some way the normal growth and development of the tissue itself. If this is true then the degree of aneuploidy would be expected to vary with the menstrual cycle. Our preliminary results suggest that this is, indeed, the case.

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SOIL SCIENCE

Influence of Ionic Phosphate Diffusion in the Soil on the Growth of *Hormidium flaccidum*

A knowledge of the ionic mobility of the essential plant nutrients in the soil is of considerable importance in determining the method and effect of particular fertilizer programmes in the eventual crop growth. Scott Russell and Newbould[1] and Schofield[2] have indicated the importance of this and the difficulties which lie behind the investigation of it. Calcium and phosphate would, for example, directly enter into exchange reactions in the soil so that the rate of ionic mobility would become limited by the degree of this interaction. Newbould[1] showed that calcium could reach a state of equilibrium in the exchange system of the soil within seven days; Fried and Shapiro[3] elaborated the factors affecting the mobility of ions by demonstrating that both the water movement in the soil, and the addition of inert material to the soil, would accelerate the diffusion of ions. Most of this work has been carried out using labelled isotopes. However, Scott Russell[1] has indicated that, even using these techniques, the knowledge of the mechanisms of diffusion was still meagre.

During an investigation of a new method of soil bioassay using algae I found[4] that *Hormidium flaccidum*, when impregnated into nutrient-deficient soils, would give, by way of its growth, indication of the distance and of the rate at which nutrient ions may diffuse in the soil.

In the method used, 10 g of dried, sieved soil with a known phosphate deficiency was added to a sterile standard bacteriological dish. An inoculum containing 5×10^5 cells/ml. of *Hormidium flaccidum* was mixed with the soil until it became saturated to a mud-like paste. To encourage algal growth throughout the soil, the soil was then incubated at 25° ± 1° C for 7 days at an even

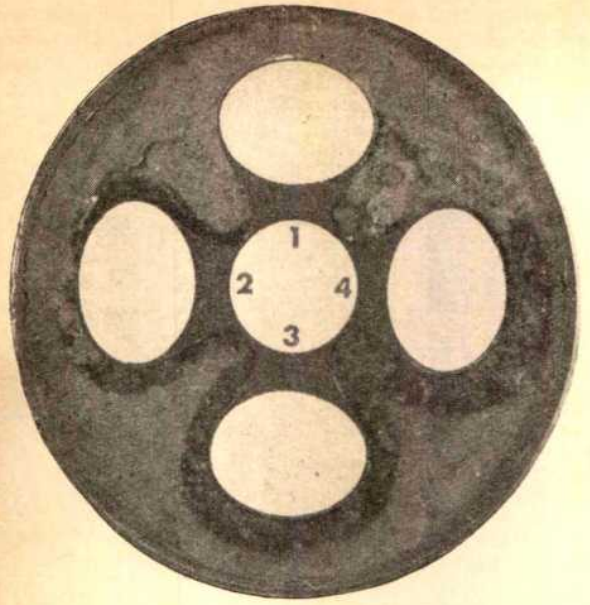


Fig. 1. Enhanced algal growth around impregnated zones on soil 35 (*R*)

illumination of 600 m-candles using 'daylight' fluorescent tubes. At the end of the 7 days, a paper rosette with four appendages cut from Whatman No. 542 double acid-washed filter paper was placed on the soil. Each of these appendages was impregnated with a different concentration of sodium dihydrogen phosphate (mg/sq. cm): zone 1, 0·00; zone 2, 0·005; zone 3, 0·0109; zone 4, 0·0219. The soil plate was now incubated for a further 6 days at 25° ± 1° C and 600 m-candles. During this period, the phosphate ions diffused out into the soil and this enhanced the algal growth around each of the treated disks (Fig. 1). At the end of incubation, the distance was measured between the edge of each of the impregnated zones and the end of the zone of enhanced algal growth. Three soil samples were used in this investigation which had been obtained from the Food and Agriculture Organization Soil Fertility Project, Teheran, Persia, through the courtesy of Dr. G. F. Hauser, the project manager. The results are recorded in Table 1.

Table 1. Distance (cm) of Phosphate Diffusion through the Soil Samples in 6 Days as shown by Enhanced Algal Growth

Zone No.:	1	2	3	4
Soil sample 26 (*SB*)	0·00	0·28	0·77	1·08
Soil sample 35 (*R*)	0·00	0·25	0·41	0·56
Soil sample 44 (*R*)	0·00	0·46	0·60	0·61
NaH_2PO_4 conc. (mg/sq.cm)	0·00	0·0051	0·0109	0·0219

In all these soils, as the concentration of phosphate in the impregnated zones increased so the diameter of enhanced algal growth increased. None of the non-impregnated zones showed any stimulated growth. The rate of phosphate diffusion varied considerably both in extent and degree for the soils used. Samples 26 (*SB*) and 35 (*R*) showed a very similar initial diffusion rate around zone 2, but after this the growth areas became far wider in the former. This would suggest that the phosphate ion may be able to diffuse more easily through this soil, due, perhaps, to the presence of a higher proportion of inert material in the soil. Soil sample 44 (*R*) initially showed a broader algal growth area, but this only increased by 0·15 cm at the highest phosphate concentration. It is probable that the phosphate diffused more quickly through this soil, due to the fact that it had a more open texture, but then became rapidly bound into the exchange complex.

The use of algae to indicate the travel of plant nutritive ions through the soil has some disadvantages. First, the algae utilize some of the ions and so reduce the overall concentration effect. Secondly, the algae only grow in light, and this limits growth to the surface, thus surface mobility can only be studied. Thirdly, the soil must be deficient in the ion which is to be studied. Despite these disadvantages, the method may provide a simple way of studying the mobility of ions in the soil under limited conditions. Work is at present in progress to investigate these conditions.

I thank Dr. G. F. Hauser for provision of soil samples.

D. R. Cullimore

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[1] Scott Russell, R., "Soil Analysis and its Relation to Plant Growth and Composition", organized by *J. Sci. Food and Agric.* at Bristol (1964); Newbould, P., *ibid.*
[2] Schofield, R. K., *Soils and Fert.*, **18**, 373 (1955).
[3] Fried, M., and Shapiro, R. E., *Ann. Rev. Plant Physiol.*, **12** 91 (1962).
[4] Cullimore, D. R., *Biologie du Sol*, 1, 10 (1964).

PSYCHOLOGY

Optical Illusions

Recent articles[1–3] in *Nature* have discussed the explanation of optical illusions and, in particular, Gregory's theory of constancy scaling. According to this theory, illusions are produced in situations where there is an inappropriate activation of a constancy scaling mechanism produced either by certain features of the two-dimensional visual pattern which normally suggests depth (primary scaling), or by apparent distance (secondary scaling). This communication presents some critical comments on this type of explanation as applied inclusively to all the optical illusions mentioned.

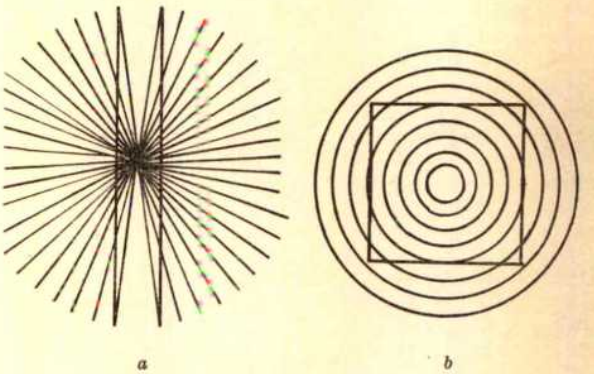


Fig. 1. Two optical illusions in which the perspective sense is the same but opposite distortions are produced

With regard to primary scaling, Gregory states that the parts of figures corresponding to distant objects are expanded and the parts corresponding to nearer objects are reduced. He also states that "the generalization seems to hold for all the visual illusions thought of in this way". In two of his own figures, however, there appears to be a discrepancy. Comparing the two illusions (Fig. 1*a* and *b*) it is clear that the perspective sense is the same in each. In *a* the centre to which the lines converge corresponds to the most distant part, as does the smallest of the concentric circles in *b*. Yet the vertical lines are distorted in opposite directions in the two cases. Of course, it would be possible, though unusual, to regard the smallest circle in *b* as the nearest part of a depth configuration, for



Fig. 2. The slight apparent bending of the horizontal line in the figure obeys the rule of the acute angles (see text). As in all such cases it is the single line which is distorted, not the repetitive stripes. This figure also contains the key configuration in Gregory's Necker cube and shows that the cube itself is unnecessary

example, as the top of a cone. To do this, however, is to make a quite arbitrary designation of the perspective sense, and the distance explanation then loses any predictive value.

A general rule which applies to all cases of figural distortion on a background field is that acute angles are perceptually enlarged (Fig. 2). This has been discussed by previous authors[4]. This rule has the merit of applying in the two cases in Fig. 1 and, in addition, since distortion is related to the acuteness of the angle of intersection, it predicts the curved distortion in these figures and the linear distortions in illusions such as the Zöllner. Unfortunately, in the majority of cases, the constancy scaling hypothesis and this angle rule for distortion predict exactly the same effects, so that it is difficult to distinguish the two experimentally. Indeed, this angular distortion is basic to Tausch's explanation of many illusions[5]. He postulates that lines forming an acute angle represent a right angle in perspective view and this cue for perspective produces the distortion, or in Gregory's terms, sets the scaling. At this extreme, Tausch's hypothesis is not useful since it is, in fact, not disprovable. Any angle can be regarded as the perspective view of a right angle, and, consequently, any pattern containing line intersections can be held to possess depth cues. The fact that the distortions observed in the illusion figures operate in the appropriate direction does not in any sense prove that they are due to the interpretation of an acute angle as a right angle. The same criticism applies to the interpretation of the bending of the line in Gregory's Necker cube figure. The bending of the line is, of course, not dependent on the presence of a Necker cube, it occurs in other configurations also (Fig. 2). All such configurations are subject to the previously mentioned ambiguities of interpretation. In further support of his depth hypothesis, Gregory reports that, in the case of the illusions where figures are distorted by a background, the illusion disappears when the affected lines are displaced in depth from the background in a stereoscope. One can produce this displacement more simply by etching the main long lines on a 'Perspex' sheet and viewing the background pattern through this at a distance (for example, at 50 cm). The Zöllner and Hering illusions are still strikingly present in such conditions. A final interesting point is that the Zöllner illusion is still present if the whole display is in three dimensions, with the pattern on the floor of a normal daylit room and the main lines extending away from the observer over a distance of 4 m. The distortion produced by the configuration thus persists even in the presence of real perceived depth. The only concrete evidence for the constancy scaling hypothesis in the context of the illusions is Gregory's experiment relating measurements of apparent distance to the size of the illusion. The correlation of 0·9 which he quotes for the Müller–Lyer is extremely convincing. In view of this, but acknowledging some of the points already mentioned, it is possible to suggest that illusions like the Müller–Lyer and Ponzo's illusion are different types of illusion from the distortion of figures on patterned fields. Such distortions remind one forcibly of some of the phenomena described by McKay[6], in particular his finding that such repetitive fields impose an orderly movement on a random noise pattern, such that the dots appear to move at right angles to the lines of the pattern. His interpretation of this and applied phenomena in terms of the satiation of certain direction analysers and of sampling of the visual field may be the correct interpretation of the distortion illusions. Certainly none of the known facts concerning these illusions appear to controvert this.

G. K. Wallace

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[1] Gregory, R. L., *Nature*, **199**, 678 (1963).
[2] Brown, L. B., and Houssiadas, L., *Nature*, **204**, 302 (1964).
[3] Gregory, R. L., *Nature*, **204**, 302 (1964).
[4] Berliner, A., and Berliner, S., *Amer. J. Psychol.*, **26**, 153 (1948).
[5] Tausch, R., and *Psychol. Forsch.*, **24**, 299 (1954).
[6] McKay, D. M., in *Sensory Communication*, edit. by Rosenblith (Wiley, New York, 1961).

I would disagree with Mr. Wallace that Figs. 1*a* and 1*b* have the same perspective sense. On my view there are gradients of expansion along each of the converging lines of 1*a*. This expansion is due to the converging perspective of these lines, so that any feature near the middle of this figure should be, and is, expanded. This gives the outward bowing of the vertical line, since the expansion is greater where these lines are near the centre of the figure. Fig. 1*b* is less obvious. If the arrangement of equally spaced circles is viewed as a luminous figure, with a single eye, to prevent the competing information that it is in fact flat, it appears flat. If, however, the spacing of the circles is non-uniform, then it will be seen as a tunnel or a cone, depending on whether the spacing increases or decreases outward from the centre. In other words, the depth of the background figure depends on the spacing of the circles, not the circles themselves.

What we must consider is why primary scaling should be set by the arcs of the circles touching, or nearly touching, the straight lines. Their distortion is compatible with the form of the Müller–Lyer illusion having curved ends. An ingoing curve produces shrinking, as does the more familiar ingoing corner. It seems that, in this Orbisson figure, the ingoing arcs produce local shrinking in the same way, giving the observed inward bowing of the superimposed lines. My hypothesis does not lose predictive power through arbitrariness, for the local depth effect can be directly measured in this, as in other, cases.

In pointing out that a line straight across a Necker cube appears bent, I realized that the whole of the Necker figure is not necessary to produce the bending. The point of using a complete Necker cube, rather than just a corner, was to show, using the reversal properties of the figure as a whole, that the bending is in the same direction whether this corner is seen in inward or outward depth. It is a crucial observation to distinguish between primary and secondary constancy. Wallace evidently fails to see the significance of this point, and his Fig. 2 does not add anything to the discussion.

With regard to illusions in binocular perception: this is very complicated, and exactly why they sometimes disappear and sometimes do not remains to be worked out. I would not agree that reference to direction analysers is helpful in explaining these illusions, for we know too little of their properties. In any event, 'satiation' can scarcely be involved, for this implies a time course; but these illusions are seen instantaneously and do not increase with prolonged viewing. The particular strength of the misplaced constancy theory is that it clearly relates well-established perceptual phenomena and does not require special or untestable assumptions.

Richard L. Gregory

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 17

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 2.30 p.m. and 5 p.m.—Colloquium on "Active Filters".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Prof. A. J. Birch: "Some Natural Anti-Fungal Agents".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5 30 p.m —Dr. D. R. Laurence: "Drug Introduction, Evaluation and Safety" (Inaugural Lecture).*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. M. W. Thring: "The Present and Future Uses of Machinery". (First of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Prof. M. G. Say: "Unusual Electrical Machines".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Major P. G. Boxall: "Island of Bliss".

Tuesday, January 18

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4.30 p.m. —Branch Annual General Meeting, followed by Mr. F. E. Warner: "The Professional Chemical Engineer and the Council of Engineering Institutions".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 5 p.m.—Symposium on "Extrusion Plant".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Human Operator Studies".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p m.—Dr. J. F. Loutit, F R.S.: "Strontium-90 and Leukaemia". (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Pressure Vessels and the Factories Act 1961".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Heavy Organic Chemicals Group, at 14 Belgrave Square, London, S W.1), at 6 p.m.—Prof. V. M. Clark "Is Organic Chemistry Dead?"

Wednesday, January 19

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p m —Mr. C. B. Winsten and Miss F Savigear. "The Use of Rooms and the Use of Land for Housing".

UNIVERSITY OF LONDON (at the Royal Free Hospital School of Medicine, 8 Hunter Street, Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. M. G. Larrabee (Johns Hopkins University) "The Metabolism of an Excised Sympathetic Ganglion at Rest and in Action".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Mr. D. Reaney: "Industrial Applications of Electroluminescence"

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m —Mr J. Croney: "Improved Radar Visibility of Small Targets in Sea Clutter (through Decorrelation by a Rapidly Rotating Antenna)".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Edmund Hirst, C B E , F.R S.: "The Role of Sugars as Energy Reserves in Nature" (Sir William Jackson Pope Memorial Lecture).

INSTITUTE OF INFORMATION SCIENTISTS (at the Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. J. B. Reed: "Spotlight on Systems of Abstracts".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (joint meeting with the Association of Clinical Biochemists, at "The Feathers", Tudor Street, London, E C 4), at 6 30 p m.—Discussion on "The Instrumental Measurement of Coloured Solutions in the Range of 200–700 mμ".

SOCIETY OF DYERS AND COLOURISTS (at the School of Pharmacy, University of London, Brunswick Square, London, W C.1), at 6.30 p.m —Sir Robert Robinson, O.M , F.R.S.: "Colour in Flowers" (Thirteenth London Lecture).*

Thursday, January 20

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. Patric Dickinson: "Computer versus Creator".*

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. O W. Nicolls, Dr. D M J. Provan, Prof. M. M. Cole and Dr. J. S. Tooms: "Geobotany and Geochemistry in Mineral Exploration in the Dugald River Area, Cloncurry District, Australia", Mr. V. E. Clifton: "The Condensation in Stages of Metal from Vertical Zinc Retorts".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m —Mr. A. D Pearson: "Lemmings—Fact and Fantasy"; Dr. C. B. Cox: "The Origin of the Modern Amphibia".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. P. M. Cohn: "Determinants".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C 2), at 5.30 p.m.—Mr. E. R. Davies, Mr. R. J. Gamble and Mr. J. B. Silleto: "Electric Process Heating for the U.K.A.E.A. Works".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. G. ff Edward: "Mycoplasma and their Role in Human Disease". (Third of fifteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation.)*

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Meeting on "The Weathering of Road Tar". Dr. E. D. Tingle and Mr. E. H. Green: "Mechanism of Changes in Tars in Road Surfacings and their Relation to Laboratory Tests": Mr. W. G. Wilman and Mr. K. D. Bartle: "Chemical Changes in Road Tars in Service".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. H. A. K. Rowlands: "Anaemia in Dar-es-Salaam and Methods for Its Investigation", followed by a Discussion.

Thursday, January 20—Friday, January 21

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Accuracy of Electronic Measurements in Internal Combustion Engine Development".

Friday, January 21

INSTITUTE OF NAVIGATION (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 5.30 p.m.—Mr. C. Powell and Mr. A. Woods: "Loran C".

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m —Prof. Saunders MacLane (University of Chicago): "Universal Elements in Algebra".*

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 5 30 p.m.—Mr. N. Burak: "Chemicals for Improving Flow Properties of Powders".

ROYAL INSTITUTION (at 21 Albermarle Street, London, W.1), at 9 p.m.— Prof. M. J. Lighthill, F.R.S.: "Waves in Fluids".

Saturday, January 22

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3 30 p.m.—Dr. W. J. Rees: "The Rocky Sea Shore".*

Monday, January 24

INSTITUTE OF ACTUARIES (at Staple Inn, High Holborn, London, W C.1), at 5 p.m.—Sir Paul Chambers, K.B E., C B., C.I.E.: "Problems in Forecasting in the Chemical Industry" (Twelfth Alfred Watson Memorial Lecture).

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m —Dr. W. N. Aldridge: "Hydrolytic Enzymes and their Inhibitors" (Further lecture on January 31).*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p m.—Dr. H Ahmed, Mr. C. E. Maloney, Mr. J. D. Sankey and Mr. A. H. Beck: "Recent Studies of Dispenser Cathodes".

INSTITUTION OF ELECTEICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5•30 p m.—Colloquium on "Gaseous Insulation".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof M. W. Thring: "Towards a Creative Society: The Philosophy of Technology". (Second of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEMONSTRATOR IN GENETICS—The Secretary to the College, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (January 20).

CHAIR OF PHYSICAL CHEMISTRY—The Registrar, The University, Sheffield (January 22).

CHAIR OF THEORETICAL CHEMISTRY—The Registrar, The University, Sheffield (January 22).

LECTURER or ASSISTANT LECTURER (preferably specialized in analysis or topology, or mathematical logic) IN PURE MATHEMATICS—The Registrar, The University, Sheffield (January 22).

LECTURER (with a degree in chemistry and good teaching experience) IN EDUCATION—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (January 25).

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 24).

LECTURERS (2) (preferably with research interests in algebra) IN PURE MATHEMATICS; and a LECTURER IN EITHER STATISTICS OR APPLIED MATHEMATICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Newcastle upon Tyne, 2 (January 28).

RESEARCH ASSISTANT (with a higher degree or equivalent experience) IN THE DEPARTMENT OF MECHANICAL ENGINEERING for a project on flame trap performance—The Secretary, University College, Gower Street, London, W.C.1 (January 30).

CHAIR OF STATISTICS—The Secretary, The University, Aberdeen (January 31).

FELLOW and TUTOR (woman) or a LECTURER (man or woman) IN PHILOSOPHY—The Principal, St. Hugh's College, Oxford (January 31).

LECTURER/ASSISTANT LECTURER IN PHYSICAL CHEMISTRY; and a RESEARCH OFFICER to assist in the operation of and development of programmes for the College's IBM 1620 tape/disc/card system—The Registrar, University College of Wales, Aberystwyth (January 31).

LECTURER IN NUCLEAR PHYSICS and a LECTURER IN THEORETICAL PHYSICS (candidates should have postgraduate experience in high energy physics or theoretical physics)—The Secretary, University of Lancaster, Bailrigg House, Lancaster (January 31).

LECTURER (with special interests in ecology) IN ZOOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (January 31).

READER IN BIOCHEMICAL ENGINEERING; and a LECTURER IN CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (February 1).

CHAIR OF THEORETICAL CHEMISTRY—The Deputy Secretary, The University, Southampton (February 4).

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER IN BOTANY at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C 1 (February 4).

LECTURER IN STATISTICS IN THE DEPARTMENT OF APPLIED STATISTICS (formerly the Unit of Biometry)—The Registrar (Room 39, O.R.B.), The University, Reading (February 5).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY—The Registrar (Room 39, O.R.B.), The University, Reading (February 5).

DEAN OF THE ROYAL MILITARY COLLEGE OF SCIENCE, Shrivenham, Wiltshire—Ministry of Defence (C.E.2 A.D.), Northumberland House, Northumberland Avenue, London, W C 2 (February 7).

CHAIR OF PSYCHIATRY at the Institute of Psychiatry—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 8).

ASSISTANT LECTURER IN MICROBIOLOGY—The Registrar, The University, Sheffield (February 12).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph D. or equivalent research experience) IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS, or related subjects—The Secretary, The University, Aberdeen (February 12).

READER IN THE PHYSICAL EXAMINATION OF MATERIALS—The Registrar, University Registry, Oxford (February 12).

CHAIR OF PURE MATHEMATICS IN THE DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (February 15)

CHAIR OF CHEMISTRY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, February 28).

CHAIR OF PHYSICS at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W.1 (Hong Kong and London, February 28).

CHIEF PHARMACIST OF THE HOSPITAL and SENIOR LECTURER IN PHARMACY TO THE MEDICAL COLLEGE (combined post)—The Dean of the Medical College, St. Bartholomew's Hospital, London, E.C 1 (February 28).

DIRECTOR (registered member of the veterinary profession and research experience relating to the improvement of health and the control of disease in horses) OF THE ANIMAL HEALTH TRUST'S EQUINE RESEARCH STATION, at Balaton Lodge, Newmarket—The General Secretary, The Animal Health Trust, 14 Ashley Place, London, S.W.1 (February 28).

LECTURER IN THE DEPARTMENT OF MATHEMATICS, University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 28).

LECTURER (specially interested in the mathematical techniques of optimization and preferably experience in these topics in an industrial context) IN THE DEPARTMENT OF MATHEMATICS and the DEPARTMENT OF SYSTEMS ENGINEERING (joint appointment)—The Secretary, The University of Lancaster, Bailrigg House, Lancaster (February 28).

READER (preferably actively engaged in research in pure mathematics) IN MATHEMATICS in the School of Physical Science in the University of Adelaide at Bedford Park, South Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, February 28).

CHAIR OF EDUCATION—The Registrar, University Senate House, Bristol, 2.

PHYSICIST or ELECTRONICS DEVELOPMENT ENGINEER (with a good knowledge of nuclear instrumentation and electronics, but previous experience of computers is not essential) to work with the recently installed PDP-7 on-line computer in the Nuclear Physics Laboratory—Prof. K. W. Allen, Nuclear Physics Laboratory, The University, 21 Banbury Road, Oxford

PHYSICISTS FOR FUNDAMENTAL STUDIES—proposed topics gas discharges, plasma diagnostics, electro-luminescence, magnetic materials, dielectrics, microwave and spectroscopic physics over all frequencies—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Cheshire, quoting Ref N/1.

SENIOR PHARMACIST (full or part-time)—The Physician Superintendent, Oakwood Hospital, Maidstone, Kent.

SENIOR RESEARCH OFFICER (biochemist) (male or female, fluent in English, with a good honours degree in biochemistry or chemistry together with at least 10 years postgraduate experience, and preferably an interest or experience in microbiology) at the National Institute for Trypanosomiasis Research, Kaduna, Northern Nigeria—The Crown Agents, M. Dept., 4 Millbank, London, S W.1, quoting Ref. M3F/54445/TA.

S R C. RESEARCH ASSISTANT to work in the field of reactions of co-ordinated ligands—Dr P. J. Staples, Department of Chemical Technology, Bradford Institute of Technology, Bradford, 7, Yorkshire.

TEMPORARY LECTURER or TEMPORARY ASSISTANT LECTURER (pure or applied mathematician) IN THE DEPARTMENT OF MATHEMATICS—The Secretary, University College, Gower Street, London, W C 1

ZOOLOGISTS (M Sc. or Ph.D with research experience, and preferably some knowledge of histology and histochemistry) to participate in experimental multi-disciplinary programmes of basic research in the field of food additive toxicology—The Director, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

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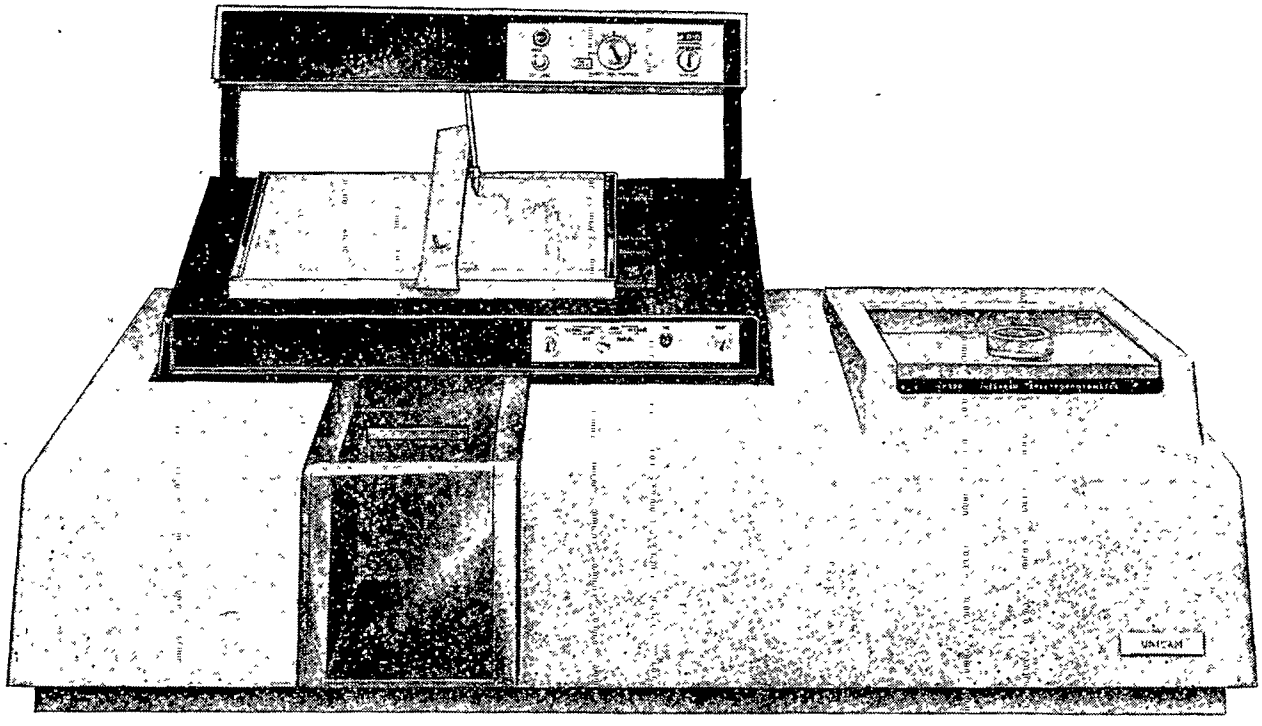
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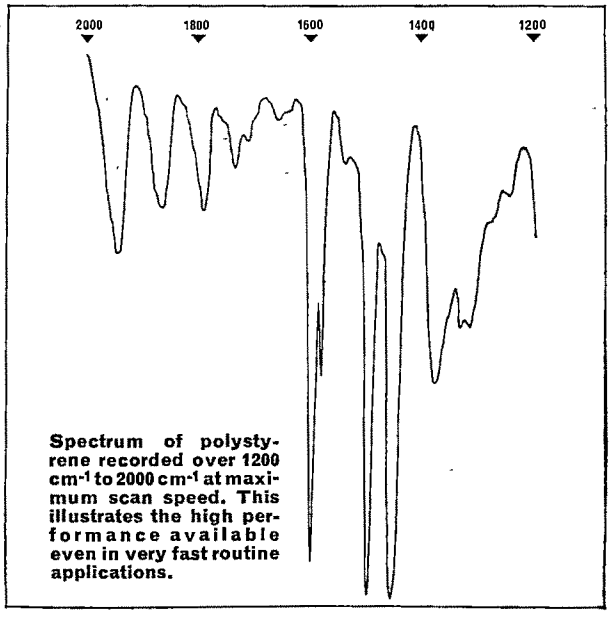


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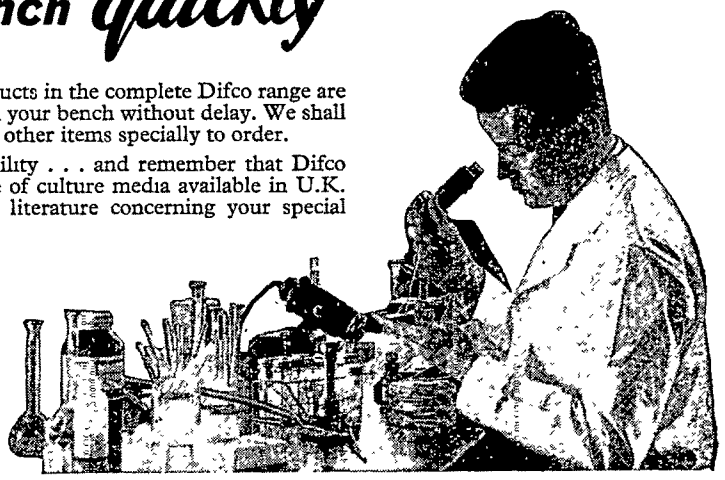
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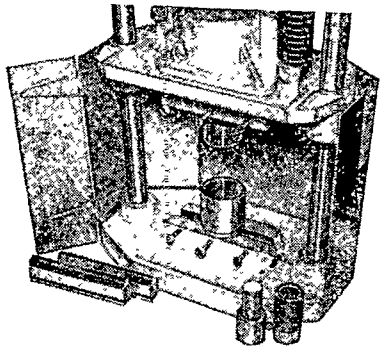
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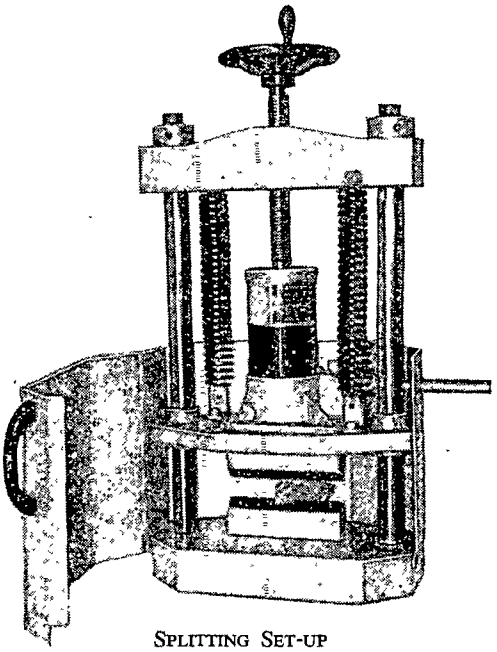
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INTENSIVE LIVESTOCK HUSBANDRY

THERE have been innumerable public complaints about intensive livestock husbandry as it has developed in Britain since the Second World War. 'Factory farming', tied as it is to 'agribusiness', is seen as inimical to the normal life of the countryside and destructive of some natural virtues necessary to our society. This view is not just an inevitable protest against progress, for this form of farming is also suspected of being economically inefficient, surviving only on subsidies and on imported grains and concentrates. Its products are mistrusted on medical grounds, and they are often found to be tasteless, or even obnoxious, and a separate industry has come into being to supply the natural, unadulterated items in their place; but at a premium. The subject clearly has strong emotional overtones, and these are most obvious when its techniques are attacked as being unnecessarily cruel, and detrimental to the welfare of the animals being exploited. These issues are interrelated, and it is to be regretted that the Brambell Committee, which has just reported *, should have been appointed to enquire only into the welfare of animals kept intensively. The roles of subsidies, of imports of feeding-stuffs, the use of food additives and drugs, and the business integration from farm to retail outlet, all require scrutiny since they determine the characteristics of intensive livestock production.

All the arguments for intensive farming are economic, and the economic argument about animal welfare is that animals looked after improperly will not grow, or produce, maximally. Economic incentives automatically ensure the well-being of the hen in her cage, or the calf in its crate. The Brambell Committee rejects this incomplete analysis, and concludes that some common practices, such as the de-beaking of poultry, the docking of pigs and the yoking or close tethering of calves, are both cruel and inefficient. The opponents of 'intensivism' therefore find their views supported in these particulars.

The critics of intensive animal husbandry have stated their case in terms of more general humanitarian arguments, too, and it is the great merit of the Brambell Committee that it has not ignored this and limited its interest only to the physical well-being of the animal. The Committee has also tried to assess the effects of present husbandry practices on the mental well-being of animals, and, in so doing, has set a most interesting and important precedent. In taking this step it considered not only the evidence from anatomy and physiology relevant to stress-causing circumstances, but also more recent researches into animal behaviour. Furthermore, it specifically states that it is "concerned with the welfare of the animal throughout what may be the duration of its life", so that what may be tolerable short-term conditions of stress, discomfort or deprivation are reviewed in their life-time context, and given as much weight, where necessary, as more acute, but transitory, suffering. Obviously our information about the mental well-being of domesticated animals is very sketchy, since many of the necessary behavioural studies have still to be made, and the Committee's conclusions with respect to such systems as battery cages for hens may seem tentative on this account. However, it also recommends, as a necessary part of a new Act to replace existing, inadequate, animal-welfare legislation, that a Statutory Farm Animals Welfare Standing Advisory Committee should be set up to advise Ministers. That is, the Brambell Committee envisages the line, which it has itself drawn, so as to include mental, as well as physical, suffering of animals, becoming more clearly defined by future behavioural researches, and it expects subsequent legislation to take account of this.

The Committee's arguments in assessing pain, stress and behavioural aspects of suffering are elegantly set out in an appendix to the report, prepared by Prof. W. H. Thorpe. This reviews recent anatomical-physiological and ethological knowledge, first in general terms and then specifically for the fowl and for the domesticated ungulates. The need for basic behavioural research is clearly indicated by this review, which emphasizes the fragmentary nature of our present knowledge about the animals most closely associated with man. Perhaps the equally pressing need for investigations on the handling of these animals without stress by developing training régimes, skilful handling procedures and new husbandry methods, is not sufficiently emphasized. But few will disagree with Prof. Thorpe's conclusion: "Whilst accepting the need for much restriction, we must draw the line at conditions which completely suppress all or nearly all the natural, instinctive urges and behaviour patterns characteristic of actions appropriate to the high degree of social organization as found in the ancestral wild species and which have been little, if at all, bred out in the process of domestication. In particular, it is clearly cruel so to restrain an animal for a large part of its life that it cannot use any of its normal locomotory behaviour patterns". Pet lovers, who have been especially vocal against 'factory farming', should pay particular attention to this humane conclusion.

Of course the Committee does not find that all intensive methods are improper, and it emphasizes that, compared with traditional practices, they frequently provide a healthier environment in which suffering from disease can be quickly and effectively controlled and where cold and damp can be minimized. Developments in intensive husbandry have brought positive achievements, which are reflected in improved yields and efficiency of food conversion. The case for retaining the battery cage is an interesting example of this, for two reasons. First, traditional 'free range' flocks are liable to chronic infection by internal parasites, are muddy and dirty, and uneconomic in the use of manpower, and to revert to them now would be a retrograde step. Secondly, the alternative intensive methods, the battery system or loose housing on deep litter, each have disadvantages: the former involves restriction of the physical movement of the birds, including such ingrained behaviour patterns as flying, ground scratching and dust bathing; the latter exposes the birds to infection (for example, coccidiosis) and to persecution by their fellows due to the inability of lower members in the peck order to escape from their social superiors. The Committee, therefore, reluctantly concludes that a modified battery system may be as good as, or even better than, loose housing from the point of view of the birds. But it then lays down more rigorous requirements of space for each bird, of birds for each cage, of

* *Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems.* Pp. v+85. (Cmnd. 2836.) (London: H.M.S.O., 1965.) 6*s.* 6*d.* net.

details of construction and siting of cages, and of ventilation and illumination of poultry houses, than have been usual in the industry. It is recognized that these changes may result in increased production costs and in higher prices to the consumer.

A great deal of attention is directed to the space needed by intensively kept animals, as well as to the types of flooring provided for them to stand or lie on and the temperatures and humidities they must tolerate. It is obvious that an ecology of farm animals is required to refine the approximate standards now laid down. Such ecological studies, like further work on the behaviour of farm animals, would be expected to improve the efficiency, as well as the humanity, of intensive production techniques. It is important that they should be initiated immediately, as intensive farming must grow to meet ever-rising food requirements.

One further point deserves comment. The Brambell Committee is greatly impressed (and rightly so) by the importance of the standard of management and stockmanship for the welfare of livestock. The amount of equipment and the numbers of animals in the care of one individual (10,000 or more laying birds in a modern, environment controlled house, for example) sets requirements of knowledge, skill and dexterity of a different order from those traditional in agriculture. At present, agricultural education does not match this requirement, and agricultural wage structure does not provide the rewards concomitant with acquisition of suitable qualifications. The Committee is right, therefore, to emphasize both needs. A more humane intensive farming may attract the necessary calibre of stockman: if the Brambell Committee's recommendations are put into effect he will also stand less chance of being degraded by some of the practices of his trade.

There have been fewer comments on this Committee's report than might have been expected, and this is probably a measure of its skill at striking a balance between the practical and the desirable. It is interesting that they have met the six points of Mrs. Harrison's charter for animal welfare†, either by calling for a modification of the present techniques (as with battery cages, the methods of 'white' veal production or the use of slatted floors), or by agreeing that they should be prohibited (as with the use of deficiency diets, permanent tethering and dim lighting). It is disturbing that research has often been used to support these practices, which are now found to be wrong. If the care which the Brambell Committee has given to its report is also given to framing the terms of reference of the much-needed future research, this error may be avoided. More research will also show whether the Committee is correct in its fundamental assumptions about the feelings and emotions of animals. Meanwhile, no humane society can do other than accept its thesis.

† Harrison, Ruth, *Animal Machines* (Vincent Stuart, London, 1964).

DIAMOND

Physical Properties of Diamond

Edited by R. Berman. Pp. xii+443. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 75*s*. net.

PHYSICAL Properties of Diamond places diamond firmly in the centre of modern solid-state physics.

More than one civilization has had its interest and imagination stirred by diamond in diverse ways. In the framework of the physical metrical approach, which characterizes our own, it is striking to find that a substance which at first sight seems uniquely simple, from a crystallographic point of view, in fact exhibits a rich complexity of properties. This receives comment by E. W. J. Mitchell concerning radiation damage in diamond: "What has been surprising, and scientifically stimulating, has been the number of effects which have a fundamental significance in solid-state physics generally. These effects are most readily seen in diamond, being a covalent structure of high Debye temperature (θ_D). In this connexion matters of particular interest include:

(*a*) The structure in absorption and emission spectra and its interpretation in terms of zero and multi-phonon interactions.

(*b*) The sharp infra-red lines associated with the acceptor lines in semiconducting diamond.

(*c*) The occurrence in the presence of defects of the normally forbidden 1-phonon vibrational absorption."

Corresponding statements might be made about many of the other fourteen chapters in this book, which constitute a unique collection of monographs by scientists working personally in the very diverse fields they discuss. The fact that they all refer to the same remarkable solid pulls the book together in a striking and suggestive way. Quite often substantial portions of the researches described in the text have not hitherto been published elsewhere. Sometimes they must even be described as obviously unfinished. As a result, a fresh and intensive discussion of many important problems in the general physics of the solid state is presented in this book, focused in terms of the properties of diamond. Furthermore, investigations of the hardness, of surface grinding and surface imperfections, and of the anisotropy of various physical properties, would, at present, be difficult to match for many other crystals. In these respects, the book should serve as a valuable study text for advanced honours and postgraduate courses on the solid state.

Various physical properties are discussed under the headings: X-ray diffraction studies on diamond and some related materials, X-ray topography, transmission electron microscopy, optical studies, ring cracks on diamond surfaces, deformation, friction and wear, hardness and wear during grinding and polishing, electronic structure, paramagnetic resonance, optical properties of natural diamonds, semiconducting diamond, the counting properties of diamonds under ionizing radiations, thermal properties, and radiation damage. These will naturally attract varied attention; possibly also some controversy, partly because their relation to cognate problems in other crystals is incompletely developed.

Such relationships are not always brought out sufficiently in the text, perhaps because of its poly-monograph structure. In fact, an additional chapter dealing with correlations in the widest sense between diamond and diamond-like assemblies of other atoms could have been very informative. Various authors discuss from different points of view substitutional inclusions of nitrogen atoms, the concentration of which may rise above 0·2 atoms per cent in certain types of diamond. But little or no mention is made of the topographical segregation of any other impurity atoms in diamond. This general problem would have been further illuminated by brief reference to segregation phenomena of hetero-atoms in other types of crystals, in which partly ordered defects may occur and may present suggestive analogies.

Modern studies of the physics of the solid state merge inextricably with much chemical physics. Tantalizing comments are to be found, here and there in the text, on various oxidative techniques of etching of diamond surfaces. Though these are used to reveal various defects of structure, there is little or no discussion of more chemical aspects of the attack, the details of which may actually help to throw light on the physical problems

envisaged. Thus much closer scrutiny seems warranted of the structure and location of the "free surface carbon" formed in the course of applying certain oxidation techniques to diamond (p. 120). Formation mechanisms of such 'carbon' may conceivably be related to basic features of the kinetics of migration of carbon atoms in diamond crystals, as well as in dislocation bundles, or in the gas/solid interface. Etching of diamond, by media which act as solvents for carbon, and not merely as oxidizing agents, could also yield complementary information about a number of structural problems.

As a development of this theme, it would clearly be useful to have more general information about the kinetics of graphitization of diamond, including experiments under conditions where the role of oxygen at the interfaces can be definitely excluded. Similarly, in this book discussion of thermal annealing of defects, whether natural or produced by irradiation, scarcely pays sufficiently detailed attention to kinetic aspects.

Since the inception, in 1949, of co-operative researches published in this book, technological mastery has been achieved of production of synthetic diamond. In view of this success, chapters on the chemical physics of its nucleation and growth from solvents or from graphite now seem definitely called for. But, despite various insufficiencies of chemical physics, the book has many admirable features. It is a worthy tribute to the originators of this programme of research on diamond, Sir Ernest Oppenheimer and Sir Francis Simon. The editor, Dr. R. Berman, also contributes a lucid chapter on the thermodynamic properties of diamond and on its thermal conductivity. This completes a fine task, in bringing a mettlesome team of contributors to such an effective goal.

A. R. Ubbelohde

UNDERSTANDING SOLIDS

Principles of the Theory of Solids

By Prof. J. M. Ziman. Pp. xiii + 360. (Cambridge: At the University Press, 1964.) 45*s*. net; 8.50 dollars.

Principles of the Theory of Solids is an admirable book. Though it will certainly reach a much wider audience, it is designed specifically as a text-book for graduate students in solid-state physics, and it is ideally suited for that purpose. As Prof. Ziman reminds us in his preface, the aim of a text-book should be to explain ideas, rather than to catalogue facts; by restricting himself to a few of the basic themes of solid-state physics—broadly speaking, electrons, phonons, and their interactions—he has been able to explore the ideas in considerable depth, explain them with admirable clarity, and bring out in full the underlying unity of the topic.

The reader is assumed to be familiar with the basic techniques of quantum mechanics, including time-dependent perturbation theory and scattering theory, and to have some idea of what creation and annihilation operators are; he is also assumed to know something of the basic experimental facts of solid-state physics. The book is exclusively concerned with theoretical concepts, rather than experimental facts: one will look in vain here for tables of Debye temperatures or electronic specific heats, and the copious figures—one hundred and seventy-four of them—are all designed to illustrate the theoretical argument; not one of them shows an experimental point. But the theoretical argument is beautifully developed, and the chapter on electron states, in particular, is a model of exposition. The approach is thoroughly contemporary throughout, so that, for example, electron–electron interactions are discussed entirely in terms of the dielectric function. No attempt is made to deal with Green's function techniques, bubble diagrams, and the other sophisticated abstractions of modern theoretical physics, but outside this highly specialized area most of the ideas now used in electron–phonon physics are discussed, analysed, and explained, so that after studying the book a student should be in a position to comprehend much of the present-day literature in this field.

Graduate text-books nowadays commonly include a set of problems at the end of each chapter, for the student to try his strength on. In the present text, there seem at first glance to be no problems, but the reader will rapidly find that the book, in fact, abounds in them: they are embedded in the text on almost every page. The author's technique is to sketch in lightly the steps in each theoretical argument, so that the line of thought is not too much obscured by algebraic detail, but to leave the reader enough clues to fill in the details for himself if he will. If he does, he will learn a great deal more about the subject.

He will also, it must be added, discover a good many obscurities and a good many minor errors in the text. Because the exposition is otherwise so excellent, these lapses may puzzle the unassisted student more than they would in a lesser book, and it may be worth giving a few examples. They are particularly frequent in the chapter on optical properties, where it is not always clear whether the author is talking about metals, or insulators, or both. If the reader has been taught to distinguish the ideas behind the Lorentz and Onsager treatments of the internal field problem, he may be a little puzzled by Fig. 114; he may or may not be puzzled by equation (8.12) and by the (spurious) argument leading to equation (8.80), but he should be. He will certainly be puzzled by equation (8.110), which—the author claims—reproduces (8.7). To see that this is so, the reader has first to detect and correct an error in sign in (8.110), and then to realize that N^2 in (8.7) becomes ε in (8.110), and ε in (8.7) becomes 1 in (8.110). All this is rather a lot to ask of the struggling graduate student. He is less likely to be puzzled by Fig. 91(*b*), which is clearly wrong.

The structural unity of the book is weakened, perhaps inevitably, by the penultimate chapter on magnetism. This discusses a whole miscellany of topics, ranging from the elementary Weiss theory of ferromagnetism through the Ising problem to a summary of the theory of ferromagnetic and antiferromagnetic spin waves. The latter topic is closest in spirit to the rest of the book, and it also serves to illustrate the use of creation and annihilation operators, in preparation for the excellent final chapter on superconductivity. But unhappily the treatment of spin waves is rather too brief to be useful, and just at this point the physical ideas, where they are most needed, tend to become submerged in the algebra: the graduate student will find this section rather heavy going.

Despite these minor criticisms, it remains true that this is an admirable book. Indeed, it scarcely needs my commendation: it is already being widely used as a graduate text on both sides of the Atlantic. It seems likely that a second edition will soon be called for, and we may hope that it will be even better than the first.

R. G. Chambers

A BIOLOGIST'S GUIDE TO TRANSISTORS AND OTHER SEMICONDUCTORS

Instrumentation with Semiconductors for Medical Researchers

By Prof. Clinton C. Brown, in consultation with George N. Webb. (A Monograph in The Bannerstone Division of American Lectures in Objective Psychiatry.) Pp. xvii + 254. (Springfield, Ill.: Charles C. Thomas, 1964.) 10.50 dollars.

A LARGE proportion of handbooks which aim to provide information over a complete field are either written by a panel of authors with carefully defined terms of reference, or, if by a single author, represent a course of

lectures which over the years has been modified and polished until the author feels it represents the perfect approach to his subject. These methods help to ensure completeness and accuracy, but too often fail to produce a readable book. In this book Clinton Brown and George Webb have followed a different course. Dr. Brown is clearly a man with wide and extensive knowledge of his subject—both biologically and electronically—as well as a rare gift for putting across his ideas. It would almost be worth having this book for the first chapter alone, for there can be few biologists who would not find something of value in this discourse on the use—and misuse—of instrumentation in biology to-day and in the foreseeable future. The whole chapter is eminently quotable, but one short passage must suffice: "Reduced power, space and weight are obvious advantages of the transistorized instrument but their greatest importance has been in the invention of new devices. The descendants of the transistor; the four layer diode, semiconductor strain element, photosensitive device, controlled rectifier, Esaki diode are such radical departures from the original transistor as to resemble new species. Commercial applications are available to-day for functions formerly considered impossible or impractical". The quotation is significant: one suspects that Mr. Webb (the electronic collaborator) made it a point of honour to see that no new device of bioelectronic value was omitted, and as the book proceeds the distinctions between, for example, different types of four layer diodes is clearly explained with ample indication of typical circuit configurations.

One inevitable flaw in a book which has virtually been written by one man—and almost certainly over a short period—is that a number of inaccuracies and inconsistencies have slipped in. Misprints such as (p. 43) $X_L = 2$ *pi fL* are not misleading, but on this side of the Atlantic some readers may be caught out by the statement that the current in a hundred-watt lamp is about one amp (p. 35). (No voltage is stated.) More misleading is the implication that capacitors in series are additive (Colpitts oscillator, p. 157), while the statement that transformer coupling is unsatisfactory "at the low power and low frequencies encountered" (p. 121) surely requires further comment since no indication is given that two quite separate problems are involved. Again in one or two places the legends on the diagrams do not agree with the text, but in one place (p. 75) both diagram and text are wrong: "In (Fig. 5: 14*B*) the signal increases smoothly from zero to some value where it drops to zero. The differential of this signal is a square wave".

It is difficult to comment on some numerical data without knowing which devices the author has in mind, but he describes a CdS cell illuminated at 400 c/s by a neon lamp (p. 140) as a form of chopper for a d.c. amplifier. I feel that this could be misleading as, while high-speed CdSe cells are available, the vast majority of photosensitive resistors would not follow this frequency satisfactorily. On the other hand, to say (p. 102) that the reverse current in a diode is "less than 5 per cent" seems very cautious, even by germanium standards. A typical silicon leakage figure would be 0·1 μamp for a diode with a maximum forward current of 100 m.amp (Mullard *OA* 200) while a forward/reverse ratio of a thousand is now common even among germanium devices.

Although, rather surprisingly, abbreviations Ω, KΩ, MΩ, A, mA, μA, nA, etc., are not explained, their omission is the more conspicuous since the development of symbolism and ideas is elsewhere excellent: the chapter on signal conditioning systems and on digital logic being particularly good.

To sum up: this book is well written, amply and skilfully illustrated and, despite numerous minor lapses, should be of great value both to bioengineers and to biologists. It does not describe any complete apparatus—that is not its object—but it does describe clearly and in simple terms the working of virtually every semiconductor device available to-day and discusses ways in which apparatus built around such devices can be profitably used in a biological laboratory.

J. R. B. Greer

SOVIET CHEMISTRY

Chemistry in the Soviet Union

By Prof. John Turkevich. Pp. ix + 566. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1965.) 93*s*.

At a meeting of the Russian Chemical Society on March 6, 1869, the periodic law was first presented to the world by Menshutkin, acting for Mendeleev, who was ill. Mendeleev later became the 'best-known Russian chemist'. In conformity with Prof. Turkevich's treatment of other topics, he should have mentioned Newlands of London, who in all essential detail presented the same concept in 1864; and these few years were critical in the matter of atomic weights. Ignored is the letter in *Chemical News* of 1882 (p. 278) in which Newlands remarks on the reception of the Davy Medal by Mendeleev "for his discovery of the periodic relation", and draws attention to his own publications of 1864 and 1865. Ignored, too, is the letter by Mendeleev himself in *Chemical News* of 1881 (p. 15), in which he admits "it is possible that Newlands has prior to me enunciated something similar to the periodic law".

Those of us brought up in the classical days will recall the full-sounding names of Lomonosov (1711–1765), Tschitschibaban and Zelinski. Klaus contributed to the knowledge of the chemistry of osmium and iridium, and in 1844 discovered ruthenium, named after the Latin name for Russia. P. Walden worked mainly on non-aqueous electrolytic solutions; but is best known for his isolated discovery of the optical inversion.

This book is mainly about Russian chemists and educational (research) institutes. The history of chemistry in Russia from the fifteenth to the middle of the nineteenth centuries is outlined in six pages, and, in 12 pages, prominent chemists in the second half of the nineteenth century are mentioned. We read that Butlerov (1826–1886) is given credit by Western authorities for introducing the expression 'structure' into organic chemistry; but the Soviet chemists now claim a greater role for him as the one who formulated the structural theory of organic chemistry. "The solution of this problem could only be the lot of a scientist that is not only of outstanding talent but also free from the enumerated (p. 36) shortcomings which were inherent in the majority of important Western European chemists." "The honour of creating a new theory which exerted a revolutionary influence on further development of organic chemistry, fell to a Russian chemist, A. M. Butlerov." "The standard text-books in the West do not mention his eminence in this role." Markovnikov (1838–1904), a student of Butlerov, was a leader of the Russian scientific circles of his period.

In the next 29 pages, chemistry in Russia in the nineteenth and twentieth centuries is summarized under the usual headings, such as organic, colloid, analytical chemistry; and similar summaries on Soviet chemistry between the two World Wars are given in 91 pages.

For those who have maturity and time for quiet thoughtful reading at home, these 140 pages can provide intensive pleasure, and a grasp of the activities of Russian chemists not otherwise so readily achieved. There are 30 helpful pages on the activities of chemical research establishments in the U.S.S.R., but the remaining 393 pages could well have been relegated to a library handbook; for these show a list of names of authors, and the titles of dissertations in chemistry at the Soviet universities, as well as a similar list of recent publications of members of the U.S.S.R. Academy of Sciences.

W. Gerrard

EPOXIDES AND THEIR DERIVATIVES

Epoxides and Their Derivatives

By M. S. Malinovskii. Translated from the Russian. Pp. xvii+493. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 144*s*.

THERE are now extant a number of reviews and monographs covering the chemistry of epoxides and their derivatives, and *Epoxides and Their Derivatives* is a commendable effort to provide chemists with a source-book wherein they might find the preparation and reactions of epoxides summarized, together with relevant references to the literature. The obvious limitation is that the literature here covered is only that up to 1958: the book was published in Moscow in 1961, and is now made available in English through the Israel Program for Scientific Translations.

The monograph is conveniently divided into three parts. The first part deals with the structure and reactivity of epoxides, their classification and nomenclature. The second covers the preparation of epoxides, and the third is an extensive review of their reactions to include their hydration, hydrogenation, isomerization, and polymerization, and reactions with alcohols, aldehydes, ketones, amines, organo-metallic compounds, mercaptans and nitrogen heterocycles. Part of the usefulness of the last two sections is that often details of the experimental procedures used are reprinted.

The book concludes with tables of known epoxides in order of their molecular formulae, giving their physical properties. The index is very good, and is not the least useful part of the monograph.

It has to be recognized that the origin of this work is a Russian one, and at times a nationalistic concern is apparent. The introduction includes a reference to Khrushchev's address to the twenty-second Congress of the Communist Party of the U.S.S.R. setting out the aims of Soviet chemical industry, and very frequently the authors attributed as having first made reference to a compound are Russian ones. There are sweeping statements such as "the preparation of ethylene oxide from ethylene chlorohydrin has been thoroughly explained in Soviet literature" (p. 69), and it appears that "Zimakov and Petrov (in 1946) were the first to explain the reactivity of ethylene oxide according to modern concepts" (p. 13): this in spite of the work of Bronsted and the Kilpatricks in 1929. Against this background it looks like wilful misinterpretation on page 113 when the conclusions of Bronsted about his own work are discussed. One of the weaknesses of the book is the lack of a thorough review of the mechanisms of the ring-opening of epoxides.

It is thus politically unfortunate that, in translation, American spellings have been adopted. It must be inevitable that certain phrases in translation appear strange to the reader, particularly when the phrase originally had to be translated into Russian. An example is the determination of bond-lengths by electron-diffraction referred to as investigation of electronograms (p. 3). But these, and other small textual points, should not be allowed to detract from the fact that this is a very useful survey which should find its place among the reference works of most research libraries.

J. Biggs

ASPECTS OF ENZYMES

An Introduction to the Study of Enzymes

By Dr. H. Gutfreund. Pp. x+335. (Oxford: Blackwell Scientific Publications, 1965.) 60*s*. net.

An Introduction to the Study of Enzymes is intended "to give an account of theoretical and experimental methods for the study of enzymes" and "to introduce the reader to the basic principles of the concepts of enzyme kinetics, to methods for kinetic investigations and to their interpretation". The author admits a bias towards the purely kinetic analysis of the behaviour of enzymes. In a sense, biochemistry is the study of enzymes, and perhaps a more accurate title for the book would be *An Introduction to the Study of Enzyme Mechanisms*. Obviously all aspects of enzymology cannot be embraced even superficially in a single short book, and the isolation of enzymes, for example, is not considered.

Dr. Gutfreund succeeds very well in his aim of giving a readable, introductory account to whet the reader's appetite, especially in those sections of the book which deal with experimental methods and the interpretation of results. He repeatedly points to limitations in the treatment and advises the reader to turn to more detailed sources. The selection of some 500 references includes some papers published in 1964, and an adequate general index is supplemented by a useful index of the enzymes discussed.

Minor blemishes include the occasional use of DPN as well as NAD, and the absence of page references when figures and tables in other parts of the book are under discussion. In the introductory chapter, which includes a helpful outline of applications of digital and analogue computors in enzyme kinetics, a steady-state definition of the Michaelis constant in the original Michaelis–Menten formulation is confusing. A suggestion that prosthetic groups as well as coenzymes should be treated as substrates is corrected later in the book, but then prosthetic groups are said to be present in 1 : 1 molar ratio to the enzyme.

The following two chapters on formal enzyme kinetics make up one-third of the book. The somewhat diffuse style of the author is perhaps less suited to these theoretical treatments than to the later, more descriptive parts of the book, although asides such as "statistical methods can never be a substitute for adequate experimental data" are illuminating. Unfortunately for the student, these chapters contain more than an acceptable number of errors and misprints in the kinetic expressions, and vagaries of punctuation and syntax largely absent from the rest of the book. Generally, adequate coverage of the usual topics and algebra are provided, together with useful sections on solvent effects, diffusion-controlled reactions and activation by metals. I found the treatment of inhibition confusing, and there seemed to be misconceptions about non-competitive inhibition. Steady-state and equilibrium treatments of a non-competitive mechanism are not equivalent, as they are said to be here, and the possibility of inferring quasi-equilibrium conditions is based on this fact.

Chapter 4 is an admirable account of special techniques for following enzyme reactions, and includes rapid reaction techniques, spectrophotometry and fluorometry, hydrogen ion, oxygen and carbon dioxide concentration measurements, and calorimetry. This lucid survey, and the following chapter on the protein chemistry of enzymes and the active site, can be recommended to both undergraduates and advanced students.

Chapter 6 brings together various applications of isotopes in a satisfactory manner, and includes, with many examples from the literature, the interpretation of isotope rate effects, stereospecificity studies and exchange reactions. In relation to this and the following two chapters, in which various types of evidence of mechanism are reviewed for a good selection of group-transfer reactions, it seems a pity that a closer correlation was not made between isotope exchange, kinetic and other means of distinguishing between the single and double displacement mechanisms, although this would require a more detailed account of the kinetics of two-substrate reactions than is given in Chapter 1. The book closes with some general remarks on the nature of enzyme action, and brief indications of some mechanisms of metabolic control.

With some reservations about the earlier chapters, this book can be recommended to students and research

workers as a personal, enjoyable and stimulating survey of the study of enzyme mechanisms, which demands little prior knowledge of the subject and is reasonable in price.

K. Dalziel

INTRACELLULAR MEMBRANES

Intracellular Membraneous Structure

(Proceedings of the 1st International Symposium for Cellular Chemistry.) Edited by S. Seno and E. V. Cowdry. Pp. xix+588. (Okayama, Japan: Japan Society for Cell Biology, 1965.) 14 dollars.

SCIENTIFIC conferences and symposia are nowadays frequently followed by publication of an edited version of the proceedings in elaborate book-form. The question arises whether these volumes are a worth-while investment bearing in mind the cost, inevitable time lag in publication, and the probability that the contributors will in any case report their observations elsewhere. In spite of these material drawbacks, the bound volume can certainly, sometimes, be justified, either through well-judged timing of the symposium or perhaps the authoritative nature of particular contributions. On both of these counts there is much to recommend the special Katsunuma Memorial issue from the Japan Society for Cell Biology, entitled *Intracellular Membraneous Structure*.

The meeting was held at Ohtsu, during March 1963, and it is clear that from a technical point of view much of the work presented reached a very high standard. The invited speakers were concerned primarily with ultrastructural aspects of cell physiology and pathology; and the papers are divided into six sections covering various categories of cell membranes. Original references, and a transcript of the discussion that followed the verbal presentations, are included. Altogether there are 43 papers (all in English) mostly emanating from laboratories in Japan and the United States. Numerous first-rate and well-reproduced electron micrographs are included as illustrations.

In the opening section, "Endoplasmic Reticulum", D. W. Fawcett describes the varying patterns of granular and agranular reticulum in cells of the mammalian pancreas, liver and testis, in different functional states. There is discussion of the possible role of the endoplasmic reticulum and the Golgi apparatus in the formation of secretory products. Several later papers touch on this question, and serve to illustrate clearly one of the difficulties most commonly met with in biological electron microscopy; namely, the interpretation of profiles that seem to suggest a dynamic inter-relation of cell components. Interesting papers by E. Yamada on membrane-limited elements in retinal cells of the cat, and by Y. Miura and H. Itoh on fibroin-synthesizing vesicles in silk-glands, are included in this section. Next, under "Mitochondria and Related Structure", two papers from D. E. Green and associates and another from F. S. Sjöstrand give an instructive view of present-day thought (much of it rather speculative) on ultrastructure, chemistry and function in mitochondrial membranes. Papers on the morphology of chloroplasts also appear in this chapter.

All microscopists who have ever been fascinated and perplexed by the Golgi controversy, dating from 1898 when Dr. Golgi reported the "apparato reticulare interno" in certain neurones, will acknowledge gratefully the contribution of electron microscopy towards recent clarification of this problem. Out of nine papers on the subject in this symposium two of special interest are by G. C. Hirsch, who strives convincingly to correlate the earlier images, based on methods of light microscopy, with the complex patterns of membranous lamellae and vesicles now familiar to electron microscopists. Further consideration of the role of the Golgi apparatus in secretory cells is presented by L. Herman and associates in a technically most impressive contribution, and the recent extension of cytochemical methods to the ultrastructural level is elegantly demonstrated by A. B. Novikoff in a comprehensive account of enzymatic activities and possible functional inter-relation of the various intracellular membrane systems. Notable in later sections of the book are an assessment by C. A. Baud of possible morphological pathways for nucleo-cytoplasmic exchange of materials; and reviews by J. D. Robertson on the present status of the unit membrane concept, and by E. V. Cowdry on the intracellular distribution of water. Finally, there are papers on the outer cell membrane itself, and mechanisms of transportation across it; these include reports on synaptic morphology, on the absorption of fat by cells of the intestinal epithelium, and on various examples of endocytotic activity.

In spite of its rather high price, this book can be recommended as a well-produced, up-to-date and potentially useful acquisition for the bookshelves of cell biologists.

J. A. Armstrong

A LAYMAN'S LOOK AT BACTERIA

Focus on Bacteria

By Emmy Klieneberger-Nobel. With a chapter on the Mycoplasmataceae by Ruth M. Lemcke. Pp. vii+145. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 32*s*. 6*d*.

IN several ways, *Focus on Bacteria* is an odd little book. The jacket flap says it "is not intended for the specialist but for the lay person with keen scientific interest". The author's foreword states: "The purpose of this book is first and foremost to show that nature has a quality of beauty even in her smallest manifestations".

The first chapter is a brief historical sketch of the development of bacteriology as a science, covering the fundamental discoveries of the father of microbiology, Antony van Leeuwenhoek, in the seventeenth century and highlighting the great names of the nineteenth century.

Chapter 2 outlines in three pages the methods of microscopical demonstration available to the microbiologist; rather too briefly in view of the fact that the book is copiously illustrated with photomicrographs and electron micrographs and the lay reader would probably like to know a little more about how they were obtained. This is followed by a short chapter (five pages) on "Diagnostic Bacteriology".

The bulk of the text is presented in Chapter 4 under the title "Bacterial Anatomy" and the various anatomical features—flagella, capsules, cell wall, internal composition and endospores—are dealt with under sub-headings.

Chapter 5 takes a brief look at bacteriophages and Chapter 6 at bacterial genetics. Chapter 7 has three sub-headings, "The Myxobacteria", "The Streptomycetes" and "Therapeutic Substances". Chapter 8 deals with the "Mycoplasmataceae". There follows a 14-page glossary, a page of references and a subject index.

There are about 60 photographic plates and about 20 line drawings. In general, the plates are of a very high standard, some of them spectacular (even though they have been printed elsewhere). It is a good thing, too, that the references to the papers from which the plates have been taken have been retained.

One of the problems about this book seems to have been an uncertainty about the level at which it should be maintained. It is written in a straightforward, sometimes over-simple way, yet the list of references includes some advanced texts that could scarcely be recommended reading for the layman. Over-simplification has also led to some odd statements, for example: "Two points closer together than half the wavelength of white light cannot be seen as

two different points; half the wavelength of white light is 0·00025 mm . . ."; and "the order of the Actinomycetaceae comprises two families, namely Actinomycetes and Streptomycetes". The experiment quoted to demonstrate that "a streptomycin-sensitive population produces as a rule a few resistant organisms, though it has never been in contact with streptomycin before" is not proof of this. Odd, too, that the chapter on the pleuropneumonia-like organisms has been contributed by Dr. Ruth Lemcke when the jacket quotes, in regard to Dr. Klieneberger-Nobel's book on these organisms, a reviewer who states: "Dr. Klieneberger-Nobel is our greatest authority on this group". The table of contents is incomplete, and surely it was unnecessary to include 'descriptions' of oxygen, hydrogen and nitrogen in the glossary.

L. B. QUESNEL

BACKGROUND TO MALARIA

Migrants and Malaria

By R. Mansell Prothero. Pp. x+142. (London: Longmans, Green and Co., Ltd., 1965.) 15*s*. net.

HERE is something new by a geographer for the epidemiologist, which the latter can study with both interest and profit, whether his concern be in malaria or in any other communicable diseases. It is not new that there should be some overlap in the fields of interest of the two, but the overlap has in the past been usually approached from the medical side, with an effort to explain known epidemiological happenings and timings on the basis of geographical factors. In *Migrants and Malaria* the approach is reversed: the geographer explains one aspect of his own subject, developing it in its own right and then examining the effect which it might have on subsequent epidemiology.

On the basis of an original interest first expanded by contact with malaria prevention in Northern Nigeria, Dr. Prothero sets out a general statement of the nature of migration in Africa, and elaborates it by special studies of the forms which it takes in the Republic of Sudan, the Horn of Africa, east and south Central Africa, West Africa and Morocco. He prefaces this with an explanation of the nature of malaria and the means by which it is prevented, and in each case studied he refers to some malaria eradication project and the ways in which migration has affected its progress.

The section on migrants classifies and describes the different types of movement, pastoral, nomadic, transhumance (movement at particular times of the year from one grazing area to another by relatively well-defined routes), pilgrimage and local population movements. Each of these aspects is not only lucidly described, but is also well illustrated by maps sufficient to give a very clear impression to the geographical novice. Some types of migration are further analysed in the local studies with special reference to their effects on malaria eradication, and even the experienced malariologist may be brought up suddenly by the realization that 85 per cent of the population of the Horn of Africa is nomadic. He may be made thereby to think hard about possible consequences: reintroduction of malaria into places from which it may have been cleared; the difficulty of spraying insecticides in houses or shelters which are in fact evanescent; and the virtual impossibility of carrying out a case-finding and treatment mechanism among populations which move overnight. The Horn of Africa is an extreme case, but the author brings out the fact that, by the standards of Europe, migration in all African countries constitutes an extreme case and must be taken into account in the special context of malaria eradication and in all epidemiological studies.

The book is well written and, though a paper-back, is well produced. It is said to be a forerunner of a series to be written by geographers and dealing essentially with their activities. If the other members of the series can keep up the standard set by Dr. Prothero, it will be a significant contribution to the understanding of geographers and of their work by followers of other disciplines.

G. MACDONALD

LABORATORY DESIGN

Chemistry and Biology Laboratories

Design-Construction-Equipment. By W. Schramm. Translated by Mrs. M. Jansen. Pp. x+255. (London: Oxford and New York: Pergamon Press, 1965.) 105*s*.

UNDOUBTEDLY there is a shortage of books on laboratory design, construction and equipment; any addition to their number, providing ideas and information which may assist in improving laboratories in these respects, is therefore welcome. *Chemistry and Biology Laboratories* is an international survey, although quite naturally Dr. W. Schramm has a predominant interest in German laboratories.

In general the translation of the text seems satisfactory; the odd strange expression does, however, creep in (such as service 'spline' by which is probably meant service duct). The use of German proprietary names for materials has also not been entirely avoided. A misleading statement is "to avoid the stroboscopic effects of fluorescent lighting the benches should be equipped with alternating current outlets".

Many interesting and highly specialized designs for benches and fume cupboards are described, including the use of movable benches with service ducts available on the walls or as floor standing columns. There is also a brief treatment of the problems and solutions of air balance difficulties in laboratories with fume cupboards. Other matters discussed include the layouts suitable for various types of laboratory and the arrangements for services. Many examples are given both of individual laboratories and specially designed buildings.

The book may well be criticized on various grounds. First, for a book selling at five guineas the quality of the reproduction of the photographs leaves much to be desired, so much so that some points they are supposed to illustrate are lost. It does not appear that any critical attention has been given in the selection of the illustrations, so that the reader is not given much help in selecting what is best, even if he is interested in designing a general purpose laboratory. While Britain is eventually to use the metric system, architects, builders and chemists still use feet and inches for measuring working space, so it is a pity that these measurements could not have been translated into familiar units.

The grading of laboratories on p. 142 is based on the curie level of radioactivity and not on the specific hazards of particular isotopes. Thus, according to the Medical Research Council memorandum, up to 10 curies of tritium or mercury-197 could be handled in a grade '*C*' laboratory (any good conventional laboratory with an efficient fume cupboard), while from this book one would expect to have to use a 'hot' laboratory. In general the stress here is not 'hot' laboratories, whereas a great deal of radiochemical work needs less-elaborate accommodation.

The photographs of shielded cupboards, etc., give no idea of dimensions, thickness of shielding, etc. This was probably included in the statistical information omitted from the English edition. Glove boxes are mentioned for β-emitters, but no mention is made of α-emitters, for example, plutonium. One might not need glove boxes for working with β-emitters in solution.

After all these criticisms have been made, the reader will nevertheless find a lot of useful information and the bibliography is considerable though mostly of German origin.

A. A. W. RUSSELL

Encyclopedia of Polymer Science and Technology

Plastics, Resins, Rubbers, Fibres. Edited by Herman F. Mark, Norman G. Gaylord, Norbet M. Bikales. Vol. 2: Amino Resins to Casein. Pp. xiii+871. (New York and London: Interscience Publications, a Division of John Wiley and Sons, Inc., 1965.) 375*s*.

THE object of this ten-volume encyclopedia is no less than the coverage of both the science and technology of polymers. Volume 1 started with "Ablative Polymers" and in some 893 pages reached "Amino Acids". Volume 2 takes us to "Casein" in a similar large number of large pages. Fifty topics are dealt with according to the space the various contributors and the editors consider they merit, from one sentence for "A-Stage" (resins) to 95 pages for "Amino Resins". The difference in page lengths is a reflexion of the efficient cross-reference system where, for example, "A-Stage" resins are dealt with in the articles dealing with the various types of resin.

These reviews can bring one from a vague background to a good general knowledge of the topics dealt with. Supplemented by the bibliographies with each article, one has enough literature background for starting original work. It so happened that I was able to test this out on a real case since antistatic agents and antiozonants are new subjects of interest to my laboratories. The articles in the *Encyclopedia* were very timely.

The articles would probably continue to serve this purpose for perhaps five years in this rapidly expanding phase of polymer work. No doubt Interscience Publishers look forward with pleasurable anticipation to the *Encyclopedia* being rather like the Forth Bridge.

The claim to cover both science and technology is met by the contents of this volume. The authors are mainly senior scientists in the industrial laboratories in the United States, where much of the fundamental science is going on and where so little opportunity for exploitation is lost. The articles vary greatly in the proportions of science and technology and have a heartening attitude not to make any artificial separation of them. Subjects such as "Amino Resins" and "Aqueous Polymerisation" are mainly organic chemistry while "Bag Molding" and "Calendering" are predominantly production technology.

Librarians serving polymer laboratories and the larger science laboratories will wish to purchase these volumes. Apart from being very handsome and beautifully produced editions for the libraries, they will be among the books most frequently not on the shelves. The integration of the fundamentals with their application should be of particular value to teachers in the polymer field, from their own experience being familiar with only some of the aspects of the subject, and to students in gaining a foretaste of the best industrial attitude.

W. F. Watson

Complement

Edited by G. E. W. Wolstenholme and Julie Knight. (Ciba Foundation Symposium.) Pp. xiii+388. (London: J. and A. Churchill, Ltd., 1965.) 60*s*.

IN 1948 Heidelberger and Mayer wrote: "It is happily a rare thing to find in scientific literature a vast body of writings so confused and so mutually contradictory as those on the manifold activities of complement". Since then, the literature on complement has become even vaster, and the symposium held in May 1964, under the auspices of the Ciba Foundation, served a most useful purpose in charting the position of research into some of the many processes in which complement plays a part.

Probably most of the work on complement has been directed to unravelling the complicated process of immune haemolysis, because this system lends itself to precise quantitative experimentation. Information gained from such studies about the many components of complement and their eventual characterization will find applications in the investigation of the action of complement in other immune processes which may be of more immediate value to the organism.

The opening paper by Mayer reviews present-day concepts of the mechanism of immune haemolysis and shows the complicated stages through which a red cell passes before its haemoglobin is finally liberated and it becomes a 'ghost'. A beautifully illustrated paper on electron-microscope studies of lysed cells complements this and shows the actual holes through which the haemoglobin presumably leaks out of the cell. Other papers in this field are on small molecular weight inhibitors of complement, and, perhaps of more interest to clinicians, on haemolytic processes *in vivo* and on the paroxysmal haemoglobinuria red cell. Of more general immunological interest are the contributions on the role of complement in bacteriolysis and phagocytic activity, immune adherence and conglutination and on the site of formation of complement components.

This symposium provides a very useful and up-to-date survey of present-day ideas on many aspects of complement activity. It is well produced and has a good index. A welcome feature is the very full reporting of the discussions which each paper generated; these add greatly to the interest and value of the papers themselves.

A. E. Wilkinson

Light: Physical and Biological Action

By Howard H. Selinger and William D. McElroy. (Monograph Series on Radiation Biology.) Pp. xi+417. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 96*s*.

LIGHT: Physical and Biological Action is one of a number of volumes designed to further the reciprocal exchange of information between the physical and biological sciences, according to the foreword by the director of this series. And it is bound to serve this function well. For this very reason, I do not think that it is particularly suited to the needs of the general student of biology with a smattering interest in photobiology, though, by picking and choosing, he may find much to interest him.

The authors have performed the service of putting together data from various fields and sources, and arranging them in tabular form, ready for use at a moment's notice. The first chapter, entitled "Measurement and Characterization of Light", is filled with tables, and six appendixes summarize the relevant physical constants and conversion factors, as well as filter data, methods for measuring light intensity, the use of firefly luminescence as an assay for ATP, and the theory and availability of various lasers.

The main text is divided into four chapters: Chapter 2, "Excitation of Molecules by Light"; Chapter 3, "Chemiluminescence—Reactions and Mechanisms"; Chapter 4, "Bioluminescence—Enzyme-catalysed Chemiluminescence"; and Chapter 5, "Biological Action of Light".

Chapter 2 is a lucid introduction to modern photochemistry and presents data in useful schematic and tabular form. Chapters 3 and 4, in which the authors are writing out of their own experience, to my mind constitute the main intellectual contribution of the book. Chapter 5 touches on such diverse topics as the anatomy of biological photoreceptors, the mechanisms of plant photosynthesis and phototropisms, vision, killing by radiation, photoreactivation, and others. Because of its breadth and the greater distance of the authors from many of the fields it touches on, this chapter is more uneven than the others. It is, however, amply supplied with references to guide the interested reader to the original literature in each of these spheres.

Ruth Hubbard

STRUCTURE OF DEOXYMYOGLOBIN: A CRYSTALLOGRAPHIC STUDY

By Dr. C. L. NOBBS, Dr. H. C. WATSON and Dr. J. C. KENDREW, C.B.E., F.R.S.
Medical Research Council Laboratory of Molecular Biology, Cambridge

THE results described in this article demonstrate that in deoxymyoglobin the iron atom is 5-co-ordinated; there is no water molecule or other group at the sixth co-ordination position as in ferrimyoglobin. They also show that, at least within the limits imposed by a study at 2·8 Å resolution, the conformation of deoxymyoglobin is the same as that of ferrimyoglobin.

X-ray studies of sperm-whale[1] myoglobin and of horse haemoglobin[2] have made it possible to describe the structures of these two proteins in detail. In myoglobin the analysis has now been extended to include all X-ray reflexions with spacings greater than about 1·4 Å (ref. 3), and this, together with the final elucidation of the amino-acid sequence (Edmundson[4]), has led to a nearly complete picture, atom by atom, of the whole molecule. In haemoglobin the analysis has not yet been carried beyond the reflexions of 5·5 Å spacing, but the low-resolution picture of the molecule thus obtained has shown that its four sub-units each bear a very close resemblance to the myoglobin molecule, and this, together with a complete knowledge of the amino-acid sequences of horse haemoglobin[5,6], has made possible the construction of an atomic model[7] which, while admittedly speculative in some details, must certainly be correct in all its main features. The way is now open to investigate the molecular basis of the physiological functions of the two proteins, their reversible combination with oxygen, and, in haemoglobin, the haem–haem interactions and other well-known phenomena such as the Bohr effect. Indeed, the oxygenation reaction has already been shown to be accompanied in haemoglobin by a gross change in the relative positions of the four sub-units[8]. The present resolution of the haemoglobin Fourier synthesis is, however, insufficient to show whether the conformations of the individual sub-units become changed in detail during oxygenation, though it is certain that if there are changes they must be small ones; nor can it reveal the mode of attachment of the oxygen molecules to the haem groups.

In myoglobin, on the other hand, the high resolution of the Fourier synthesis would be sufficient to reveal detailed changes in conformation (if they occur) and to investigate the nature of the iron-oxygen binding. Even though myoglobin does not exhibit the same complexity of behaviour as haemoglobin, it is clear from spectral and other comparisons that the mechanism of the oxygen binding itself must be very similar in the two proteins, so conclusions reached about the one must also apply to the other.

The main studies of myoglobin have been carried out with ferrimyoglobin (met-myoglobin) at about *p*H 6·8, and have shown that in this derivative a water molecule is attached to the iron atom, in the sixth co-ordination position. Similar methods might be used to determine independently the complete structures of deoxy- and oxy-myoglobins, which are the two forms directly concerned in the oxygenation reaction. Recent investigations[9] of yet another derivative, myoglobin azide, have shown, however, that useful results can be obtained by a much more rapid method, that of the difference-Fourier synthesis. In this method a Fourier synthesis is computed using as its terms the differences between the amplitudes of the reflexions from the derivative and from the native protein, associated with the phases characteristic of the native protein. To a good approximation the resulting synthesis shows as its only features differences in structure between derivative and native structure, in the form of positive peaks if additional atoms have been introduced, and of negative peaks if atoms have been removed. In the present study a three-dimensional difference Fourier synthesis has been used to investigate the differences in structure between deoxymyoglobin and ferrimyoglobin of sperm whale, and hence to draw conclusions about the structure of the former.

Experimental

Deoxymyoglobin is very easily oxidized *in vitro* to the ferric form, especially in the concentrated salt solutions required for crystallization. The main difficulty in the present investigation was to prevent oxidation during the preparation of crystals and their subsequent exposure to the X-ray beam.

Ferrimyoglobin was prepared from sperm whale meat and purified by the method described by Kendrew and Parrish[10]. The solution used for crystallization consisted of saturated ammonium sulphate mixed with 5 per cent by volume of 4 M mixed phosphate buffer (K_2HPO_4 and NaH_2PO_4) at a *p*H of 5·6–5·8 in the final crystallizing solution, and solid ethylenediaminetetraacetic acid to a concentration of 0·01 M to eliminate metal ions which might catalyse oxidation processes. This solution was carefully added to samples of a concentrated salt-free solution of ferrimyoglobin (at least 5 mM) until the mixture was slightly turbid; a fifty-fold excess of solid sodium dithionite (see following) was then added. These additions were made, and crystallization subsequently took place in an atmosphere of nitrogen (containing traces of hydrogen). Diamond-shaped crystals up to 5 × 3 × 1 mm were formed in about a week, and the *p*H of the solution was then adjusted to 6·3–6·4 (that used in X-ray studies of haemoglobin).

For X-ray photography, crystals about 0·6 × 0·4 × 0·1 mm were mounted in sealed quartz capillary tubes containing a drop of mother liquor and a nitrogen atmosphere. A microscope with a hand spectroscope replacing the eyepiece was used for observation of the absorption spectrum of mounted crystals both before and after the X-ray exposure; control experiments with solutions indicated that this technique could detect some 25 per cent of oxy- or ferri-myoglobin in the presence of deoxymyoglobin. During irradiation, oxidation (but not oxygenation) was liable to occur, and to prevent detectable changes in the spectrum during a normal exposure (12 h) it was found necessary to use a fifty-fold excess of sodium dithionite. Data from crystals showing any detectable change in spectrum were discarded.

In contrast to haemoglobin, but in agreement with earlier studies of myoglobin[11], crystals of deoxymyoglobin were found to be isomorphous with those of ferrimyoglobin, the cell dimensions agreeing within 1 per cent. Conventional methods, using the Buerger precession camera and copper $K\alpha$ radiation from a rotating anode X-ray tube, were utilized for recording photographically all reflexions with spacings greater than 2·8 Å. The intensities were measured using a Joyce–Loebl automatic recording densitometer and corrected by the usual factors and placed on an absolute scale by comparison with standard data for ferrimyoglobin. A total of 3,212 reflexions were used to calculate a three-dimensional difference Fourier synthesis: the mean amplitude of the reflexions was 266 electrons, the mean amplitude difference $\overline{||F_{deoxy}| - |F_{ferri}||}$ was 24 electrons and the largest difference 135 electrons.

Results

The most striking characteristic of the difference Fourier synthesis (Fig. 1) is its almost complete lack of features; for the most part it is completely devoid of detail, and it

Table 1. Significant Peaks in the Difference Fourier Synthesis between Deoxy- and Ferri-myoglobin

Peak No.	Co-ordinates (fractional) x	y	z	Maximum excess density (el. Å^{-3})	Location
1	0·38	0·06	0·27	−0·68	At site of sulphate ion near N_δ of *E*7(His)
2	0·28	0·89	0·15	−0·42	At site of haem-linked water molecule
3	0·40	0·42	0·48	+0·32	At site of $AuCl_4^{2-}$ substitution
4	0·28	0·26	0·15	+0·25	At site of *p*-chloromercuribenzene sulphonate substitution
5	0·24	0·89	0·17	+0·24	Between haem Fe and N_ε of *F*8(His)
6	0·07	0·75	0·54	−0·26	Near *EF*4(His) and *EF*3(Gly)
7	0·12	0·72	0·73	−0·21	Near *EF*4(His)
8	0·07	0·74	0·88	+0·20	Near *G*1(Pro), *G*2(Ile), *HC*4(Gln) and HC5(Gly)
9	0·29	0·20	0·29	+0·20	Near *G*17(His) and *H*4(Gln)

contains very few difference peaks which appear to be significant. The standard deviation of the electron density differences, taken over all points in the unit cell, is only 0·06 el. Å^{-3}. We have considered that any peak exceeding three times this value, namely, 0·18 el. Å^{-3} or more, is probably significant; there are only nine such peaks, which are listed in Table 1.

Peak 1. The largest difference peak, a negative one of −0·68 el. Å^{-3}, occurs at the site of a positive peak in the ferrimyoglobin structure near N_δ of the imidazole ring of the 'distal' histidine *E*7. The latter peak is paralleled by a negative difference peak of nearly the same depth (−0·75 el. Å^{-3}) and at the same place in the difference synthesis of azide myoglobin[9]. This difference peak was interpreted as being due to the partial loss of a negative ion, for example, sulphate, when a negatively charged azide group replaces water in forming azide myoglobin; we interpret the negative peak in the present synthesis in exactly the same way, as representing the loss of the negative ion from N_δ of histidine *E*7 during conversion of ferrimyoglobin to deoxymyoglobin.

Peak 2. The next largest difference peak, of −0·42 el. Å^{-3}, is exactly at the site of the peak in the ferrimyoglobin Fourier synthesis which is interpreted as a water molecule attached to the iron atom at its sixth co-ordination position. In the azide derivative this water molecule is replaced by N_3^-; the net addition of two atoms excluding hydrogens (three nitrogens added, one oxygen removed) gave a positive difference peak of +0·75 el. Å^{-3}. In the present case a negative peak of −0·42 el. Å^{-3}, half the absolute magnitude of the former peak within our limits of accuracy, must correspond to the removal of a single atom. The conclusion is inescapable, that during the formation of deoxymyoglobin from ferrimyoglobin the haem-linked water molecule is lost, and nothing takes its place.

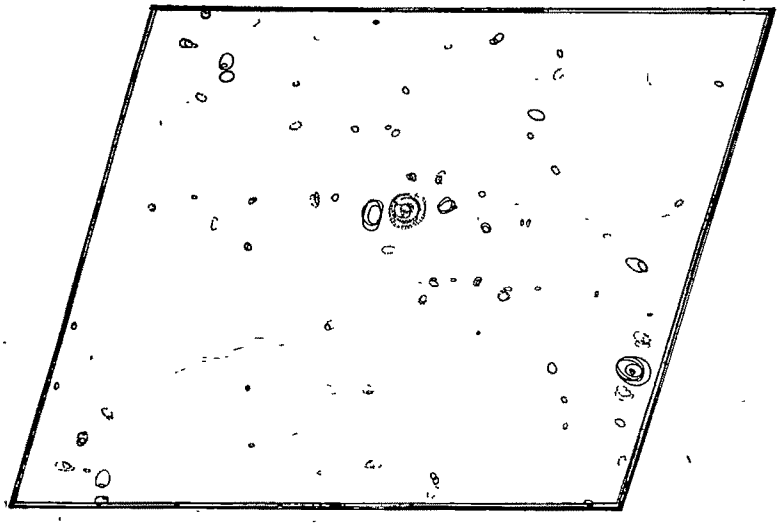


Fig. 1. Slice through the difference Fourier synthesis perpendicular to (010) and lying between $y = 0{\cdot}85$ and $y = 0{\cdot}94$; x axis horizontal. Contours are drawn at multiples of 0·125 el. Å^{-3}, full lines positive and dashed lines negative. The large negative peak in the upper centre is at the site vacated by the haem-linked molecule (peak 2 in Table 1); to its left is the positive peak near the iron atom (peak 5). Near the right-hand edge of the slice is the peak at the gold site (peak 3)

Peaks 3 *and* 4. The next two difference peaks, of +0·32 and +0·25 el. Å^{-3}, are respectively at the site of the gold atom in the aurichloride derivative, and of the mercury atom in the *p*-chloromercuribenzene sulphonate derivative, used for phase determination by isomorphous replacement[12]. Disturbances are found to occur at the heavy atom sites in the ferrimyoglobin Fourier synthesis, and were presumed to be due to residual errors in phase determination. The difference peaks at these sites in the present synthesis may be assumed to have a similar origin, and to be without structural significance.

Peak 5. This small peak lies between the iron atom of the haem group and the N_ε of the haem-linked histidine, 1·6 Å from the former and 1·9 Å from the latter, but displaced 1·4 Å from the line joining these two atoms. While an actual shift of the iron atom cannot be excluded as the cause of this peak[13], its position relative to the haem group and the lack of a complementary negative peak make this extremely unlikely. We prefer to assume that this peak is produced by errors in either the original phase information or in the present measurements.

Peaks 6 *to* 9. These peaks, the only others significantly above the background, all lie outside the boundaries of the molecule. They are all small, and it is not possible to give any precise interpretation of them. They may well represent slight changes in the distribution of water or ions bound to the protein, due to differences in the crystallization medium used in preparing deoxymyoglobin crystals in the present experiments.

Discussion

The almost complete absence of features in the difference Fourier synthesis (apart from the immediate neighbourhood of the iron atom) indicates that the structure of deoxymyoglobin is extremely similar to that of ferrimyoglobin; there is no evidence of conformational changes or of movements of the haem group. It should be noted, however, that slight changes in the orientation of isolated groups would be undetected; some side-chains are partly invisible in the ferrimyoglobin Fourier synthesis (presumably because their conformations are variable), but even in a well-resolved one some degree of rotation would not be detected at the present resolution—for example, it is improbable that at the resolution of 2·8 Å used in the present investigation, we could detect with confidence a rotation of as much as 45° about the Cα—Cβ bond of serine, though movements of more bulky side-chains such as leucine or phenylalanine would be readily visible. No such movements have been observed. Incidentally, it may be noted that the conformation of the molecule has been preserved unchanged within these limits, in spite of the use of a very large excess of sodium dithionite in preparing deoxymyoglobin crystals; this is possibly because any molecules damaged by this reagent were selectively excluded by the process of crystallization.

The most striking conclusion to be derived from the present results is that in deoxymyoglobin the iron atom is 5-co-ordinated; there is no water molecule or other group attached in a sixth co-ordination position as in ferrimyoglobin. We have already indicated that the same must surely be true of deoxyhaemoglobin. In this respect deoxymyoglobin and deoxyhaemoglobin differ from all the other derivatives. Though the absence of a sixth group might be thought to be the most natural explanation of the many unique properties of the deoxy form, especially its paramagnetism[14], and though a structure of this kind was proposed by Pauling[15], it has generally been supposed that a water molecule was present ever since the

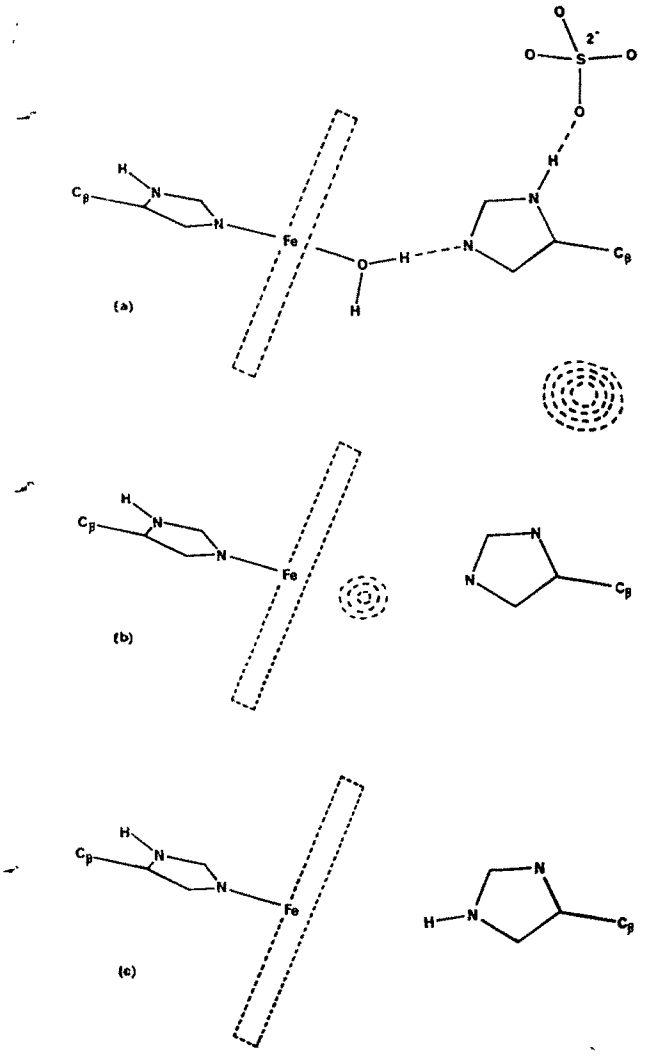


Fig. 2. Schematic representation of the environment of the iron atom in myoglobin derivatives; (*a*) ferrimyoglobin, (*b*) observed negative peaks in difference Fourier synthesis, (*c*) deoxymyoglobin

publication of Haurowitz's ingenious experiments on the spectral changes induced by thorough drying[16].

No evidence can be found from our difference synthesis of any shifts in the position of the iron atom, the haem group, or either of the associated histidine residues *E*7 and *F*8 (quite small shifts of a few tenths of an Angstrom unit should have been detectable). It follows that the environment of the iron atom is the same, apart from the missing water molecule, in deoxymyoglobin as it is in ferrimyoglobin, and in turn the environment in the latter is within the limits of error identical with that in α-chlorohaemin, the crystal structure of which has been accurately determined by Koenig[17].

We have noted the ejection of a sulphate or other ion from its position bonded to N_δ of the distal histidine residue *E*7, as also occurs in the formation of azide myoglobin from ferrimyoglobin. This ion has no physiological significance (the sulphate concentration in a myoglobin crystal has the quite non-physiological value of about 4 M): but its presence or absence is certainly indicative of the distribution of charge around the imidazole ring of the histidine residue. In azide myoglobin its ejection indicated that N_δ has become more negative, and N_ε more positive, in consequence of the introduction of the negatively charged N_3^- in place of a neutral water molecule. In the present case it is the iron atom itself which becomes less positive, that is to say, more negative, in consequence of being reduced from ferric to ferrous, and the result is the same. Our interpretation of the changes which occur is shown schematically in Fig. 2.

Finally, these results show that under good conditions it is possible to detect the loss of a single oxygen atom from a protein molecule, without going through the lengthy process of a complete structure determination of the derivative under examination. Provided that the structure of the parent substance has been determined to a resolution of 2 Å or better, and provided that the derivative is isomorphous with it, the technique of difference Fourier synthesis should be capable of elucidating the details of interactions between proteins and small molecules such as enzyme substrates, inhibitors and coenzymes. It should be a valuable tool for the investigation of the detailed mechanism of enzyme reactions.

We thank Miss Eva Rowland and Mr. M. Fletcher for able technical assistance. One of us (C. L. N.) thanks Shell Oil (New Zealand), Ltd., for the award of a Shell postgraduate scholarship, Mr. C. J. Mackay of Shell International Petroleum Co. (London), Ltd., for his interest and encouragement, and the Rockefeller Foundation for a grant.

This work was first reported by one of us (J. C. K.) at the Cambridge meeting of the British Biophysical Society, April 1965.

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PERMANENCY OF THE CONTINENTS

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EITHER the continents have moved relative to one another or they have stayed generally in one position. The theories relating to these opposed views are known, respectively, as 'continental drift' and the 'permanency of continents'. Early evidence which led to the development of the theory of continental drift was the apparent similarity of the east coast-line of South America and the west coast-line of Africa. However, the theory of continental drift does not offer a unique explanation of the similarities of the two coast-lines and the associated Mid-Atlantic Ridge. A second explanation of the similarities may be derived from the theory of permanency of continents when combined with a world-wide shear pattern such as developed by Vening Meinesz in 1947 (ref. 1).

The purpose of this article is to evaluate Vening Meinesz's crustal shear pattern on a world-wide basis.

It not only presents evidence regarding the relative strength of the two opposing theories, but also offers the first explanation of the world-wide heat-flow data (heat flow through the crust). Information is also included that will be of importance in developing an optimum technique for mapping ocean bottom topography.

Origin of the Shear Pattern

Vening Meinesz[1], after investigating the results of the then new method of ocean bottom mapping by echo sounding, noticed more or less regular linear topographic patterns, mostly in two directions, over extended parts of ocean floors. He associated this work with the shear plane evidence brought out by the old 'lineament' tectonicians. Since there was an apparent world-wide distribution of shear planes, Vening Meinesz thought it likely that there was a world-wide cause responsible for the development of a world-wide shear pattern as opposed to a world-wide distribution of random shears.

Vening Meinesz investigated stresses in the Earth's crust brought about by a shift of the crust with respect to the axis of rotation. The basic theory is that the Earth rotates about an axis, and is flattened at the poles and bulges at the equator. If the Earth's crust were to be shifted, *in toto*, the new crust at the poles would become flattened and the new crust at the equator would bulge outwards. Such a change in the relative shape of the crust would produce stresses in the Earth's crust, and these stresses, if great enough, would cause a 'breaking' or 'cracking' of the crust; in other words, a shear pattern would develop.

Mathematical shear patterns were obtained by Vening Meinesz for various shifts in the Earth's crust relative to the axis of rotation and by considering changes in the Earth's shape caused by changes in the speed of rotation. The pattern which most closely matches the topographic features of the Earth is shown in Fig. 1. This is the shear pattern that would develop if the Earth's crust shifted relative to the axis of rotation for a distance of 70° along a line 90° E. from the prime meridian. That is, the north pole of rotation makes an apparent shift 70° along the line 90° E., and the south pole makes an apparent shift 70° along the line 90° W. into their present positions. Note that the axis of rotation must remain fixed in space and it is the crust that shifts.

The postulated cause of this shift in the Earth's crust is either convection currents in the Pre-Cambrian acting directly on the crust itself, or else redistribution of mass (continental growth) associated with these Pre-Cambrian convection currents. This latter mechanism is very simple to picture. Imagine a child's top rotating about a vertical axis. Now, if a lump of clay is added to the exterior portion of the top, or if a piece of the top is removed, then it is easily seen that on rotation the top still possesses its vertical axis but the mass of the top now re-aligns itself with respect to the axis.

It should be noted that the postulated convection currents are not the convection currents existing at present, but Pre-Cambrian convection currents. Jeffreys[2] directs attention to the difficulties of maintaining convection in the Earth as we know it to-day, but he also points out that convection seems to be likely during a certain stage in solidification of the Earth. As this article is intended to examine the postulated shear pattern and not the mechanism behind, it, the reader is referred elsewhere for further discussion of convection in the mantle[1–3].

Comparison of the Shear Pattern with Ocean Bottom Topography

To evaluate the shear pattern shown in Fig. 1, two maps were used. The first map, entitled *The World*, was published by the U.S. Navy Hydrographic Office at a scale of 1 : 12,233,000 at the equator. This map measures 8 × 12 ft. and presents the bottom topography of the Indian, Atlantic and Pacific oceans as it was known in 1961. The second map, prepared by the University of Wisconsin, entitled *Bathymetric Chart of the Arctic Basin*, was used for evaluation of the topography of the Arctic Ocean[4]. These maps allow extension of the original work into areas not previously examined with respect to the shear pattern. The new maps increase coverage so that all the floor of the ocean is now evaluated instead of only the portion outlined in the original study. This means

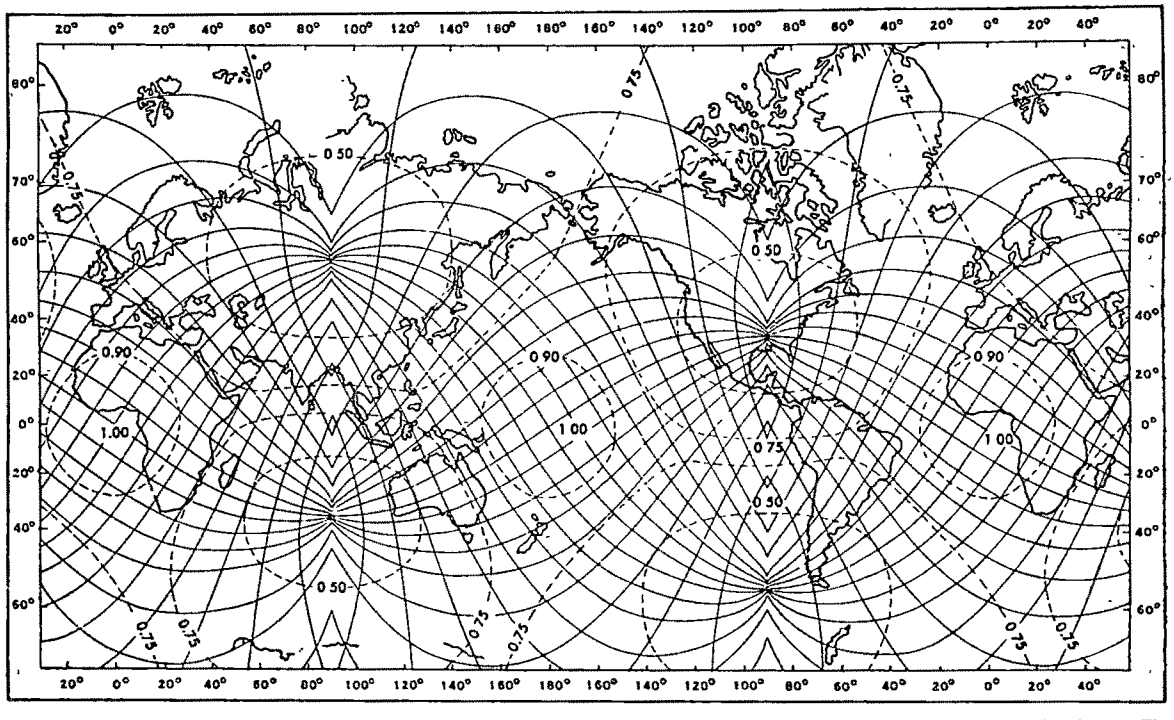


Fig. 1. Theoretical shear pattern in Mercator's projection for an apparent shift of the poles over 70° along the meridian of 90° longitude. The solid lines show the expected directions of shear and the dashed lines show the relative shear intensity which reaches a minimum where the shear pattern concentrates and a maximum in the areas labelled '1·00'

that the coverage of the Earth is increased from 50 per cent to 70 per cent. This figure of 70 per cent will be raised later in this article as some structures of the Canadian Shield and almost all of Africa are also evaluated.

Secondly, these new maps contain many data that were not available during the original investigation. Examples of large tectonic features, that are not in evidence on the original maps evaluated, are: (1) the Chile Rise; (2) the portions of the mid-ocean ridges south of Africa and Australia; (3) the Emperor Seamount Chain; (4) the Mendocino Seascarp; (5) the Murry Fracture Zone; (6) the deep trench located at 44° N., 174° W. Not only were these new features delineated but also the previously known features are more accurately defined and substructures are now apparent in many cases.

At the start of this investigation, the azimuths (measured in degrees east or west of geographic north) of all major oceanic structures were determined and compared with the corresponding azimuths of the theoretical shear pattern at the location being evaluated. Data points were taken approximately every 300 miles: (1) along borders of continents; (2) along island arcs; (3) along ocean trenches; (4) along the world-wide system of mid-ocean ridges; (5) along major fault structures; (6) along island chains; (7) along sea mount chains. The only areas of the world excluded were the borders of Antarctica (but the Palmer Peninsula was included) and the ocean areas inside the Sea of Okhotsk and the Sea of Japan. These latter areas were excluded because Japan, the Kuril Islands, and Kamchatka were taken to be the eastern border of Asia rather than the Chinese mainland.

The frequency distributions for the two sets of azimuths are shown in Figs. 2 and 3. The fact that the ocean structures fail to show a random azimuthal distribution reinforces the basic concept of a world-wide control of tectonic features. A comparison of the two curves shows that the distribution of the Earth's features does not exactly follow the predicted shear patterns. This is to be expected for four reasons: (1) the Earth's crust was probably not homogeneous and isotropic at the time of the generation of the shear pattern (for example, there may have already been some continental growth); (2) the sampling of the world used in this investigation was restricted to ocean areas, and the ocean basins are not symmetric about the Earth; (3) the theoretical shear pattern is not symmetric about the poles because the

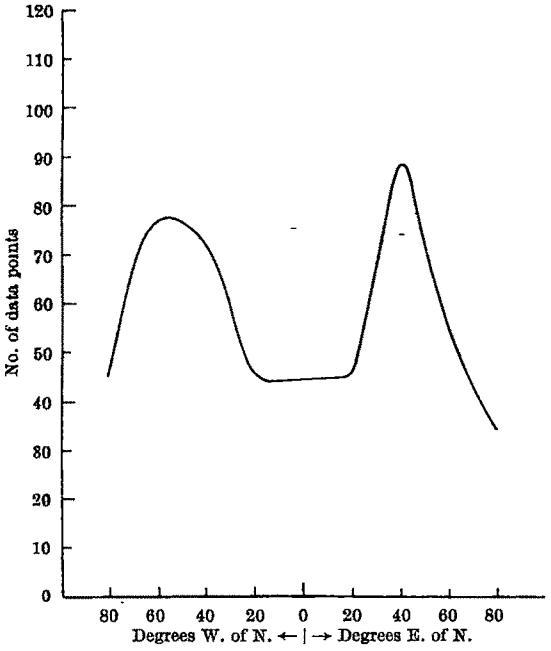


Fig 2. Azimuthal frequency distribution of the Earth's oceanic features

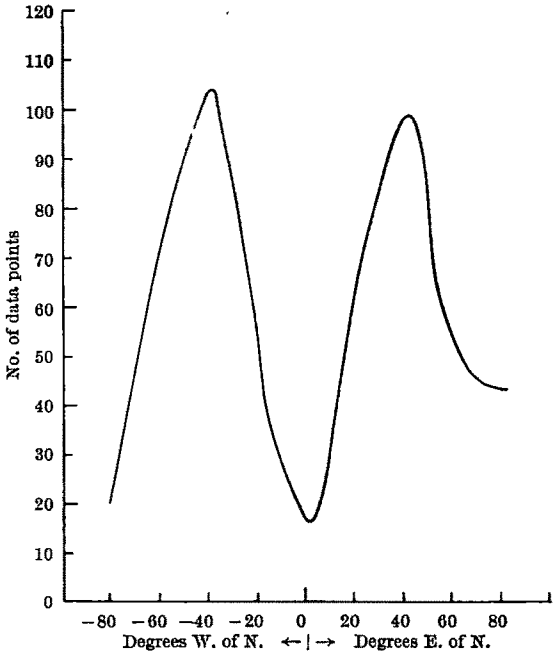


Fig. 3. Azimuthal frequency distribution of the theoretical shear pattern; that is, frequency distribution of the direction of expected shear at all locations of observed ocean bottom features. Note that the frequency distribution shown in Fig. 2 is a damped model of the foregoing frequency distribution

postulated change in axis of rotation is 70° and not a convenient 90°; (4) the local stresses applied to the Earth's crust at any time before the time of shearing should offer zones of weakness in random directions, which would modify the expected shear pattern or which would be co-existent with the shear pattern and tend to 'damp out' the peaks and troughs of the frequency distribution. Also, local stresses applied to the Earth's crust after the shear pattern has developed may not be able to 'take advantage' of the pre-existing zones of weakness and therefore may generate new lines of shear independent of the world-wide 'caused' shear pattern. These new lines of shear will also act to 'damp out' the effects of any world-wide cause of crustal shearing.

Notwithstanding all these difficulties, the remarkable similarity of the two frequency distributions suggests that serious consideration should be given to further investigation of Vening Meinesz's crustal shear pattern.

After comparing frequency distributions of the topography and the theoretical shear pattern, correlation coefficients between the observed azimuths and the theoretical azimuths were computed. The resulting correlation coefficients are presented in Table 1. Four important points should be noticed: (1) that more than 50 per cent of the correlation coefficients are greater than 0·90 and that 90 per cent are greater than 0·80; (2) that both areas where correlation reaches 0·94 are located in

Table 1. Correlation Coefficients as computed between the Theoretical Shear Patterns and the Ocean-bottom Topography and Continental Borders

Structures in the Indian Ocean excluding its borders	0·87
Structures in the Atlantic Ocean excluding its borders	0·94
Structures in the Pacific Ocean excluding its borders	0·90
Southern border of Asia	0·92
All structures in the Caribbean Sea, including the border of the Gulf of Mexico	0·92
North America excluding Arctic Ocean	0·92
Border of Arctic Ocean and its internal structure	0·78
Atlantic coast of South America	0·94
Pacific coast of South America	0·80
Mediterranean Sea area	0·92
South American border *in toto*	0·91
African borders excluding Mediterranean area	0·87
European border excluding Arctic Ocean borders	0·87
All major island arcs regardless of location	0·90
Australia, excluding northern border	0·91
Borders of the Indian Ocean	0·68
Pacific Ocean border excluding Asia	0·86
Indonesia, entire area, all island chains, etc.	0·89
Atlantic Ocean boundaries	0·90
Average world-wide correlation (average of above figures)	0·88

the Atlantic Ocean and on the Atlantic Coast of South America; (3) that the lowest correlation is located around the Indian Ocean; (4) that the last-mentioned two areas are predicted to have, respectively, high and low relative shear intensity by the dashed lines in Fig. 1. Thus, the theory behind Vening Meinesz's crustal shear pattern not only predicts the directions that most of the large-scale features will take but also predicts where correlation will be high and where it will be low.

Again, in Table 1, it should be noted how highly the borders of continents correlate with the theoretical shear pattern. This strongly implies that the shear pattern developed before the continents completed their growth. Continental growth (defined as the areal expansion of continents with time) is known to be complete by the start of the Cambrian: Fig. 4 shows North America at this time[5]. There is, in fact, much more evidence that the continents were already completed by Cambrian time, but this one figure will save belabouring the point. (Note it is not suggested that the continents were 'volumetrically' complete. There is ample evidence that new material is being added to the continents from the upper mantle, but it is repeated that the areal extension of continents has remained essentially unchanged since the Cambrian period.)

Since the continents were complete at the start of the Cambrian, and since there is a strong correlation between the borders of continents and the theoretical shear pattern, then it is concluded that the shear pattern developed in the Pre-Cambrian. If this age were correct, and if the shear pattern at present observed on the floor of the ocean were to match the pattern generated by the theoretical model, then continental drift would become a complete impossibility, as any motion of the ocean floor would destroy the correlation between ocean bottom topography and the theoretical shear pattern.

Comparison of the Shear Pattern with Other Geophysical Evidence

Now that it has been established that the shear pattern does indeed correlate well with the ocean bottom topography over the entire world, it becomes worth while to examine the relationship between three parameters that have been evaluated on a world-wide basis. These are: (1) the relative shear intensity of Vening Meinesz's crustal shear pattern (as shown by the dashed lines in Fig. 1); (2) the deviation of the Earth's crust from the theoretical geoid (hereafter referred to as geoid heights); (3) the heat-flow data. (The data for these last-mentioned two parameters were taken from Lee and MacDonald[6].) For all correlations computed in the following paragraphs the same sampling procedure was used to establish the degree of representation and homogeneous treatment of the data.

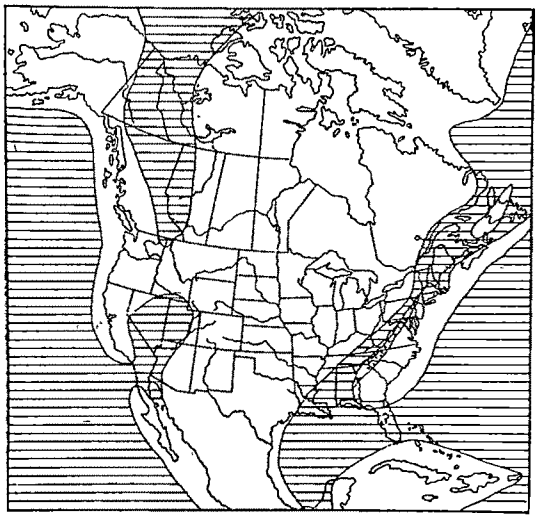


Fig. 4. Generalized palaeogeographic map of early Cambrian of North America. The ruled area shows sea and the clear area shows land. The dotted area was also possibly sea

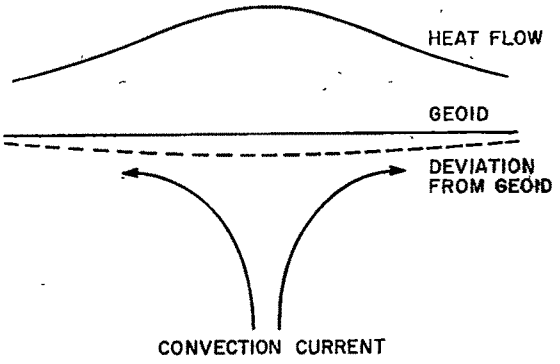


Fig. 5. Relationships between convection currents, heat flow and deviations from the geoid as postulated by the theory of continental drift

First, let us consider the correlation between the geoid heights and the heat-flow data. The heat-flow data, as a whole, show that higher values exist over continental rather than oceanic areas. As these values should reflect any convection currents at present active in the Earth's mantle or lower crust, it appears that convection currents (if they exist to-day) flow in directions opposite to those required by the theory of continental drift. But convection currents, if they exist, should also affect geoid heights. The theory of continental drift postulates that above a rising component of a convection cell the heat flow will be enhanced and the geoid will be depressed (see Fig. 5). For descending components of the convection cells, the opposite is expected. Therefore, one would expect to see a good correlation between the heat-flow values and the geoid heights if convection currents exist to-day. The data show a linear relationship with a correlation coefficient of $-0{\cdot}33$ and with large variance. This relationship is at least of the right sign, but it can only explain 10 per cent of the variability of the heat-flow data and thus most probably indicates only chance relationship.

It is also reasonable to expect a chance relationship between geoid heights and the relative shear intensity, as a mere cracking or breaking of the Earth's crust would not be expected to affect the shape of the geoid. The correlation coefficient between these two parameters was found to be 0·15, which indicates the expected chance relationship.

However, correlation between heat flow and the relative shear intensity is a different matter. The calculation of the correlation coefficient between the relative shear intensity and the heat-flow data gave a result of $-0{\cdot}52$. This figure is an improvement over the $-0{\cdot}33$ computed for the linear relationship between heat flow and geoid heights. However, on plotting the heat-flow data versus the relative shear intensity, it was found that the relationship between these parameters is bi-linear, not linear. In Fig. 6 it is seen that there is no relationship between these two parameters for values of low shear intensity, but that at high values of shear intensity there is a good relationship. The correlation coefficient between these two parameters for values of high-shear intensity is $-0{\cdot}72$, and this means that the variability of high-shear intensity can explain 50 per cent of the variability of low heat-flow data.

In Fig. 7, the two sets of data have been mapped. It should be noted that: (1) the areas of low heat flow coincide with areas of high shear intensity; (2) the heat flow is found to be symmetric about 90°, as is the shear intensity; (3) 90° is the line along which the poles shifted as the generating mechanism for the shear pattern. Thus, even the general pattern and symmetry of the heat-flow

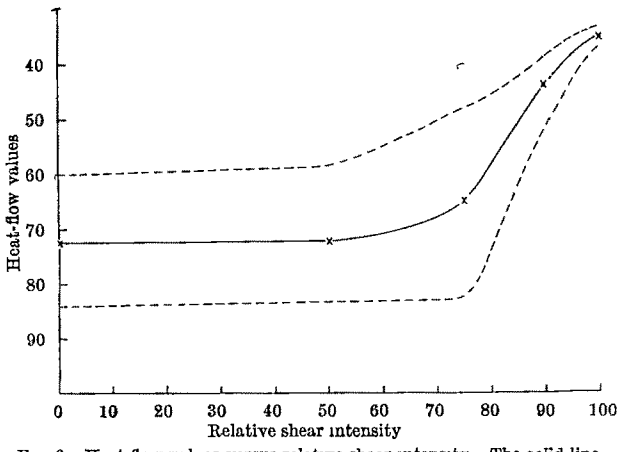


Fig. 6. Heat-flow values versus relative shear intensity. The solid line is drawn through average heat-flow values (denoted by X) and the dashed line represents the envelope enclosing all data points. Note that the solid line could be represented by two linear curves intersecting at the point where the heat flow equals 72 and the relative shear intensity equals 0 65

data reinforce the concept and mechanism of Vening Meinesz's world-wide crustal shear pattern.

Now to explain the negative value of the correlation between the heat flow and the relative shear intensity. Consider an area of high-shear intensity with its corresponding 'breaking' of the Earth's crust. This area offers paths for advection of molten mantle material along the 'zones of weakness' or 'breaks' in the Earth's crust. Thus this area of high shear intensity offers two mechanisms for heat loss: (1) conduction and (2) advection through to the Earth's surface. However, areas of low shear intensity will offer conduction alone or conduction with lesser amounts of heat loss by advection. Since over long periods of time heat has been lost by advection through the crust in areas of high shear intensity, and since heat loss by advection would be smaller in areas of low shear intensity, then obviously the sub-crustal temperature will remain 'higher' in areas of low shear intensity and higher heat-flow values will be observed to-day in those areas of low shear intensity—all other factors being equal. Therefore, there is indeed a reasonable and logical reason for the existing relation between high shear intensity and low heat flow.

Thus the theory of permanency of continents with the associated postulated movement of the axis of rotation not only explains the distribution of features on the ocean bottom and the shapes of the continents, but also offers the first theory for explanation of the gross features of heat flow through the Earth's crust. It should be noted that this is a considerable improvement over the theory of continental drift which requires doubtful convection currents to exist in the Earth's mantle and which requires them to flow 'backwards'.

There is additional evidence supporting the permanency of continents and the theoretical shear pattern. Tatsch[7], using a three-dimensional least-squares analysis, points out that about 93 per cent of all active volcanoes lie along a world-wide system of belts which may be defined approximately by the great circle traces of three mutually orthogonal planes passing through the centre of the Earth. These three planes are symmetric about 90°, as is the shear intensity and the world-wide heat flow.

Now it is seen that the shear pattern correlates well with two independent geophysical parameters, namely, heat flow and distribution of volcanoes. Most of the data for these parameters were gathered and reduced after the theoretical shear pattern was proposed. When one uses this form of 'predictability' or 'later day verification' as a method of comparing theories, then it should be noted that the theoretical shear pattern when combined with the permanency of continents offers a considerable improvement over the theory of continental drift which seems to be 'modified' almost every time some new 'evidence' is uncovered.

Comparison of the Shear Pattern with Continents

North America and Africa are the two continents to be evaluated. Before we go into the actual evaluation, it would be wise to point out a major difference between these two continents. This major difference in the continents is the apparent difference in their growth patterns. North America presents a growth pattern in which new areas of continental growth appear to be added to the

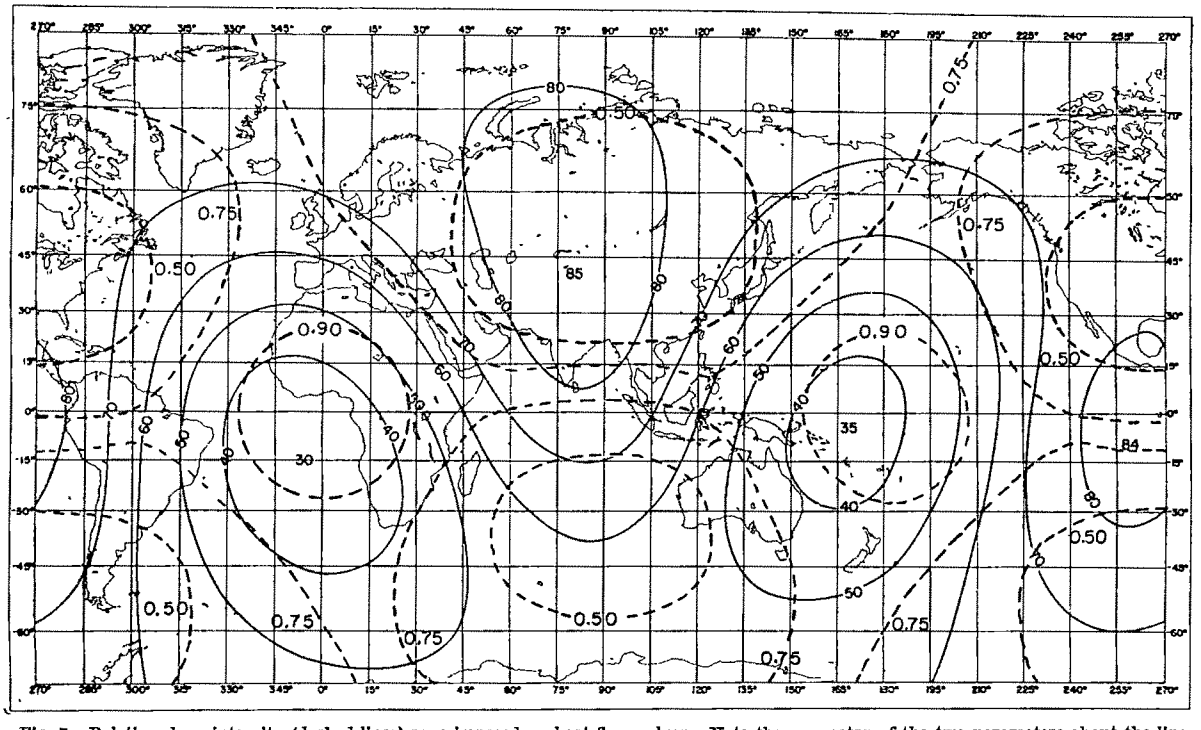


Fig 7. Relative shear intensity (dashed lines) superimposed on heat-flow values. Note the symmetry of the two parameters about the line 90° E. 90° E. is the line along which the north pole made its apparent movement into its present position

borders of the old areas. Thus, North America appears to have grown about some centre or nucleus. Africa, however, presents an entirely different picture. The orogenic patterns in the shield areas of Africa seem to cross one another in an apparently random pattern with no obvious nucleus present. These differences in continental growth patterns have led to some conceptual difficulties between geologists from different areas of the world. If Vening Meinesz's crustal shear pattern can explain some of the obvious differences between the two different continental types, then the shear pattern when combined with the permanency of continents will be helping to solve a problem that continental drift by its very nature will be unable to explain.

Table 2. Average Potassium–Argon Ages from the Dike Swarms as shown and numbered in Figs. 8 and 9

Dike swarm No.	Age in million years	Matches theoretical shear pattern
1	2,485	Yes
2	2,150	No
3	2,165	No
4	2,090	No
5	1,675	No
6	1,230	Yes
7	1,315	Yes
8	1,220	Yes
9	1,030	Yes
10	875	No
11	790	No
12	700	Yes
13	640	No

The data to be evaluated for North America are the distribution of diabase dike swarms of the Canadian Shield[8]. These dike swarms are shown in Figs. 8 and 9, and their isotopic ages are shown in Table 2.

The importance of the results shown in Table 2 is that there is a period from 1,315 million years to 1,030 million years where the correlation between the azimuths of the dikes and the azimuths of the theoretical shear pattern is fairly good. This implies that the shear pattern affected the continental regions as well as the oceanic regions and that the shift of the pole may very well have occurred at, or before, 1,315 million years ago. This evidence, on a different scale of structures from those used to evaluate the oceans, is important and the shear pattern theory is reinforced by the fact that the general trend of the Grenville Province, as shown in Fig. 9, seems to be well controlled by the theoretical shear pattern. Therefore, the shear pattern should have occurred before the development of the Grenville Province (950 million years ago) and more probably before the development of dike swarms, dated 1,315 million years. It is also reasonable to expect that local stresses applied to the Earth's crust after the time of shearing may be able to 'take advantage' of the pre-existing shears. Thus many young geological structures will be found to match the pre-existing shear pattern.

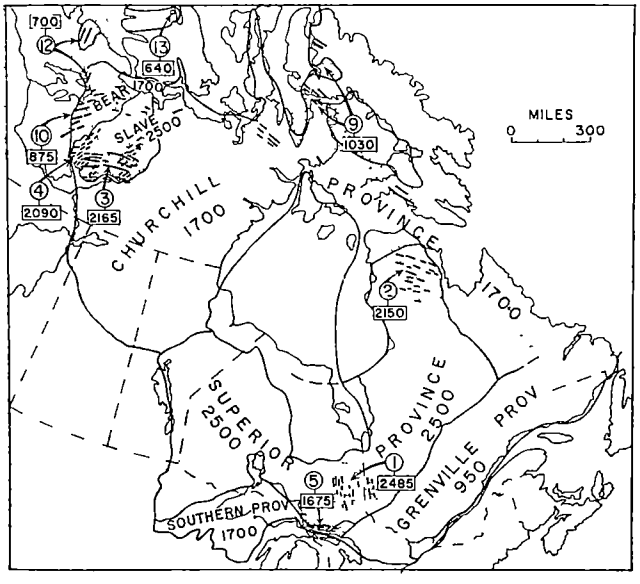


Fig. 8. The diabase dike swarms of the Canadian Shield. These swarms are shown in two figures for the sake of clarity. The geological provinces of the Canadian Shield are outlined by the solid lines and their approximate ages are given (in million years) below the name. The diabase dike swarms are indicated by the arrows pointing to them and the potassium-argon age is indicated in the boxed number below the encircled swarm number

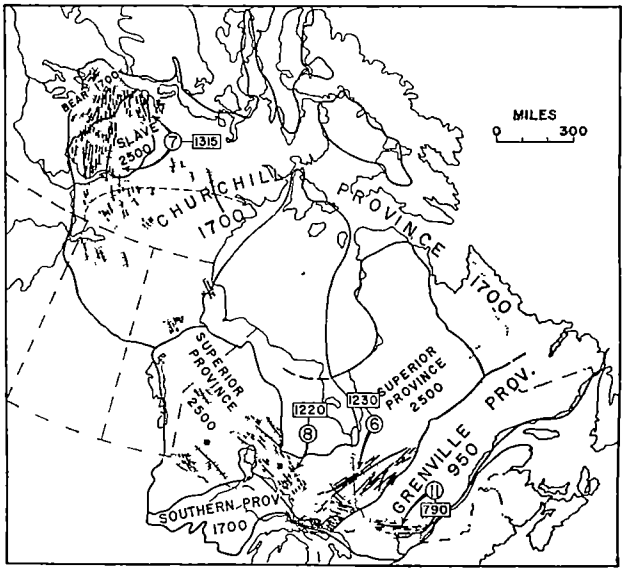


Fig. 9. The diabase dike swarms of the Canadian Shield (continued from Fig. 8)

Now, to test for significance of the foregoing data, consider the azimuth of any random shear. Azimuths of any such shear may be measured from 0° to 90° west of north and from 0° to 90° east of north. Thus a span of 180° will include all possible shears. Any shear the general trend of which is within 10° of the theoretical shear is considered to 'fit' the theoretical shear pattern. It should be borne in mind, however, that in any location it is possible to have two shear directions. Thus the probability of any random shear following the theoretical shear pattern is 2 (10° E. + 10° W.)/180° or 2/9. Treating the azimuths of dikes as a binomial distribution, the probability of six 'fits' out of thirteen dike swarms is 0·032. Since the probability is so low for the six 'fits' that are observed (see Table 2), then quite obviously the azimuths of dikes in North America are not random, but there appears to be an underlying control for many of the dikes and this control appears to be Vening Meinesz's crustal shear pattern.

Note that the North American data show: (1) that the trend of the Grenville Province follows the theoretical

Table 3. Dated Pre-Cambrian Structural Trends of Africa

Structure No	Age in million years	Matches theoretical shear patterns
3	3,400	No
18	2,944	No
8	2,800	Yes
17	2,550	Yes
9	2,250	Yes
4	1,850	Yes
19	1,650	No
7	1,600	Yes
15	1,500	No
16	1,500	Yes
6	1,000	Yes
14	660	Yes
10	625	No

Table 4. Undated Pre-Cambrian or Post-Cambrian Structural Trends of Africa

Structure No.	Age in million years	Matches theoretical shear pattern
1	?	Yes
2	?	No
5	?	Yes
11	?	No
12	?	Yes
13	?	Yes
20	?	No

shear and is dated at 950 million years ago; (2) that there are four 'fits' in a row, which implies the shift of the poles occurred before 1,315 million years ago, and (3) there is one 'fit' dated at 2,485 million years ago. The significance of this 'oldest fit' will be clarified by considering the results obtained from the African data.

The major structural trends for Africa are shown in Fig. 10 (ref. 9). Those structures that have been dated by isotopic means are listed in chronological order in Table 3 and the structures that have not been accurately dated by 1961 are listed in Table 4.

The probability of the twelve 'fits' out of twenty African structures being due to a random process is 0·00022. This makes Africa one continent out of 4,545 (based on a random distribution) while North America is only one continent out of 31. Thus the control exerted by Vening Meinesz's crustal shear pattern in Africa is much greater than is the control in North America. Note that this is predicted by the relative shear intensity (the dashed lines in Fig. 1) of the theoretical shear pattern.

Now, to return to the differences in growth patterns between Africa and North America, we note that (see Fig. 1) the shear pattern in Africa produces a cross-hatch pattern, while in North America the lines of shear converge to a point or are symmetrical about the point of convergence. Thus, if the shear pattern controlled continental growth, we would expect growth about a centre in North America or at least a lack of crossing structure due to the low values of the relative shear intensity. We would also expect Africa to have a very intense cross-hatch structure due to the high relative shear intensity and the nature of the shear pattern in that area. This is exactly what we see in the structure of these continents. Thus, the shear pattern is also capable of explaining the development of two apparent grossly different continental types which have long puzzled geologists. Perhaps when all the continents have been evaluated we shall know which of the two parameters plays the controlling part in continental growth, that is, the direction of shear in the shear pattern or the relative shear intensity.

To find out if that odd fit at 2,485 million years in North America has any significance, let us look at Table 3, which shows that there are 4 fits in a row in Africa starting out at 2,800 million years ago. Thus, it appears that the North American fit at 2,485 million years is not an accident but that the time of occurrence of the re-

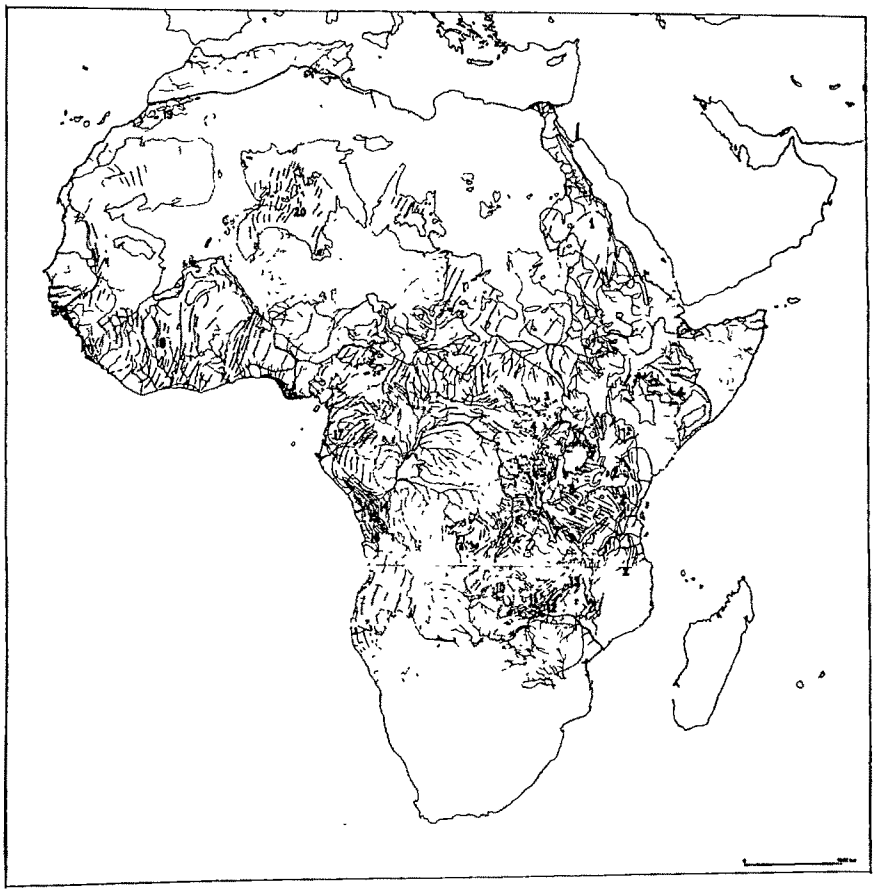


Fig. 10. Structural sketch of middle and northern Africa. In equatorial Africa the trends have been taken chiefly from original documents; in west and northern Africa, from River Furon, *Esquisse structurale provisoire de l'Afrique*, Paris (1959), Assoc. Serv. géol. africains. (1) Egypt and Sudan. Last events of a polycyclic evolution have been dated in Egypt (435–540 m.y.). (2) Mozambique belt. Last events of a possibly polycyclic evolution have been dated (465–636 m.y.). (3) West Nile belt. Considered extremely old; probably older than *c*. 3,400 m.y. (4) Groupe du Kibali. Probably older than *c*. 1,850 m.y. and probably linked with Buganda series to the north of Lake Victoria, which are certainly older than 1,850 m.y. (5) Groupe de l'Urundi and Karagqe Ankolean system, imprecisely dated but in physical continuity with No. 6. (6) Groupe des Kibara. Late orogenic events are dated at 1,000 ± 50 m.y. (7) Ubendian: Groupe de la Ruzizi. Postorogenic phase is dated at *c*. 1,600 m.y. (8) Nyanzian and Kavirondian. Common lead model ages which may date the Nyanzian are *c*. 2,800 m.y. (9) Dodoman. Older than 2,250–2,440 m.y. (10) Katangan belt. Late orogenic uranium mineralization (Shinkolobwe) at 625 m.y., postorogenic uranium mineralization (Zambia) at 500 m.y. (11) Irumi belt, undated, pre-Katanda. (12) Rufunza belt, older than Irumi. (13) Mafingi belt, probably part of the Late Pre-Cambrian–Early Palaeozoic activity. One age *c*. 440 m.y. (14) Groupe du Congo Occidental. Activity at *c*. 660 and *c* 475 m.y. (15) Groupe des Monts de Cristal (Système du Mayumbe); older than *c*. 1,500 m.y (16) So-called Basement of Northern Angola. Some of this belongs to the Système du Mayumbe (see No. 15). Other crystalline rocks are or may be much younger (17) Basement Complex of North Gabon and Southern Cameroons, *c* 2,550 m.y. or older. (18) West Africa; see text. (19) Anti-Atlas-Morocco. One age post-dates the earliest Pre-Cambrian in this area, 1,650 m.y (20) Hoggar. Undated area, generally correlated with the west African section

lative shifting of the poles occurred at least 2,800 million years ago. Confirmation or further pushing back of this date should occur as other continents are evaluated.

The inferences that can be drawn from the results of this research are: (1) That the shear pattern can be used to predict the directions of ocean bottom features that should be discovered in ocean areas so far unexplored. In fact, this is a method of testing the theoretical shear pattern. (2) That because certain directions are expected in ocean bottom mapping, optimum mapping procedures could be developed to uncover fully the ocean bottom topography in any ocean area. The Arctic Ocean will be the most difficult region in which to predict directions of topography (see Table 1). (3) That a drilling operation in a true ocean basin will uncover Pre-Cambrian rocks once the drill has passed through the layer of sediments and lava flows. The ages of these rocks could quite possibly be older than any known continental rocks. Even the finding of early Palaeozoic rocks will weaken the present theory of continental drift, because the theory postulates that the rock of the ocean basins is continually returned to the mantle by convection currents.

It should be noted that some gabbro from St. Paul's rocks on the Mid-Atlantic Ridge has been tentatively dated at 4,500 million years old[10]. Thus a region of an upward component of a convection current postulated by continental drift, which should be a region of very young rocks, possesses rocks which are older than any known continental rock.

In the light of (1) the new ocean bottom data confirming existence of the world-wide shear pattern, (2) the structural data of continental interiors dating the shear pattern as at least 2,800 million years old, and (3) the independent heat-flow data and the distribution of volcanoes reinforcing the generating mechanism of the shear pattern, it is concluded that the majority of the Earth's major topographic and geological features are controlled by, or grew along, major zones of weakness generated as part of the world-wide shear pattern. The preservation of this shear pattern on the ocean bottom for a period of 2,800 million years firmly enforces the theory of the permanency of continents.

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OBITUARIES

Dr. P. L. E. Rothschild

Dr. Paul Rothschild, who died on November 11, 1965, was born in Mannheim in 1901 and graduated M.D. at Freiburg in 1926. With the coming of the Nazi régime in 1933 he saw no future for himself in Germany, and moved at once, with his family, to England, and was naturalized in 1938. He obtained a British qualification in 1934 and from then on practised medicine in Britain: in a Welsh mining village (1934–37), in London (1937–42), in the Royal Army Medical Corps (1942–46), and again in London until his death.

It is written in *Ecclesiasticus*: "Honour a physician with the honour due unto him . . . for the Lord hath created him". Paul Rothschild was honoured by all his many patients, not only for his skill and wisdom but also because he knew that the Lord had created them, too, and he treated them so. One of his treasured possessions was a miner's lamp, the one he had used in the pit in his Welsh village when emergency called him. A patient once asked him what he would do if he learnt he would die at 3 p.m. next day. His answer, "I should see my last patient at 2.30", was true; he had known the precarious state of his heart for a long time, but continued to communicate his courage, by work and example, to others.

It was a good many years, after his M.D. in 1926, before he turned over finally to medicine. Two years were spent in a pathological institute in Berlin, then two years with Otto Meyerhof, first at Berlin-Dahlem and later at Heidelberg. That is how I came to know him, and also because of an admirable paper on the physiology of muscle, published in 1930 in the *Biochemische Zeitschrift*. Then he came to England to extend his scientific experience, working with Verney at University College, London, with Barcroft at Cambridge, and with Thomas Lewis and his colleagues at University College Hospital. Between 1927 and 1934 there are sixteen of his published papers referring to all this (published in the *Journal of Physiology*, *Heart*, *The Proceedings of the Royal Society* (B), *Biochemische Zeitschrift*, etc.).

After this scientific pilgrimage to England, Rothschild returned to Germany, as "Assistent" with the late Prof. Volhard at the University Hospital in Frankfurt. It was Volhard who had advised him originally to undertake all those years of scientific training, and but for Hitler he would probably have continued in clinical medicine in Germany. But he knew already that he would be welcome and happy in England, and the variety of his earlier scientific experience proved an important element in his later activities here.

During the earlier years of the Second World War, I saw him often at the Royal Society (I was then Biological Secretary). I cannot remember well how it happened—it may have been because of his concern for refugees—but somehow he was adopted by the officers and staff of the Society as a sort of unofficial medical adviser; and as time went on he became the personal friend and physician of many Fellows. At the time of the Tercentenary of the Society in 1960 his name was printed in the programme as "Honorary Physician", in case any of the guests needed help.

In the autumn of 1942 his long ambition to serve his adopted country more directly was satisfied and he was commissioned in the Royal Army Medical Corps. He served as adviser in medicine to the Persia and Iraq force, becoming a lieutenant-colonel, and retired in 1946. A pleasant story is told (by Sir Reader Bullard, in *The Times*) of an American officer in Iran, who had a bad attack of poliomyelitis, from which Rothschild's swift diagnosis and skilled and devoted care (and an iron lung supplied by Lord Nuffield) saved him. Whenever this officer visited England (and Rothschild) later, only a slight limp recalled the danger he had passed through.

From 1946 until his death, Rothschild worked in a large general practice in London. He is remembered by his medical colleagues for the breadth of his knowledge and the wisdom of his advice. And the 'honorary physician' of the Royal Society will be missed by his scientific friends at the functions there to which he regularly, and proudly, went.

His wife, and a son and a daughter, both married, survive him. A. V. HILL

Prof. E. Stiasny

PROF. EDMUND STIASNY, the venerable doyen of leather chemists, died at his home in Helsingborg, Sweden, on October 17 at the age of ninety-three. He was best known to chemists at large for his invention some fifty years ago of the so-called synthetic tannins. These 'syntans' have, structurally, little in common with the natural tannins. However, like these latter, they possess tanning properties, being condensed phenolsulphonic acids.

Stiasny's life-work was dedicated to the introduction of scientific principles into leather manufacture and to the elucidation of the intricate chemical reactions in the tanning process. Together with the late Prof. H. R. Procter, his predecessor as professor of leather chemistry at the University of Leeds, he was a pioneer in the application of science to an ancient art, which was becoming an industry.

Stiasny was born in 1872 in Vienna. He obtained his doctorate at the University of Zürich in 1898. His head professor was Alfred Werner, the originator of co-ordination theory. Stiasny entered on his life's work in 1899 at the Viennese Research Station. At this time little attention was given to the theory of tanning. However, the young scientist could not resist the urge to elucidate the chemistry of these mysterious processes.

His results were very favourably received internationally. Thus, the University of Leeds, seeking a successor to Prof. H. R. Procter, the father of leather chemistry, asked Stiasny to accept this chair. Prof. Stiasny's association with Leeds was of short duration (1909–14), because of the First World War, but it became exceedingly fruitful. While the discovery of the syntans takes the most conspicuous place, the renaissance of teaching and research under Stiasny was perhaps still more important for the Department. He used to dwell on this happy period in his life with pleasure, recalling incidents with his enthusiastic students, the Atkin-Thompson team and Donald Burton, who were to follow him as incumbents of his chair.

He served throughout the First World War as an artillery captain in the Austrian Army. Afterwards he found refuge in Helsingborg, his wife's home town, until, in 1920, he was offered the chair at the Technische Hochschule, Darmstadt. A period of great activity followed (1920–33). An international élite of enthusiastic graduate students flocked about this charming, inspired and inspiring master, who was such a salutary contrast with the formal and stereotyped German professor of those days. Here he endeared himself to his disciples for ever. His studies of protein chemistry problems during the early years were evidently stimulated by Jacques Loeb's *Proteins and the Theory of Colloidal Behavior*. The effect of lyotropic neutral salts on gelatin was investigated using optical rotation measurements, one of the earliest studies using this now-favoured technique. However, his main research concerned the chemistry of basic chromium salts and their tanning potency, resulting in a score of dissertations. By uniting the Ol-structure of basic salts (conceived by Pfeiffer, his life-long friend) and the Bjerrum concept of the protolysis of chromium salts, Stiasny and his school extended our knowledge of these compounds fundamentally. In Stiasny's main work, *Chromgerbung* (1931), the substance of these researches is lucidly presented.

Noticing the *mene tekel* of the approaching deluge, Stiasny resigned from his chair. From 1934 he made his home in Helsingborg. It became a place of pilgrimage for his former students, other friends and colleagues of many lands.

During this period of enforced idleness a welcome interregnum of creative work came with the outbreak of the Second World War. With the supply of tannins becoming critical due to the blockade, the Swedish industry induced Stiasny to develop a domestic syntan manufacture. The research resulted in an industrial production of high-grade syntans. Stiasny's contribution to his adopted country was recognized by the Government with the award of a decoration. By the vicissitudes of fate, the invention of a person exiled by the Nazis came to play an important part in the war effort.

Stiasny was a man of stately exterior, engaging manners and wide culture, and with a warm humanity. As a lecturer and teacher, he was brilliant. His linguistic sense and versatility were exceptional. His writing was lucid, concise and unaffected. The victim of military and political upheavals in the prime of his scientific sphere of activity, with severe personal tribulations as a result, his optimistic way of looking at life, fortified with a dose of stoicism, stood him in good stead. He leaves no near relatives. In the relief of refugee children, he found his last mission in life. K. H. GUSTAVSON

NEWS and VIEWS

Editor of *Nature*: Mr. J. Maddox

JOHN MADDOX, an assistant director of the Nuffield Foundation, has been appointed editor of *Nature* in succession to the late L. J. F. Brimble. It is intended that he shall take up his appointment as soon as he can be released from his present duties as co-ordinator of the Nuffield Science Teaching Project—probably at the end of the present academic year. In the meantime, he will spend some of his spare time helping with the production of *Nature*. Mr. Maddox, who was born in Swansea in 1925, read chemistry at Christ Church, Oxford, before turning to theoretical physics as a research student at King's College, London, under Prof. (then Dr.) C. A. Coulson. In 1949 he was appointed to the University of Manchester as an assistant lecturer and later lecturer in theoretical physics and was chiefly engaged on problems of thermodynamics and statistical mechanics. In 1955, Mr. Maddox joined the *Guardian* (then still the *Manchester Guardian*) as science correspondent, first in Manchester and then London. In that capacity he worked as a reporter of events, a popularizer and as a writer of leaders. Until a few years ago he had been a regular contributor to the *New Scientist*—in fact, he had been associated with that journal since its inception. He worked as science correspondent of the *Washington Post* for several months in 1960, and was chairman of the Association of British Science Writers for two separate terms. In 1962 Mr. Maddox joined the faculty of the Rockefeller Institute, New York, for an academic year. During the past two years he has administered the Nuffield Science Teaching Project—the programme of curriculum develop-

ment begun in 1961 and intended to lead to the design of materials and methods to help with the teaching of science in British schools. Material for use in Ordinary-Level courses for the General Certificate of Education is to be published in May, and the programme as a whole will be completed in 1968.

Prof. J. H. Quastel, F.R.S.: Sixty-fifth Birthday Tribute

THE July 1965 issue of the *Canadian Journal of Biochemistry* consists of scientific papers dedicated as a sixty-fifth birthday tribute to Prof. J. H. Quastel by present and former colleagues in Canada. In brief introductory biographies, Drs. R. M. Johnstone, P. G. Scholefield and K. A. C. Elliott mention some of the major biochemical research fields which have been opened up by Quastel. These include his well-known work at Cambridge on the nature of enzymes and enzyme inhibition, at Cardiff on the biochemistry of the brain and the action of narcotics, at Rothamsted on soil metabolism, soil texture and weed killer, and at Montreal on the biochemistry of cancer, on the action of drugs and on the mechanism of transport across membranes. Sixty-six authors from eight different countries have taken part in this birthday tribute. On retirement from his administrative duties as a professor in the Department of Biochemistry at McGill University, Dr. Quastel will continue his research work as director of the McGill Unit of Cell Metabolism.

Science Administration in the National Institute of General Medical Sciences, National Institutes of Health

Dr. Robert S. Melville and Dr. Carl A. Kuether have been appointed scientist administrators with the Research Grants Branch of the National Institute of General Medical Sciences, National Institutes of Health. The National Institute of General Medical Sciences administers the majority of the basic science research grant projects of the National Institutes of Health. Both Dr. Melville and Dr. Kuether will serve in the Biochemistry Section of the Research Grants Branch, and will be responsible for handling the reviews of applications and the administration of grants for a major segment of the Institute's programme.

Dr. R. S. Melville

Since 1963, Dr. Melville has been chief biochemist and laboratory requirements specialist in the Veterans Administration Central Office. He was previously the chief biochemist in the Veterans Administration Hospital in Iowa City from 1954 until 1963, and a clinical biochemist at St. Luke's Hospital, Chicago, during 1950–54. During the Second World War, Dr. Melville was chief chemist in the Sixth General Hospital Laboratory, United States Army. From 1939 until 1942 he was a research chemist with the Massachusetts General Hospital. Dr. Melville received an A.B. degree from Clark University, Worcester, Massachusetts, in 1937. In 1950 he was awarded a Ph.D. in biochemistry from the State University of Iowa. In July 1965, Dr. Melville was appointed chairman of the Committee on Clinical Chemistry for the Chemistry and Chemistry Technology Division of the National Research Council–National Academy of Sciences. He is a member of the American Association for the Advancement of Science, the American Chemical Society, the Instrument Society of America, Sigma Xi, Phi Lambda Upsilon, Gamma Alpha, a director of the American Board of Clinical Chemists, and a Fellow of the American Association of Clinical Chemists.

Dr. C. A. Kuether

Since 1962, Dr. Kuether has been with the National Science Foundation. Previously, he was associate professor of chemistry at Youngstown University, Ohio, during 1960–62. He has also worked as a senior research biochemist at Eli Lilly and Company, Indianapolis (1951–60), assistant professor of biochemistry at the University of Washington, Seattle (1946–51), instructor and senior instructor in biochemistry at Western Reserve University (1943–46), and teaching Fellow and instructor in biochemistry at the George Washington University (1940–43). Dr. Kuether received an A.B. degree from Miami University, Oxford, Ohio, in 1936, and an M.S. from Wayne State University in 1949. In 1943 he was awarded a Ph.D. in biochemistry from the George Washington University. He is a member of the American Chemical Society, the American Association for the Advancement of Science, the American Institute of Biological Sciences, the Ohio Academy of Science, Phi Eta Sigma, and Sigma Xi, and is a professional lecturer in biochemistry at the George Washington University.

Crop and Animal Husbandry in the Queen's University of Belfast : Prof. J. Morrison, O.B.E.

PROF. JAMES MORRISON retired from the chair of crop and animal husbandry at the Queen's University of Belfast on December 31, 1965, having been specially asked to act until this date to allow for the appointment of his successor, Dr. J. C. Murdoch, by the joint board of the Civil Service and the University. Morrison had a good practical background as an agriculturist, having been directed to work on his home farm in Banffshire during the First World War. Thus he did not graduate, after a brilliant student career at Aberdeen, until 1924. He went to Northern Ireland in 1925 and rose rapidly in the county agricultural service and the Ministry of Agriculture before accepting a transfer in 1933 to the joint appointment in the University and Ministry's Research Division. His keen and practical approach to the teaching of husbandry and his interest in his students were notable from the start. During the Second World War, he was the Ministry's principal officer in charge of the tillage campaign in Northern Ireland, while continuing University work, and in 1947 he toured Poland with the Food and Agriculture Organization mission. Morrison was well known as a leader of agricultural developments, through his numerous extra-mural activities and especially for his work as director of the Agricultural Research Institute at Hillsborough. Here his own special interests have given emphasis to the production, conservation and utilization of grass, illustrating again his practical approach to the special problems of farming in Northern Ireland.

Prof. J. C. Murdoch

DR. J. C. MURDOCH has been appointed to the chair of crop and animal husbandry in Queen's University, Belfast, in succession to Prof. J. Morrison. Dr. Murdoch was born in Ayr. He graduated in agriculture at the University of Glasgow in 1947 and was awarded a Ph.D. in 1950 for work on the growing of sugar beet in Scotland at the University of Edinburgh. Since 1951, as a member of the staff of the National Institute for Research in Dairying, his work has been concerned with the conservation and use of grass products. He has made a special investigation of the factors affecting the losses involved in ensiling grass and their effect on the value of grass silage for milk production. His investigations have ranged from grass quality and field losses through problems of conservation and feeding to the effects of grass products on milk yield and milk quality. His work on silage making has been particularly useful to milk producers in providing evidence on the importance of the basic principles involved in the ensiling process and on the relative insignificance of some of the more glamorous additives which are from time to time recommended for inclusion in silage. His experiments on the factors in silage which affect intake and digestibility and the effect of feed supplements to grass products have found direct application in newer systems of cattle feeding involving *ad libitum* feeding of grass products. Apart from many technical papers on the

production and use of grass products, Dr. Murdoch is the author of a text-book on *Making and Feeding Silage*.

Physical Chemistry at the National Bureau of Standards : Dr. H. White

Dr. Howard White has joined the Institute for Basic Standards of the National Bureau of Standards as assistant chief of the Physical Chemistry Division. In this post, he will direct the work of the Division, particularly as it applies to the physical chemistry of liquids and solutions. Before joining the Bureau, Dr. White was special assistant to the Assistant Secretary of the Navy for Research and Development, where he provided staff functions relating to the provision of funds for, and management of, research in the Navy. From 1959 until 1961, he carried out a research programme in polymers and textiles at Stanford Research Institute. Before this he worked at the Textile Research Institute, where he became director of research and helped to develop and maintain a graduate educational programme in co-operation with Princeton University. Born in Batavia, New York, Dr. White did undergraduate work at Princeton University, where he received an A.B. in 1942. His postgraduate studies took place at the University of Wisconsin, which granted him an M.S. in 1944, and Princeton, where he took his Ph.D. in 1947.

The Royal Society

The report of the Council of the Royal Society for the year ended September 30, 1965, records that progress in planning, etc., concerned with the reconstruction of Nos. 6–9 Carlton House Terrace was slower than expected, and although reconstruction began before September 30, 1965, furnishing and fitting out for occupation will only be completed by July 1967. Besides two additional research professors appointed in November 1964, the first three Leverhulme scholars were also appointed in that month, and in June 1965 the second S. C. Brown Award and Medal was given to Mr. F. T. Bacon for his work on the development of fuel cells. The Royal Society's expedition to the Solomon Islands commenced its field work on July 1, 1965, and a Tristan da Cunha study group was set up to consider the need for continuing facility in the Tristan group of islands to promote biological and geological studies. After examination of a proposal to set up study groups which would enable scientists from a wide range of disciplines to discuss subjects of topical and national interest, a population study group was appointed in April 1965, and an *ad hoc* committee is considering the desirability of establishing a group to deal with long-term questions concerned with water as a resource. The Scientific Research in Schools Committee is at present assisting one hundred and seventeen research projects, and supported, for the second year, a project at the Procter Department of Food and Leather Science, University of Leeds, in which two science masters joined a research team in food science research. The report also summarizes briefly the relations of the Society with the international scientific unions and with antarctic, oceanic, geophysical and space research as well as with the International Biological Programme. Parliamentary grants administered by the Society for 1965–66 totalled £398,000 compared with £251,000 in 1964–65. Research professorships accounted for £82,500, scientific investigations for £113,000, and £124,250 went to international research and scientific congresses, with another £43,000 for the International Biological Programme: £13,450 was spent on scientific publications and £2,500 on libraries assistance.

National Research Council of Canada

The *NRC Review, 1965* (National Research Council of Canada, Ottawa. Pp. 428. NRC No. 8597. Ottawa: National Research Council of Canada, 1965. 2 dollars), provides the usual comprehensive reference work on the activities of the National Research Council of Canada in 1964–65, including those of Canadian Patents and Development, Ltd. Together with the financial report, organization chart and list of members of the Council 1965–66, it includes details of membership of the numerous committees of the Council and a Directory of Staff. There are brief reports from the several divisions on work in progress and to each of these is appended a list of publications during the year. A brief review of the work of the Medical Research Council is also included.

National Museum of Canada : Anthropology Papers

In Anthropology Papers No. 10, Mr. Donald H. Mitchell gives the results of preliminary excavations at a cobble tool site in the Fraser Canyon, British Columbia (National Museum of Canada. Anthropology Papers, No. 10: *Preliminary Excavations at a Cobble Tool Site (DjRi 7) in the Fraser Canyon, British Columbia*, by Donald H. Mitchell. Pp. 20+11 plates. Ottawa: Queen's Printer, 1965). For several years surface finds of early artefacts have been made in this area and the excavations were undertaken to determine if the earlier surface collections with their absence of bi-facially worked artefacts and projectile points were truly representative of the assemblage; to locate artefacts *in situ*; to determine the depth of culture-bearing deposits; and to locate areas of artefact concentration. In general, it was found that the character of the assemblage is essentially the same as depicted by the earlier surface collections: on the first terrace, artefacts and clipping detritus were found to a depth of 44 in. below the surface, and significant clustering of the artefacts is not apparent. The age of the finds is, as yet, uncertain, but according to our present understanding of Fraser Canyon glacial history, the deposits were all river-laid during Post-Pleistocene times.

Salisbury and South Wiltshire Museum

The annual report of the Salisbury and South Wiltshire Museum 1964–65 is a convincing denial of the rumour that the Museum would have to close unless substantially greater funds became available (Pp. 24+4 plates. Salisbury, Wilts.: The Museum, 1965). Now, it is not so much a question of whether it will survive, but in what form it will survive. The report states that steps are actively being taken to increase the revenue from public sources, to increase the membership, and to appeal for capital funds if the first and second aims are successful. Both the County Council and City Council have increased their grants and the local rural district councils have made grants for the first time. Two research projects include the investigation of a possible new alignment for the Roman road Old Sarum to Dorchester and the continuing excavation of the deserted medieval village site of Gomeldon. The amount of voluntary help given to this Museum on the inspiration of the curator continues to be very impressive.

Salamandra

A new journal, *Salamandra*, to deal with the biology of Amphibia and Reptilia, is being published in German (*Salamandra*, Band 1, Helft 1/2; September 1965. Herausgegeben von der Deutschen Gesellschaft für Herpetologie und Terrarienkunde. Pp. 52. Frankfurt/Main-Niederrad: Deutsche Gesellschaft für Herpetologie und Terrarienkunde, 1965). It will be issued quarterly and each article will contain an English summary; all illustrations and photographs will have an English caption. *Salamandra* will also publish reviews of new books and papers on herpetology, including those published in Russia, China, and other Eastern countries. The first issue (September 1965; 1, Parts 1/2) contains an article to celebrate the seventieth birthday of Prof. Robert Mertens. Other articles are by Gerhard Benl,

Pure Chemistry Library

"Neotenie und Albinismus bei *Triturus vulgaris vulgaris*"; Hehuda L. Werner, "Uber die israelischen Geckos der Gattung *Ptyodactylus* und ihre Biologie"; "Leuchtsfflammpen Gro Lux in der Reptilienpflege", by John Merhtens; "Gegen *Strongyloides-Infektionen* bei Reptilien", by Dieter Backhause; and "Zur Biologie der Rassen von *Echis carinatus* (Schneider) 1801", by Othmar Stemmler-Gyger. The journal is mailed only to members of the Society Deutsche Gesellschaft für Herpetologie und Terrarienkunde e V. Membership of the Society is open to all interested individuals, other societies and institutions. Annual subscriptions are DM 12 (plus DM 2 for entrance fees) within Europe, and 4 dollars (plus 0·5 dollar for entrance fee) abroad. Further information can be obtained from Dr. D. Backhaus, 6 Frankfurt/Main-Niederrad, Gerauer Strade 69b, or from Alfred A. Schmidt, 6 Bergen-Enkeim bei Frankfurt/Main, Muhibachstrade 5.

Freshwater Habitats of *Culex pipiens fatigans* in Rangoon

C. H. Fernando, of the Department of Zoology, University of Singapore, investigated twenty-one freshwater habitats in Rangoon during February 1963 in relation to the ecology of *Culex pipiens fatigans* (Wiedemann) (*Ceylon Journal of Medical Science*, 13; December 1964). Ten habitats were positive and eleven negative at the time of the investigation. They represent a fair range of freshwater habitats in Rangoon and show a range of pollution conditions. The fauna and flora show an overall reduction with increase of pollution and, among the fauna, a high proportion of 'pan South-East Asian' species occur in polluted habitats. The bacterial flora was studied very cursorily and needs more detailed investigation. In *C. fatigans* the tolerance for most natural organic and inorganic materials is high, though physical factors may influence densities. Oviposition sites may be of considerable importance for high densities of *C. fatigans*. High densities of this insect are associated with decaying organic matter, unsuitability for other fauna and flora in general and the accompanying reduction of predatory animals, especially insects. The Notonectidae are perhaps the most important predators of *C. fatigans* and these penetrate into polluted waters also. In the most heavily polluted habitats, *Armigeres* might well prove a competitor and even a predator of *C. fatigans*.

Microwaves in Action

Many phenomena affecting our everyday life are manifest in the form of radiant energy or electromagnetic waves. These vary greatly in length and in frequency of vibration; the longest are more than 6,000 miles from crest to crest with vibration only 30 times per sec; at the other extreme are cosmic rays as short as $1{\cdot}5 \times 10^{-9}$ in., with vibrations more than 30×10^{21} times per sec. An informative chart in colour and entitled "The Radiant Energy Spectrum", covering the full range of wavelengths and frequencies, is featured in *Vectors*, a quarterly publication of the Hughes Aircraft Company of California (7, No. 3; third quarter, 1965. Hughes Aircraft Co., Culver City, California). Selected in this issue for special discussion are the micro-waves, and in an account of their uses and potentialities, in an article entitled "Microwaves in Action", some interesting details of their place in the radiant energy spectrum and in industry are given. The microwave region is now generally considered to span waves from about 30 cm down to about 0·1 mm in length, with frequencies between 1,000 and 5×10^6 megacycles; the part below 1 cm wave-length is known as the millimetre-wave region.

Among some of the valuable properties of microwaves are that they can penetrate fog or haze; this form of radiation can be sent out and controlled like a searchlight beam, and if part of that beam strikes an object, it is reflected back; this is the basis of the ranging and detecting devices called 'radar'. Microwaves are well adapted for communications work; they can be transmitted in a line-of-sight pencil beam from one place to another, and in this application they permit many simultaneous channels of communication. A more recent application of microwaves—one destined to grow in importance—is for cooking foods; here the waves penetrate the food, which absorbs them in the form of heat. They are used not only for roasting, but for drying, defrosting and frying. In industry, microwaves find many outlets, such as control of materials, including moisture content; in printing they are used for drying inks in colour lithography, reducing delays of hours to a matter of minutes. Diathermy has long been used for the relief of pain by dielectric heating of body tissue, frequencies for this purpose being about 10 Mc/s; it has been found, however, that microwaves of 2,400 to 2,500 Mc/s may be more efficient because they can be directed to specific areas of the body without contact. It is predicted that microwaves have a brilliant future; microwave receivers in homes and schools receiving television direct from *Early Bird* satellites is one of the most exciting possibilities suggested.

Concrete Staved Silos for storing High-moisture Barley

Among its many and varied activities, the Agricultural Research Council sponsors an Experimental Farm Buildings Scheme which provides for co-operation with farmers in investigations of experimental farm buildings and of experimental ideas affecting buildings. Grants are made towards the capital cost of these buildings and in return farmers provide data for a limited period about the buildings and generally about the operations conducted therein. The practical application of this scheme is well illustrated by the publication of a recent report entitled: "The Use of Concrete Staved Silos for Storing High-Moisture Barley" (*Experimental Farm Buildings—Report No. 4*. By H. J. M. Messer, J. M. Hill, Dr. R. Whittenbury and Dr. J. Lacey. Pp. 24. London: Agricultural Research Council. Obtainable from H.M.S.O., 1965. 3*s*. 6*d*. net). This report is in three parts; (*a*) effect on grain temperature, nutrient content, *p*H value and the composition of the intergranular air, by H. J. M. Messer; (*b*) microbiological changes in ensiled grain, by R. Whittenbury; and (*c*) interim report on the micro flora of airborne dust from moist-stored barley grain, by J. Lacey. In summary, seven concrete staved silos designed for unloading from the top were filled with damp barley from the 1964 harvest. Records of harvesting, filling, unloading and rolling the barley were kept throughout the experiment; changes in the stored grain and in the atmosphere in the silos were determined. It was found that conditions in the silos were sufficiently anaerobic to prevent serious deterioration of the grain so long as it was removed to a greater depth than 3 in. each day. No difficulties were experienced with unloading from the top. Trials of feeding and digestibility with dairy cows indicated that there was no significant nutritional difference between this barley and conventionally dried barley. From the biological point of view, it was found that yeasts and lactic acid bacteria were the dominant micro-organisms in anaerobically stored barley carrying 18–40 per cent moisture. Potentially pathogenic fungi and actinomycetes were found in all silos, but their numbers were minimized by efficient top sealing and by using the barley at a sufficiently fast rate to avoid self-heating. A precautionary measure is noted: efficient dust respirators should be worn when entering silos containing high-moisture grain.

Royal Society of South Africa

The following have been elected Fellows of the Royal Society of South Africa: Dr. L. H. Ahrens, Dr. D. H. Davies, Dr. V. Fitzsimons, Dr. E. S. W. Simpson and Dr. E. M. van Zinderen Bakker.

Fisheries Division of the Food and Agriculture Organization of the United Nations

On January 1 the Fisheries Division of the Food and Agriculture Organization of the United Nations became a department, consisting of two divisions, a Fishery Resources and Exploitation Division, the acting director of which is Mr. S. J. Holt, and a Fishery Economics and Products Division, the acting director of which is Mr. R. W. Harrison. Mr. R. I. Jackson, director of the former Fisheries Division, has been appointed assistant director-general, and Mr. F. E. Popper has been appointed director of programme co-ordination and operations for the new department, and will act as deputy to Mr. Jackson.

Academy of Sciences of the Socialist Republic of Romania

The following have been elected honorary members of the Academy of Sciences of the Socialist Republic of Romania: Prof. J. D. Bernal (professor of crystallography at Birkbeck College, London); Prof. A. V. Hill (emeritus professor of physiology in the University of London); Sir Robert Robinson (emeritus professor of chemistry in the University of Oxford); Dr. G. Constantinescu. Sir Rudolph Peters (professor emeritus of biochemistry at the University of Oxford) has been elected a corresponding member of the Academy.

Institution of Mechanical Engineers : James Clayton Fellowship

A James Clayton Fellowship, tenable for 2 years, to enable an engineer to work in collaboration with surgeons and doctors on a suitable project, is to be awarded by the Institution of Mechanical Engineers on the recommendation of its working party on medical engineering. The sum granted will be adequate to cover living expenses during the tenure of the award and the value of the Fellowship will be assessed accordingly. Applications, on forms available from the Institution, 1 Birdcage Walk, London, S.W.1, are required not later than April 13, 1966, from candidates, who must be more than 23 and have obtained an engineering degree or have satisfied the Institution's examination requirements for graduate membership by some other means. They should preferably have had not less than 18 months' practical experience in industry. Normally, applicants for the James Clayton Fellowship must present a planned project for approval, but in this case a successful applicant who has a strong inclination towards medical work but no direct contacts in that field will be helped to find a suitable project.

A. A. Benedetti-Pichler Memorial Award in Microchemical Techniques

The American Microchemical Society has created a fund to commemorate the work of the late Dr. A. A. Benedetti-Pichler. The award will be made to an individual who has made outstanding contributions to the practice or teaching of micro-techniques. The award will be given at regular intervals with the understanding that the recipient will give a lecture at a gathering of analytical chemists covering the field for which the award has been designed. Nominations are now being sought and must be in the hands of the committee by April 1, 1966. A letter of 300 words or less, giving the name and address of the nominee and citing, in general, his work, should be sent to the chairman of the 1966 committee, Mr. David B. Sabine, U.S. Vitamin and Pharmaceutical Corporation, Yonkers, New York. The Fund is not closed, and gifts payable to the A. A. Benedetti-Pichler Memorial Fund may be sent to the chairman.

University News:

Belfast

Dr. F. A. Vick, former director of the Atomic Energy Research Establishment, Harwell, has been appointed vice-chancellor.

Birmingham

Dr. T. S. M. Maclean has been appointed senior lecturer in the Department of Electronic and Electrical Engineering. The following Research Fellows have also been appointed: Dr. S. M. Amir and P. J. Somers (chemistry); Dr. I. E. Harris (physical metallurgy). The title of reader has been conferred on the following: Dr. J. S. Hough (applied biochemistry and brewing science); Dr. D. G. Walker (biochemistry); Dr. J. H. Edwards (human genetics).

London

The title of professor of geology has been conferred on Dr. J. G. Ramsay in respect of his post at the Imperial College of Science and Technology. The title of reader in geography has been conferred on Dr. J. H. G. Lebon in respect of his post at the School of Oriental and African Studies.

Announcements

The following have been elected honorary members of the Genetical Society: Prof. N. P. Dubinin, Dr. M. Demerec and Prof. C. D. Darlington.

Dr. B. Benjamin has been elected president of the Institute of Actuaries in succession to Sir Herbert Tetley, whose term of office will expire on June 13.

Dr. W. Tuve has been appointed chief of the Research Training Grants Branch, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland.

The fourteenth annual general meeting of the Society for Water Treatment and Examination will be held in London on March 11. Further information can be obtained from the Secretary, Society for Water Treatment and Examination, 41 Carshalton Road, Sutton, Surrey.

An exhibit featuring the meteorite which fell at Barwell, Leicestershire, on December 24, 1965, is now mounted at the Geological Museum, London. The exhibit includes fragments of the meteorite, an account of its fall and photographs and maps of the locations where fragments have been found.

A symposium on "Non-linear Optics", organized by the National Academy of Sciences of India, will be held at Agra during February 26–28. Further information can be obtained from Prof. Vachaspati, Department of Physics, University of Allahabad, Allahabad.

A conference on "Stresses in Structures and Machinery", arranged under the auspices of the Institution of Civil Engineers, will be held at the Institution during March 3–5. Further information can be obtained from the Institution of Civil Engineers, Great George Street, London, S.W.1.

The twentieth annual symposium on fundamental cancer research entitled "Carcinogenesis: A Broad Critique", arranged by the University of Texas M. D. Anderson Hospital and Tumor Institute, will be held in Houston during March 7–9. Further information can be obtained from the Symposium Committee, University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas.

A celebration to mark the centenary of the birth of Sir Arthur Keith (1866–1955), conservator of the Hunterian Museum and master of the Buckston Browne Farm, will be held at the Royal College of Surgeons of England on February 14. An introductory address will be given by Lord Brock and short papers delivered on aspects of Sir Arthur's work at the Royal College of Surgeons. The Sir Arthur Keith Memorial Lecture will be delivered by Sir Wilfrid Le Gros Clark. Further information can be obtained from the Secretary, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2.

UNITED KINGDOM ATOMIC ENERGY AUTHORITY

THE eleventh annual report of the Atomic Energy Authority covers the year ended March 31, 1965*. During this period the Authority's staff continued to decrease, but only by about 3 per cent compared with 5 per cent in the previous year, the reduction in the civil research and development programmes being very slight. By March 31, 1965, nuclear reactors in the United Kingdom had fed some 2,600 million units of electricity to the grid, substantially more than the total for the whole of the West outside Britain, and shortly before the end of the year the prototype advanced gas-cooled reactor at Windscale completed two years' operation, in which it had produced more than 350 million units of electricity for the grid and demonstrated a plant availability of 85 per cent. It is already clear from tenders for the Dungeness *B* station of the Central Electricity Generating Board that, whichever reactor station is chosen, nuclear power will be fully competitive from the beginning of the second nuclear power programme.

In 1964–65 expenditure from parliamentary grants on the Authority's programme of civil research and development was £47·5 million, of which £37·5 million was current expenditure and £10 million was for capital facilities. Some 2,840 graduate or professional engineers and scientists were employed on the programme on March 31, 1965, of whom 160 were working on projects for other organizations; 360 were employed on basic research, for which expenditure at present is estimated at £5 million and capital expenditure at £1·5 million; 80 on isotope research, for which expenditure at present is estimated at £500,000; 120 on basic research into health and safety, for which the expenditure at present is £1 million; and 200 on plasma physics and fusion research, for which expenditure at present is £3·5 million and capital expenditure is £1·5 million. In the reactor research and development programme 500 were employed in work on gas-cooled systems (current expenditure £9 million, capital £1 million); 480 in work on water-moderated systems (£5·5 million and £4 million, respectively); 575 on fast systems (£9 million and £1·5 million, respectively); and 365 on general reactor technology (£4 million and £500,000, respectively). The report refers to the new requirement under the Science and Technology Act, 1965, for the Authority to undertake research and development in science and technology outside the nuclear energy field, and the first major application is expected to be a programme of development of desalination techniques.

Research on reactor systems includes a continuing programme of nuclear measurements to provide data for calculating the physical characteristics of proposed reactors, and equipment has been installed which stores and processes the experimental information obtained from a modern high-intensity machine, and at the same time allows the experimenter to inspect the results as they come in, by displaying them on a cathode ray oscilloscope. The installation is probably the first of its kind in Europe. General investigations of the physics of reactor systems have included measurements in thermal systems of neutron energy spectra to discover whether the processes of energy transfer between neutrons and moderator atoms are correctly described by proposed scattering laws. The novel reactor types and applications being investigated include the fused salt reactors, which show long-term promise. Progress has been made in the field of control science, with the ultimate aim of achieving computer control of nuclear power plants, the main areas of progress being in optimal theory, studies of experimental dynamics and analytical techniques. Investigations into the behaviour of ceramic fuels at high burn-up included investigations of uranium dioxide and the mixed oxides of uranium and plutonium. Research on canning and structural materials has been concerned with the effects of irradiation, with corrosion and with methods of fabrication. To improve understanding of the effects of irradiating graphite, further work has been carried out on the formation and growth of defects in this material, and the investigation of the reaction between irradiated carbon dioxide and graphite has been continued. Research on heat transfer is now concentrated on problems connected with water and steam coolants.

General research has covered work on materials and their properties, and the effect of irradiation on them; on processes, metallurgical and chemical; nuclear and theoretical physics; instruments and techniques and the direct conversion of heat to electricity; and stress is laid on close collaboration with the university research departments, as well as with industry and overseas agencies. Experiments on the chemical effects of irradiation have been supported by researches into the basic principles underlining these effects. That on ceramic fuels is supported by research into the basic properties of ceramic materials generally. Work on the physical properties of plutonium has included measurements in the alpha phase and on stabilized alloys of some of the other phases. The reasons for the brittle nature of beryllium are now beginning to be understood and research has continued on fluidization, the process in which solids are made to behave to some extent like liquids by blowing gas through a bed of fine particles, and it is now possible to predict, on a logical basis, the behaviour of a fluidized bed in which a chemical reaction is occurring, at least in simple systems.

Research in experimental nuclear physics is directed to the elucidation of nuclear structure and of the basic nuclear interaction forces, while a major effort at Harwell is devoted to studying solid-state physics and the theory of radiation damage. Research on solid-state particle detectors has also continued, while the exploratory programme at Harwell on methods for directly converting heat to electrical energy has continued, with further examinations of the feasibility of the magneto-hydrodynamic method using non-equilibrium ionization of the hot gas. Research on non-fissile ceramics appears likely to have applications in other industries, and the production of high-density, fine-grained alumina bodies has been investigated. Attention is being paid to developing fibre structures by normal processes of fabrication and heat treatment, and, in an aluminium-based material, strength at high temperatures was comparable with or better than that of other high-strength materials such as sintered aluminium powder. Research on organic high polymers has been directed towards improving properties, and a fire-retardant, polyurethane foam, has been developed which also has higher compressive strength and will work at higher temperatures than is possible with other materials at present available. In a programme of work on non-destructive testing, a method has been perfected for obtaining a detailed survey of the distribution of fuel in a plate-type fuel element.

In fusion research, the theoretical and experimental development of magnetic wells has been a main feature of the work during the past year. Two sets of experiments have been used to examine the behaviour of low-density plasma in open-ended magnetic traps, and two other experimental groups are studying hot dense plasma in open-ended magnetic traps. Fundamental investigations of specific aspects of plasma behaviour have continued

* United Kingdom Atomic Energy Authority. Eleventh Annual Report for the period 1st April, 1964–31st March, 1965. Pp. x+102+8 photographs. (London: H.M.S O , 1965.) 8s. net.

in carefully controlled environments, free from many of the complexities which would accompany them in containment experiments. Solid dielectric switches have been developed, which have proved to be capable of consistent operation under the onerous conditions needed for controlling high-energy discharges. Research concerned with health and safety has included the development and testing of a 'cascade centripeter' for the determination of aerosol activities as a function of particle size, and a series of reports is being issued on the marine radiobiological investigations at Chapel Cross. Good progress was made in safety studies of the steam-generating heavy-water reactor, and an experimental programme to provide data checks for calculations of mixed plutonium and uranium systems has been initiated in which results of theoretical calculations agreed closely with experimental data. A team has been set up at Harwell to conduct inhalation investigations on human volunteers using radioactive vapours and labelled aerosols. Some account is given in the report of the Authority's relations with industry and with the universities. Extra-mural agreements between the Authority and universities and colleges already involve some 250 research workers and there is frequent interchange of staff between the Authority and universities, home and overseas, in the course of normal appointments as well as by exchange and attachment. During the long vacations in any year there are usually some 400 university students, research workers and staff working in or in close contact with Authority establishments.

Major changes in the programme of the Postgraduate Education Centre, Harwell, included the ending of the 14-week courses on radiation potential and the 20-week standard courses on radiation technology. A total of 858 students attended the 30 full-time courses, of whom 782 were British, 448 being from Authority staff. The 16 part-time courses were attended by about 1,100 students, mainly from the Atomic Energy Research Establishments. An innovation was a 4-day introduction to technology for 25 pupils from the sixth forms of grammar schools. By the end of 1964, 3,638 students had attended 165 courses at the Isotope School, the 15 courses held during the year having an attendance of 307. Seven 3-week courses at the Calder Operation School on the operation of magnox reactors were attended by 58 British and 9 overseas students and the four 2-week courses for Authority staff were attended by 32 staff.

An illustrated summary of the eleventh annual report has also been issued by the Authority, and this also includes a summary of the Central Electricity Generating Board's assessment of the Advanced Gas-cooled Reactor.

UNIVERSITY FINANCE IN BRITAIN

AS with the previous *Returns from Universities and University Colleges in receipt of Exchequer Grant**, those for the academic year 1963–64 are not accompanied by a general survey of activities, which is now issued as a separate publication. The grant list for 1963–64 covered 30 universities and 2 colleges, 25 of the former being in England, 1 in Wales and 4 in Scotland. Of the 2 colleges, the Royal College of Science and Technology, Glasgow, was granted a Charter as the University of Strathclyde in August 1964. The Universities of Kent and Lancaster received grants for the first time.

The number of full-time students in British universities increased to 126,445, compared with 119,004 in 1962–63, and of these 99,469 were reading for a first degree, 3,390 for a first diploma, 22,555 were engaged on research or other advanced work, and 1,031 were attending courses not leading to a degree or diploma. Of the full-time students, 73·3 per cent were men and 26·7 per cent were women, the small but steady annual increase in the proportion of women since 1959–60 continuing. 18,133 were in the Universities of Oxford and Cambridge, 23,955 in the University of London, 53,989 in other English universities and colleges, 8,447 in the University of Wales, and 21,921 in the Scottish universities. There were 13,786 students from overseas, 7,271 being postgraduate, of whom 7,562 were from within the Commonwealth; the percentage of overseas students was 10·9 compared with a peak of 11·5 per cent in 1961–62. Of the full-time students, 31·1 per cent were in colleges or halls of residence, 50·4 per cent in lodgings and 18·5 per cent at home, compared with 28·1, 40·9 and 31·0, respectively, in 1953–54. The proportions continue to vary widely for men and women, and in different universities: in Scotland only 11·7 per cent of men and 20·8 per cent of women are in colleges or halls of residence, while 45·2 per cent of men and 41·6 per cent of women are at home; for the Universities of Oxford and Cambridge the corresponding percentages are 56·0 and 74·4 and 2·0 and 3·0, respectively.

Of the 92,636 full-time men students, 23·4 per cent were in arts, 12·5 per cent in social studies, 28·3 per cent in science, 19·5 per cent in applied science, and 10·6 per cent in medicine; for the 33,809 women students the corresponding figures are 51·4, 11·6, 24·0, 1·2 and 9·0. Of the 22,555 postgraduate students, 3,801 were taking postgraduate courses in teacher training; 4,001 of the remainder were in arts, 2,493 in social studies, 6,882 in science, 3,551 in applied science, 1,300 in medicine, and 364 in agriculture. Of the 34,773 full-time students entered for the first time in 1963–64, 33,147 were reading for a first degree and 1,626 for a first diploma; 10,107 were women; 11,016 were in arts, 4,773 in social studies, 9,458 in science, 5,249 in applied science, and 2,770 in medicine. The figures for science and applied science compare with 9,224 and 4,965 in 1962–63, and over the past decade the intake in science has climbed steeply, whereas that in medicine has only increased slightly, and that in agriculture and forestry and veterinary science has remained relatively static.

The proportion of State-assisted students again increased, from 88·4 per cent in 1962–63 to 89·9 per cent in 1963–64, compared with 71·9 per cent in 1953–54, ranging from 94·5 per cent in Wales, 93·4 per cent at English universities other than London, Oxford or Cambridge, 91·4 per cent at Oxford and 85·7 per cent in Scotland to 83·5 per cent for the University of London. There were 16,528 part-time students (4,555 from overseas) and, of these, 13,116 were in English and 3,205 in Scottish universities. Almost half the part-time students were in six institutions of the University of London; 6,814 were postgraduate, 2,379 being in arts, 838 in science, 333 in applied science, 2,431 in medicine, and 629 in social studies. Of the 25,954 first degrees obtained, 18,186 were honours; and of the total 30·5 per cent were in science, 13·3 per cent in applied science, 6·9 per cent in medicine, and 13·3 per cent in social studies. Full-time teaching and research staff, excluding Oxford and Cambridge, increased to 14,939 (13,801 in 1962–63), and 27·8 per cent are now in science, 14·3 per cent in applied science, 15·8 per cent in medicine and 9·3 per cent in social studies: 11·6 per cent were professors, 6·5 per cent readers, 12·7 per cent senior lecturers and 46 per cent lecturers. Including Oxford and Cambridge, the full-time academic staff totalled 15,252, of whom 4,250 were in science, 2,156 in applied science, and 2,397 in medicine.

* University Grants Committee. *Returns from Universities and University Colleges in receipt of Exchequer Grant, Academic Year 1963–1964*. Pp. v+46. (London: H.M.S.O., 1965.) (Cmnd. 2778) 6*s*. 6*d*. net.

Of the recurrent income of £104,441,063 (£86,402,542 in 1962–63), £74,500,135 was from Treasury grants (70·5 per cent) and £888,277 from grants from Government departments; £9,156,682 (8·8 per cent) from fees; £2,188,214 (2·1 per cent) from endowments; £1,734,077 (1·7 per cent) from local authority grants; £520,699 from donations and subscriptions; and £12,043,222 (11·5 per cent) from payments for research. For English, Welsh and Scottish institutions the respective total incomes were: £83,656,716, £5,668,742, and £15,115,605. Non-recurrent Treasury grants totalled £43,295,545 (£36,481,424 in 1962–63), and of this £26,869,345 was in respect of building work, £4,133,745 professional fees, £10,334,363 furniture and equipment, and £1,958,092 purchases of sites and property. Since 1952, grants for furniture and equipment have been confined to new accommodation, including buildings adapted for new purposes. Of the recurrent expenditure of £102,301,794, 6·7 per cent was on administration, 43·4 per cent on salaries and superannuation of teaching staff, 12·1 per cent on departmental wages (technicians and laboratory assistants), 16·6 per cent on departmental and laboratory maintenance, 2·9 per cent on repairs and maintenance of buildings, and 11·1 per cent on rates, insurance, heat, light, water, etc. Of the expenditure of £3,946,646 on libraries, £2,093,719 was on salaries and wages, etc., of staff, and only £957,017 on books and £461,855 on periodicals; only London (£155,596), Oxford (£106,421), Cambridge (£71,074) and Leeds (£61,840) spent more than £50,000 on books, and only London (£115,663), Cambridge (£35,407), Oxford (£34,570), Edinburgh (£23,291) and Glasgow (£20,553) more than £20,000 on periodicals.

EMPLOYMENT OF GRADUATES IN BIOLOGICAL SUBJECTS 1962/63

WHILE covering much of the ground dealt with in its first report, the recently published second annual report (1962–63) of the University Grants Committee on the first employment of university graduates* includes an analysis of the employment of men and women who have graduated with Masters' or Doctors' degrees by thesis and/or examination (Table 1).

Table 1. Employment of Graduates in Biological Subjects (1962–63)

Degree subjects	Total graduating	Research training	Teacher training	Available for employment	Gained employment home and abroad	Seeking posts 31.12.63
Biochemistry	195 (163)	98 (80)	6 (6)	81 (68)	76 (58)	4 (4)
Botany	258 (289)	93 (111)	66 (55)	89 (112)	77 (91)	7 (12)
Pharmacy/pharmacology	278 (250)	54 (36)	0 (0)	209 (209)	203 (199)	1 (2)
Physiology and/or anatomy	386 (336)	76 (52)	6 (4)	33 (25)	30 (15)	1 (3)
Zoology	375 (335)	124 (110)	66 (82)	170 (120)	138 (97)	18 (15)
General biology	248 (264)	19 (31)	74 (70)	128 (125)	103 (101)	12 (11)
Agriculture	345 (393)	98 (105)	11 (10)	192 (122)	163 (193)	19 (27)
Totals	2,085(2,030)	562 (525)	229 (227)	902 (892)	792 (754)	62 (74)

Table 1, abstracted by A. C. Simpson from the two reports (the 1961–62 figures being given in brackets), shows surprising differences between the various sections of biology when the figures for the two years are compared. Thus, while the numbers graduating in zoology and biochemistry increased 12 per cent and 20 per cent, respectively, the numbers graduating in botany and agriculture decreased by 10 per cent and 12 per cent. However, the number of zoology graduates going on to teacher training fell by 20 per cent while the number of botanists increased by 20 per cent. To the numbers of graduates that were still seeking employment 6 months after graduating, the figure for all biologists covered by Table 1 for 1962/3 was 3·0 per cent of those graduating compared with 2·5 per cent for chemists and 1 8 per cent for physicists. The figure is not so satisfactory when those continuing study are excluded and the numbers available for employment in the various sections of biology are considered separately; for botany, zoology and general biology respectively, the percentages of those available for employment who were still seeking employment were 8 per cent, 11 per cent and 9 per cent, respectively. On the other hand, only two of the 434 biologists graduating with higher degrees failed to get employment within six months of graduation.

Table 2 gives an analysis of the employment categories of men and women who qualified for first and higher degrees in biochemistry, botany and zoology in 1962/63 and obtained employment in Britain.

Table 2. Graduates in Biochemistry, Botany and Zoology who obtained Employment in Britain (1962–63)

Degree:	Biochemistry First	Biochemistry Higher	Botany First	Botany Higher	Zoology First	Zoology Higher	Totals First	Totals Higher
Civil service	5	2	3	8	14	5	22	15
Local government	17	—	5	1	20	2	42	3
Universities and technical colleges	23	26	22	28	26	33	71	87
Schools (excluding those having taken teacher training)	7	—	26	9	37	4	70	13
Industry and commerce	13	8	14	5	13	2	40	15
Others	8	4	6	1	18	3	32	8
	73	40	76	52	128	49	277	141

It is seen that more than half those employed who obtained higher degrees in biochemistry, botany or zoology found their first employment within the universities and technical colleges, that schools took on 70 graduates immediately after their first degrees, apart from the 138 who went into teacher training courses with biochemistry, botany and zoology degrees, and that industry and commerce took nearly three times as many graduates straight from their first degrees than straight after taking higher degrees.

* *J. Inst Biol.*, 18, No. 3 (1965).

SCIENTIFIC POLICY IN BELGIUM

THE fifth annual report of the National Council for Scientific Policy, Belgium, covers the activities of the Council during 1964*. Its seven chapters review in succession the development of scientific policy at the national and international level; the budget for science; the organization of research; the expansion of higher education; the scientific establishments of the State; the distribution of scientific manpower; and the level of scientific potential in 1961. Reports or recommendations were presented to the Government on the expansion or adaptation of higher education at university level; the State scientific establishments and services; the development and finance of nuclear research; the status of the teaching hospitals; and the budget for science for 1965. At the international level, besides investigating the structure of scientific policy, the Council was concerned with the support of Euratom and the European Space Research Organization. The budget for science has

* Conseil National de la Politique Scientifique. *Rapport Annuel, 1964*. Pp. 200. (Bruxelles: Conseil National de la Politique Scientifique, 1965.)

grown from 2,622 million francs in 1960 to 4,690 million francs in 1964, 85 per cent of which is for the Departments of Education (58 per cent) and Economic Affairs (27 per cent), and, of this, 24·5 per cent and 26·5 per cent, respectively, are for research. Universities and institutions of higher education received 2,714 million francs; Government scientific establishments, 1,472 million francs; while 298 million francs went to the private sector.

Expenditure on fundamental research is estimated at 1,500 million francs and on applied research at 1,446 million francs. The Council recommends an annual growth of 15 per cent in the scientific budget as essential to provide normal development of higher education and research; grants for particular fields of science should be weighted within this limit.

The distribution of the scientific budget between different fields of science and institutions is analysed in some detail in the following chapter, which also discusses the reorganization of nuclear research and space research. The Government has accepted the Council's recommendation for the establishment of new university institutions at Anvers and Mons. The Council, after examining the work of the teaching hospitals, has recommended drawing a distinction between them and certain specialized and other university services and is now considering a general programme of development for the biological and medical services, having regard to the legal and social as well as the financial aspects. Some integration and regrouping of some of the scientific services and establishments of the State is also recommended and there is also a brief discussion of the status and organization of scientific staff in the Government service and in the State universities.

The 2,059 professionally qualified scientific staff, the 2,334 possessing technical diplomas and the 7,470 technicians and others engaged in scientific activities in 1961 were employed in 1,377 institutions. Of these latter, 180 were concerned with chemistry, rubber or pharmacy, 58 with metallurgy, 435 with metal manufacture, and 176 with textiles; the corresponding figures for qualified scientific staff are 874, 203, 450, and 73, respectively; for technical staff, 791, 219, 755 and 122; and for technicians, etc., 2,932, 1,000, 2,359, and 238. The corresponding distribution of the total expenditure of 2,882 million francs was 1,282 million francs, 235·5 million francs, 1,029 million francs, and 47 million francs, respectively. There is also a brief account of the activities of the National Foundation for the Finance of Scientific Research.

ASSESSMENT PROCEDURES IN SECONDARY SCHOOL SCIENCE

J. F. EGGLESTON holds a research fellowship in the School of Education, University of Leicester, where special thought is being given to the teaching of science in schools along with related questions of syllabuses and examinations. His paper* is mainly concerned with the examinations for the Certificate of Secondary Education, which in the past few years have been taken by an increasing number of boys and girls of Newsom quality, that is, those not normally expected to become sixth-form specialists. His essay merits attention, for it amounts to an interim report on a problem which is entering on a period of rapid change, while with varying impact all examinations in science are subject to the same considerations.

As Eggleston points out, in the past, examinations have served a dual purpose, providing measurements of achievement and predictions for the future. Up to now the first of these aims has been predominant, with its emphasis on the factual content of the syllabus to the detriment of original enquiry and practical experience. In support of this contention he draws on a survey of examinations at 'O' level, by R. W. Crossland and R. Amos, recently published in *Biology and Human Affairs*†.

The Boards which have been established to conduct examinations for the Certificate of Secondary Education have attempted to devise courses of work and tests to meet a wider range of requirements, as, for example, to make observations, develop manipulative skill, foster the ability to solve problems, and develop the pupils' ability to 'think scientifically'. Following R. L. Ebel and B. S. Bloom in America, and J. Furst in England, Eggleston maintains that aims like these are much too general; to increase their validity they need to be defined in more precise terms. He also thinks that no count should be taken of pupils' interests and enthusiasms, partly because as attributes they are subjective, and also because in many cases they are ephemeral.

He does not deal with oral examinations, which raise problems of a different kind. As for practical tests, he holds that under favourable conditions they measure qualities not disclosed by other procedures. Biology teachers appear to be most convinced of their validity, at the level of the Certificate of Secondary Education, although, owing to difficulties in arranging for them, some Boards do not make use of them at all. Similarly, a good case can be made for supplementary assessments based on course work, made by teachers who are in constant touch with their pupils, but again, subjective elements are involved. Altogether, in reading this essay, one is impressed by the number of questions which remain wide open, and by the spirit of amateurism which still prevails in a situation which urgently calls for hard scientific experiment and thought.

Even when some of these questions come nearer to solution, others of comparable import will remain. One, for example, is the advisability of holding any examinations at all at Certificate of Secondary Education level since they draw a line between the pupils who pass and the large number of children who fail to make the grade. Still, since this system is now in being it may call for modification rather than immediate abolition. Another question is the narrowing of interest and reduction in the teacher's initiative brought about by any examination syllabus; while a third, of similar classroom applicability, is that of the sophistication introduced by teachers who seek to anticipate examiners' requirements, as in question-spotting. This consideration produces more distortion in the simpler kinds of tests.

Beyond the schoolroom other questions await attention. For example, scientific ability is protean in its diversity; it cannot be reduced to a tidy single entity; and most of the outstanding discoveries in science cut across current techniques and systems of reasoning, they cannot be prepared for by training in making observations, and the spirit of enquiry is not a kind of searchlight which can be switched on and off at will. Scientists need time to brood, and their single-mindedness is something of spontaneous growth. How is it to be fostered? How is it to be assessed?

Then, no course in science can claim to be truly educational unless thought is given to its informational content, including appreciation of some of the major applications which provide the material fabric of our civilization; and at a higher level, all young people should be given some

* *A Critical Review of Assessment Procedures in Secondary School Science (with Special Reference to C.S.E.).* By J. F. Eggleston. Pp. 56. (School of Education, University of Leicester, 1965.) 5*s*.

† Crossland, R. W., and Amos, R., *Biol and Hum. Affairs*, **30**, 35 (1965).

understanding of the part science plays in cultural life, including moral and even, perhaps, spiritual issues. In this sense the ideological content of syllabuses will have to be brought under review. Points like these pose difficult questions for teachers and examiners, but if in their training young scientists are to be treated wholly as specialists they will remain nothing but specialists when they grow up. R. Weatherall

REPORT OF THE AGRICULTURAL RESEARCH COUNCIL 1963–65

THE Report of the Agricultural Research Council for the years 1963–65 (Cmnd. 2792, H.M. Stationery Office, 8*s*.) describes the work of twelve out of the forty-seven institutes and units for which the council is, in effect, responsible; summarizes some of the major developments in the two years under review; and, in so doing, demonstrates the wide range of modern agricultural research. Thus, in soils, work (some of which may be relevant to public health) on the persistence of organo-chlorine insecticides shows that a single dressing of dieldrin has effectively controlled carrot fly, *Psila rosae*, for nine years, while very low residues of this compound reduced the natural enemies of the cabbage root fly, *Erioischia brassicae*, and thus aided the survival of this pest. If the placement of fertilizers at depths of up to 2·5 ft. can be made practicable, then sub-soil water could be more effectively used by pastures, and irrigation needs reduced.

In crops, the influence of kinins on root growth, germination, bud growth and leaf-senescence (which affects the effective photosynthetic area) is noted, while revolutionary possibilities are opened up by complete chemical control of vegetation which permits 'minimal cultivation' and even brings into question the necessity for ploughing.

In animals, research on the metabolism of clinical disorders associated with calcium, phosphorus, iron, copper, magnesium, zinc and selenium illustrates the biochemical complexities of intensive animal production, while work at the Animal Diseases Research Association in Scotland on that elusive disease of sheep, scrapie, exemplifies the value of multi-discipline research. A link between reproduction research in humans and livestock is seen in the use, on pigs, of a temporary ovulation inhibitor which opens up the prospect of synchronized 'batch farrowing' by pig producers.

The Council (which now reports to the Department of Education and Science) is, of course, primarily concerned with scientific and engineering research. This may explain why there is no mention of the tentative, but potentially very important, link between scientific and farm management research through the creation, by multi-variable experiments, of the multi-dimensional input–output curves so badly needed for the effective application to farming of modern business mathematics.

The report concludes with administrative and financial data. The total spent each year by or on behalf of the Council is about £10 million in Great Britain. This excludes research at official husbandry farms, on farm management, by agricultural economists, by the official veterinary service, by the supporting chemical and engineering industries and by agricultural scientists at universities. Even if this group spent as much as the Agricultural Research Council, the total research expenditure on and in the agricultural industry would only be about 1 per cent of the value of its gross annual sales. In farming in the United Kingdom, productivity per man is increasing at about 7 per cent per annum—one of the highest rates in the national economy. What further contribution could agriculture make to national economic efficiency if research expenditure was doubled? A. N. Duckham

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

VARIOUS aspects of antimicrobial agents, infectious diseases, and chemotherapy were discussed during October 17–21, 1965, in the 237 papers and 11 round tables and conferences presented at the fifth Interscience Conference on Antimicrobial Agents and Chemotherapy—Fourth International Congress for Chemotherapy. This meeting was held in Washington, under the sponsorship of the American Society for Microbiology and the International Society for Chemotherapy, and was organized with the co-operation of the Infectious Diseases Society of America. 1,422 interested scientists attended this meeting; 228, representing 26 countries, came from outside the United States.

The Congress opened with addresses by Prof. E. B. Chain, Prof. S. A. Waksman, and Prof. J. F. Enders, discussing the past 25 years of antibiotic therapy and prospects for the future. This theme of reviewing the accomplishment of the past and problems of the immediate future was carried further in a round table on "Are New Antibiotics Needed?" organized by Prof. M. Finland. The consensus of the panel members, including Profs. W. M. M. Kirby, H. F. Dowling, A. C. Todd and L. P. Garrod, and Prof. E. B. Chain, Dr. Y. Chabbert and Dr. C. W. Pettinga, was that new and specialized antibiotics are still needed. There are many infections which are not adequately treated by the available antimicrobial agents and there is a need for a 'good' antifungal agent, a better anti-Gram-negative bacterial agent, and agents to treat a host of 'uncovered diseases' including the viral diseases and those caused by protozoa and other parasites. Other round tables were concerned with "Gram-negative Infections", "Antibiotic Assay Methods", "Bacterial Sensitivity Testing Methods", and the "Chemotherapy of Venereal Diseases". In each of these panel discussions, experts from many countries reviewed their findings in the particular subject being discussed, and presented new information on the prospects for the immediate future. The need for non-toxic and effective antibiotics in the treatment of Gram-negative infections, the fungal infections, and in venereal diseases was repeatedly emphasized.

Among the topics discussed at the 'informal discussions' were: practical methods for handling information on antibiotics and chemotherapy; the activities of the International Center for Antibiotic Information (Liège): antibiotic nomenclature; and ecological roles of antibiotics The following rules were proposed for choosing antibiotic names: (*a*) an antibiotic's name should be chosen so that, if it is later shown to be a member of a family, the root-name can be modified to show members of the related series. (*b*) The name should be based on chemical structure, and, except where the structure is known, the name should be based on the generic class of the producing organism. (*c*) The name chosen should be euphonious.

A symposium on the chemotherapy of tuberculosis (organized by Dr. G. Brouet, Paris) included discussion of experimental bases of chemotherapy (by Dr. F. Grumbach), therapeutic possibilities with minor antibacterial drugs in infections with *Mycobacterium tuberculosis* (Prof.

K. L. Radenbach), and the United States Public Health Service trials of isoniazid prophylaxis. In the latter, Mrs. Shirley Ferebee summarized the experience with trials involving 73,000 participants, and concluded that in the 4–9 years of observation after the prophylaxis year, isoniazid reduced the incidence of tuberculosis by 50 per cent in all trials.

Cancer chemotherapy was the subject of another symposium (convened by Prof. C. Heidelberger). Among the subjects reviewed were the antitumour antibiotics (by Prof. H. Umezawa), immunological approaches to host-tumour interactions (by Dr. M. Feldman), the relationships of pharmacology and cancer chemotherapy (by Dr. D. P. Rall, National Institutes of Health), and the present status of clinical cancer chemotherapy (by Prof. F. J. Ansfield). Of particular interest was Dr. Feldman's presentation of the value of the immunological approach in experimental cancer chemotherapy.

The pharmacology and pharmaco-kinetics of chemotherapeutic agents were discussed in a variety of the contributions. Of noteworthy interest was a discussion of the antibiotic absorption following otic administration as observed by M. J. Weinstein and colleagues (Schering Corporation). (This approach reminded the audience of Shakespeare's treatment of the subject in *Hamlet* (Act I, scene 5).) The pharmaco-kinetic discussions included experimental models, the logistics of chemotherapy, the pharmaco-kinetic studies using sulphonamides and ABOB, and protein binding studies. The latter subject was treated more fully in a symposium on penetration of drugs into tissues (convened by Prof. W. F. Verwey), where special attention was given to serum protein binding of penicillins and other drugs, the penetration of chemotherapeutic agents into cells, passage of drugs into and out of the central nervous system, and mechanisms of drug transfer across the placenta.

As more is known of the mechanisms of action of various antibiotics, it is possible to use these inhibitory agents as biochemical tools. Prof. J. T. Park led a symposium which discussed the use of chloramphenicol in the investigation of the synthesis of lysine peptides by enzyme systems, antibiotics as inhibitors of cell wall synthesis, the use of aminoglycoside antibiotics in studying the genetic code, and the antifungal antibiotics as agents in observing the physiology of moulds and yeasts.

The first day of the Congress was devoted to a series of papers on problems of infectious disease. These included: a discussion of endotoxin; clinical significance of the occurrence of various Gram-negative organisms; infections following cardiac surgery; a series of papers on Mycoplasma as infectious agents; and immunization using adenovirus vaccines and parainfluenza virus vaccines. New penicillins were discussed in other clinically oriented discussions, with special emphasis on cephaloridine in therapy of a variety of bacterial infections. The differences in the β-lactamases hydrolysing penicillins and those splitting cephalosporin *C* were summarized by Sabath and Abraham, who noted a zinc co-factor requirement for the latter, as well as differences in heat stability in these enzymes from *Bacillus cereus*.

The chemistry of antimicrobial agents was discussed in a symposium (organized by Prof. K. L. Rinehart, University of Illinois) and in a series of contributed papers. Among the especially noteworthy reports were:

(1) The presentation of the structure of the iron-containing antibiotic, ferrimycin A_1, and the disclosure that a 'minor change' of an amide group changes this from an antibiotic to a growth factor. (This work was done at the Federal Institute of Technology, Zurich, under the direction of Prof. V. Prelog.)

(2) A study of the structure-activity relationships among the depsipeptide antibiotics (with special reference to valinomycin) was summarized by Academician M. M. Shemyakin (Institute for Chemistry of Natural Products, Moscow), who concluded that there is a parallelism between their anti-fungal activity and their ability selectively to induce active K^+ transport in mitochondria.

(3) Proof of the structure of the antibiotic verrucarin *A* was used by Prof. G. A. Sim (University of Illinois) to demonstrate the usefulness of X-ray crystallography in determining the structures of antibiotics and other natural products.

(4) The mode of anti-tumour action of the copper-containing phleomycins, and the new antibiotics, bleomycins, was reported by Prof. H. Umezawa (Institute of Applied Microbiology, Tokyo) to involve inhibition of DNA synthesis as with pluramycin. On the other hand, the high molecular weight enomycin was found to have a selective action on ascites cells and was more rapidly absorbed by these cells than by normal cells.

(5) A series of antibiotics, the moenomycins, were described by Schmidt-Thome (Hoechst) as having the empirical formula: $(C_{70}H_{124}N_7O_{40}P)_n^1$. Hydrolyses showed the presence of: glucose; glucosamine; quinovosamine; phosphoric acid monoesters; a terpene-like carbon chain; and a chromophore. These antibiotics are active against Gram-positive bacteria. Very slow elimination was noted on subcutaneous administration, and blood levels of moenomycins were demonstrated 47 days after administration. The moenomycins have been widely tested as feed supplements, and were found more useful than the tetracyclines in growth promotion tests with broilers, pigs and calves.

(6) Kasugamycin, an aminoglycoside antibiotic, was originally selected on the basis of antifungal activity against rice blast disease. It was reported by Prof. H. Umezawa and Dr. T. Ichikawa (First National Hospital, Tokyo) to be useful in controlling *Pseudomonas* infections in clinical trials.

(7) Desdamine, desdamethine and ethesdamine are new antibiotics formed when *S*-methyl cysteine or *S*-ethyl cysteine was added to celesticetin producing fermentations.

(8) Everninomicin *B* is an antibiotic produced by *Micromonospora carbonacea*. It is active *in vivo* against Gram-positive bacteria.

(9) A group of antibiotics called the sugordomycins were described by Berger *et al.* (Hoffmann-LaRoche) as chemically related to the coumermycins, and having favourable *in vivo* potentialities against Gram-positive infections.

(10) Wiley (The Upjohn Company) reported on the identification of a cytotoxic agent *U*-20,904 as *cis*-β-acrylamidine.

(11) The structure of alazopeptin was reported by Patterson (Lederle Laboratories) to be L-alanyl-(6-diazo-5-oxo)-L-norleucyl-(6-diazo-5-oxo)-L-norleucine.

Most of the papers presented at this Congress will appear in *Antimicrobial Agents and Chemotherapy—1965*, which will be published in June 1966, by the American Society for Microbiology. The book will be distributed to all registrants at the meeting, and will be available from the headquarters of the Society, 115 Huron View Boulevard, Ann Arbor, Michigan.

Plans for the 1966 Interscience Conference on Antimicrobial Agents and Chemotherapy are already under way. This conference will be sponsored by the American Society for Microbiology and organized with the co-operation of the Infectious Diseases Society of America. The sessions will be held during October 26–28, 1966, in the Sheraton Hotel, Philadelphia. Among the topics which will probably be discussed in symposia and round tables are: identification of antibiotics; chemical modification of antibiotics; adverse reactions to drugs; chemotherapy under immunosuppressive conditions; epidemiology of antibiotic-resistant infections; and non-pharmaceutical uses of antibiotics. Further information on the meeting and abstract forms can be obtained from Mr. R. W. Sarber, executive secretary, American Society for Microbiology, 115 Huron View Boulevard, Ann Arbor, Michigan.

SUPERNOVA PHENOMENA IN OB ASSOCIATIONS AND COSMIC X-RAY SOURCES

By L. L. E. BRAES and J. W. HOVENIER
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WE wish to direct attention to the fact that most of the cosmic X-ray sources so far observed[1-3] are located close to OB associations, and that three of them are situated between associations and 'runaway' stars which originated from them (Fig. 1). In view of the probable relation between OB associations, 'runaway' stars and supernovae on one hand[4], and supernovae and X-ray sources on the other[1,5-8], it is tempting to suggest that supernova phenomena in these associations may be the origin of the X-ray emission. It is not necessary that these X-ray sources be associated with the particular supernova outbursts that released the 'runaway' stars. The presence of such stars should be regarded only as an indication of former supernova activity.

Most striking is the proximity of the strongest X-ray source, Sco*XR*-1, to the nearest association Scorpius II. Situated at $\alpha = 16h\ 15m$, $\delta = -15{\cdot}2°$ ($l^{II} = 359°$, $b^{II} = +24°$), its position is nearly midway between the centre of Sco II ($l^{II} = 341°$ to $2°$, $b^{II} = +10°$ to $+30°$) and the 'runaway' star ζ Oph, which originated from it $1{\cdot}1 \times 10^6$ years ago[4]. It has already been suggested that Sco*XR*-1 is a nearby object because it is the most intense source, at the highest galactic latitude. If identified with a supernova remnant associated with Sco II, it would indeed be a nearby object with a distance of 170 parsec (pc) (ref. 9).

The X-ray source Sco*X*-2, located at $\alpha = 16h\ 50m$, $\delta = -39{\cdot}6°$ ($l^{II} = 345°$, $b^{II} = +3°$), lies between the emission nebula *IC*4628 in the Sco I association ($l^{II} = 343°$ to $345°$, $b^{II} = +1°$ to $+2°$) and the 'runaway' star *HD*151397. There is some evidence that several supernova explosions did occur in this association[4], for which a distance of 1,800 pc is adopted[10].

Cyg*XR*-2 at $\alpha = 21h\ 43m$, $\delta = +38{\cdot}8°$ ($l^{II} = 88°$, $b^{II} = -11°$) is an X-ray source situated between the association Lacerta I ($l^{II} = 92°$ to $107°$, $b^{II} = -7°$ to $-20°$), at a distance of 600 pc (ref. 11), and the two 'runaway' stars *HD*197419 and *HD*201910 (ref. 4). Because of its relatively high galactic latitude the relation to Lac I seems probable.

In summary, these three X-ray sources lie near the apparent edges of the stellar associations, but since it is certain that the 'runaway' stars belong to the associations, some members may be beyond these edges. This fact, combined with the uncertainty in the adopted positions of the X-ray sources, makes it possible to regard them as association members as well.

Without considering Sco*XR*-1 and Cyg*XR*-2, we should now like to direct attention to the resemblance between the general distribution of the X-ray emission along the galactic equator[2], and that of associations and clusters of early-type stars within 2 kpc (ref. 12).

The complex of sources and the regions from which X-ray signals were clearly observed above the background between $l^{II} = 330°$ and $40°$ may be associated with the groupings of early-type stars in the Sagittarius spiral arm, at a distance of about 1·5 kpc. The gap in the distribution of X-ray emission between $l^{II} = 40°$ and $60°$ may correspond to the region between the Sagittarius and Orion arms. Finally, the emission from the Cygnus region between $l^{II} = 60°$ and $80°$ may originate from the Orion arm associations between 1 and 2 kpc from the Sun. If these identifications are accepted, the X-ray sources in the two regions mentioned would be at the same distance of about 1·5 kpc. If equal distances in general involve equal fluxes for X-ray sources, then the fluxes of the sources concerned would also be about the same and equal to that of Tau*XR*-1 (the Crab Nebula). This is indeed in accordance with observations[2].

Apart from Tau*XR*-1, no X-ray sources have been detected near the galactic equator between $l^{II} = 80°$ and $300°$. This may be partly due to the fact that the search

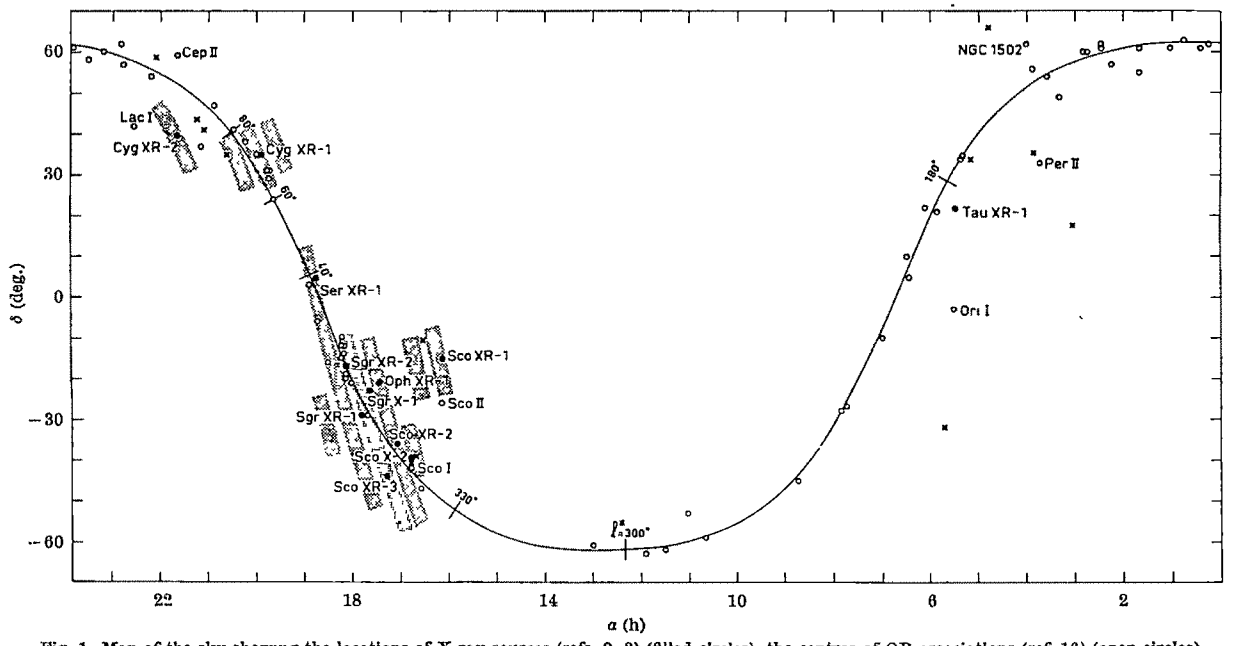


Fig. 1. Map of the sky showing the locations of X-ray sources (refs. 2, 3) (filled circles), the centres of OB associations (ref. 16) (open circles), and 'runaway' stars (ref. 4) (crosses). Shaded areas indicate regions in which X-ray emission in excess of the background radiation has been observed (ref 2). The full line is the galactic equator

for X-ray emission has not yet been made as thoroughly in this region as elsewhere. In addition, most of the associations between $l^{II} = 80°$ and 180° are in the Perseus arm, which is more than 2 kpc from the Sun, and therefore associated X-ray radiation might be too weak to be detected. Also the region along the galactic equator between $l^{II} = 180°$ and 300° is sparsely populated with nearby OB associations. Finally, between $l^{II} = 300°$ and 330°—where associations are missing—no search for X-rays has been made.

Provided that all supernova remnants in nearby associations emit detectable X-rays, which need not be true, X-ray emission would be expected in the direction of Orion I ($l^{II} = 207°$, $b^{II} = -18°$), from which three 'runaway' stars are known to have originated. Other nearby groups of early-type stars that are accompanied by 'runaway' stars, but apparently not by X-ray sources, are Cepheus II ($l^{II} = 101°$, $b^{II} = +5°$), *NGC*1502 ($l^{II} = 144°$, $b^{II} = +8°$) and Perseus II ($l^{II} = 161°$, $b^{II} = -16°$).

The distribution of X-ray sources over the sky can thus be explained by a relation with supernovae in associations within ~ 2 kpc. We shall now show that the observational data on their number, spectra and angular diameters may also be consistent with this picture.

Hayakawa *et al.*[6] suggested that in general the observed X-ray sources may be attributed to relatively nearby galactic supernovae. They found, by comparing the rate of supernova outbursts as derived by Hsi and Po with the number of observed X-ray sources, that the X-ray emission of a source should be appreciable for as long as $\sim 4 \times 10^4$ year. This was based on the assumption that these sources were not more distant than about 1 kpc. However, if we change this limit to about 2 kpc. as we do here, an X-ray lifetime of $\sim 1 \times 10^4$ year follows.

A supernova remnant may emit X-rays by several processes, but we shall limit our considerations to the thermal radiation of an optically thin hot plasma, since the spectra of the Sco*XR*-1 source and the sources in the region near the galactic centre can be fitted very well with a spectrum of such radiation if the temperature is $3{\cdot}8 \times 10^7$–$5{\cdot}8 \times 10^7$ °K (refs. 13 and 14). The 20–40 keV photons of the Crab Nebula found by Clark[15] may then be due to a high-energy tail in the distribution of electron velocities.

Using the results of Gould and Burbidge[6] and adopting a temperature $T = 5 \times 10^7$ °K, we find for the rate of loss of the free-electron kinetic-energy density 1×10^{-23} n_e^2 erg cm^{-3} sec^{-1}, where n_e is the electron density. Hence the cooling time may be defined as:

$$\frac{3kT}{10^{-23} n_e} \text{ sec}$$

where k is the Boltzmann constant. If this is taken to be 10^4 years we obtain $n_e = 5 \times 10^3$ cm^{-3}.

The X-ray flux of a source received on Earth is given by:

$$F = \frac{p n_e^2 V}{4\pi d^2}$$

where pn_e^2 is the X-ray emission (erg cm^{-3} sec^{-1}), V is the emitting volume, and d is the distance of the source. Taking the observed flux in the 1–10 keV range to be $F = 10^{-7}$ erg cm^{-2} sec^{-1}, and adopting $p = 9 \times 10^{-24}$ erg cm^3 sec^{-1} (ref. 6) and $d = 170$ pc, we find for Sco*XR*-1 an X-ray emitting volume $V = 1 \times 10^{51}$ cm^3. If this is to be thought of as a sphere its radius will be 2×10^{-2} pc, corresponding to an angular diameter of ~ 1′. This would be well within the upper limit of 7′ found for this source even if the volume were not exactly spherical.

The flux in the 1–10 keV range of the other sources is lower by about a factor of 10 than that of Sco*XR*-1, whereas in our opinion their distances are larger by about a factor of 9. Consequently, the difference in flux cannot be ascribed to a distance effect only, but could be due, for example, to the emitting volume of these more distant sources being larger. Other things being equal, their angular diameters would be smaller by a factor of about 4, which again is small enough to allow for the observational evidence.

We think that the existence of a hot plasma of the kind considered above in a supernova remnant cannot be excluded. The less so because, in the case of the Crab Nebula, Hayakawa *et al.*[8] have shown that the emitted 1–10 keV photons may be due to one or more hot condensations with $n_e \approx 10^3$ cm^{-3} and $T \approx 1 \times 10^8$ °K, having a total volume of ~ 10^{53} cm^3. These values do not differ very much from those considered in this article.

We conclude that there may be a close relationship between observed X-ray sources and supernova remnants in OB associations nearer than 2 kpc.

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IONIZING RADIATION AND THE INTRACELLULAR OXIDATION-REDUCTION STATE

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SIGNIFICANT changes in activity of oxidases and other enzyme systems after exposure of animals or cells to ionizing radiations have been described by many investigators. However, many reports have also appeared which failed to confirm these changes in the same systems. For example, Warburg *et al.*[1] reported that 2,000 r. markedly reduced the rate of glycolysis in ascites tumour cells, while Coe *et al.*[2], using somewhat different incubation conditions, found no such effect in these cells. Barron *et al.*[3] reported inhibition of respiration and oxidation of substrates requiring sulphydryl enzymes in tissue slices of various organs, but Le May[4] found that the succinoxidase system of the rat kidney homogenates was unaffected by 400–800 r. Ashwell and Hickman[5] found no effect on

spleen succinic dehydrogenase or cytochrome oxidase after an LD_{90} dose of whole-body irradiation to mice, yet Fischer *et al.*[6], using rat spleen, reported the opposite result. More recently, significant decreases in Krebs cycle dehydrogenase systems were reported in liver and spleen of rats immediately following moderate doses of whole-body irradiation, and with increasing effect with time after irradiation[7].

Most of the investigations which demonstrated changes in the activity of respiratory enzymes were carried out hours, or days, after irradiation, by which time the cell population in the 'radiosensitivie' tissues (such as spleen, gastro-intestinal mucosa, thymus, lymph nodes) has grossly altered—and thus alterations in metabolic activity at such times are of dubious importance in evaluating the primary effects of irradiation at the cellular and sub-cellular level. In addition some of the observed effects on tissue enzymes after whole-body exposure have later been ascribed not to direct radiation effects on the tissues *per se* but to indirect (abscopal) mechanisms—since local irradiation may fail to produce the changes which result from whole-body exposures[8,9].

Oxidative phosphorylation studies after irradiation are also difficult to interpret in terms of primary effects. Thus Potter and Bethel[9,10] reported a depression of oxidative phosphorylation in rat spleen, confirmed by van Bekkum[11], while other workers interpret such changes as most likely due to preparation artefacts[12]. For the irradiation of isolated mitochondria *in vitro*, fairly general agreement exists that no effect on oxidative phosphorylation is produced by doses up to 30 krads[10,11,13–15].

Recent detailed investigations on the respiration and phosphorylation in isolated mitochondria after irradiation *in vitro* show that succinate or β-hydroxybutyrate oxidation was not altered by radiation doses of less than about 100 krads, while oxidative phosphorylation was somewhat more sensitive[15]. The study of coenzyme-levels, principally NAD and NADH, after irradiation has led to similarly conflicting reports for the different systems investigated[16,17].

The various contradictory reports described here cover only a limited amount of work in this field, but serve to exemplify the difficulties involved in an attempt to determine whether irradiation can primarily disturb respiratory metabolism and whether such effects contribute to cellular radiation damage. As pointed out by Dale[18] and Krebs[19] in discussing the effects of irradiation on cell metabolism, a depletion or activation of stored enzymes in a cell may not be a true indication of changes in the functional capacity of the enzyme. Changes in levels of enzymes which are not essential control sites of cellular function may also cause little disturbance of such normal function[19]. Indeed, Dale[18] asks: "Can one expect from an analysis of tissue extracts or homogenates irradiated as such, or made from irradiated tissues, any answer to the question of the participation of enzymes in biological radiations effects ?".

A different approach to this problem was used in the present investigation where the level of reduced pyridine nucleotides was monitored during and up to 1 h after irradiation in several organs of the rat *in vivo*, and in ascites cell and thymocyte suspensions *in vitro*. Continual monitoring of the intracellular oxidation-reduction state as reflected by the ratio of oxidized pyridine nucleotides (NAD and NADP) to reduced pyridine nucleotides (NADH and NADPH) was possible with the fluorometric techniques developed by Chance *et al.*[20–22]. Decreases in dehydrogenase activity cause oxidation of NADH, while inactivation of the electron transport chain between pyridine nucleotides and the final electron acceptor, oxygen, produces reduction of pyridine nucleotide[23]. This technique can provide a continuous monitor of 'functional' activity of the respiratory system in the intact cell, thus removing tissue preparation artefacts and also allowing measurement of any changes in the system during the actual irradiation of cell suspensions or tissues.

The apparatus used for *in vivo* fluorometric measurements was as described by Chance *et al.*[24]. Rats (150–220 g Canberra Black males) were anaesthetized with 1·2 g of urethane per kg body-weight, a trachael cannula inserted, and the organ under investigation exposed. As most of the fluorescence measured *in vivo* is emitted from the exposed surface of the tissue, to a depth of about 0·5 mm[24], large doses of radiation could be given in a short time, by using a Phillips beryllium window source operated at 50 kV and 2 m.amp, with no added filtration. The remainder of the animal was lead-shielded during irradiation. For cell suspension work the fluorometer was mounted above a small gassing (pressure) chamber, mounted over a magnetic stirrer, as described by Chance *et al.*[25].

In vivo experiments. The tissues investigated were kidney and ileum, the latter with either serosal or mucosal surfaces uppermost. The kidney has previously been used for the study of *in vivo* oxidation-reduction states[20,21,24] and its responses are well characterized. Thus increases in fluorescence, due to pyridine nucleotide reduction, are observed in the aerobic–anaerobic transition *in vivo* and similar fluorescence responses to inhibitors of electron transport are obtained in the kidney[20], as have been shown to occur in isolated mitochondria[26]. The use of ileum for such fluorometric measurements has not been reported previously but it proved to be a very suitable tissue; large consistent fluorescence responses were obtained with changes in the steady state, and the emission spectrum of the isolated perfused tissue under aerobic or anaerobic conditions was similar to that of the other organs previously reported[21]. Cyclic changes in fluorescence were often observed in the ileum for both mucosal and serosal surfaces, but particularly for the latter. Their origin has not yet been investigated but, by analogy with results obtained with skeletal[27] and cardiac muscle[28], it is possible that they are due to cyclic activity of the intestinal smooth muscle, such as pendulum movements. Examples of such cyclic activity, together with the responses of the ileal smooth muscle *in vivo* to injections of amytal and sodium sulphide, are shown in Fig. 1. It can be seen in Fig. 1*A* and *B* that anoxia, amytal and sulphide cause approximately the same increase in fluorescence, due to pyridine nucleotide reduction, and the responses are similar to those described for kidney *in vivo*[20]. Results in this respect for the mucosa were similar. Thus ileum behaves in a 'predictable' fashion. Liver, particularly in the aerobic–anaerobic transition[29],

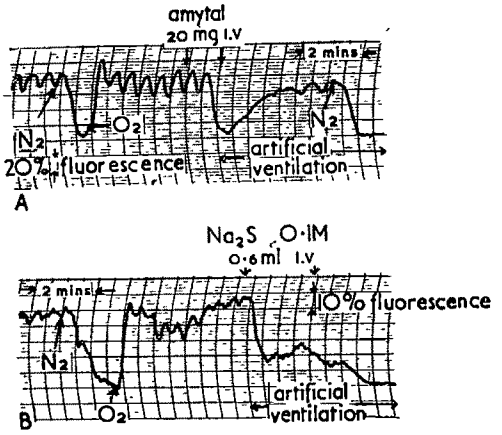


Fig. 1. *A*, Comparison of the effect of anoxia and an intravenous injection of amytal on pyridine nucleotide fluorescence in rat ileum (serosa) *in vivo*. *B*, Effect of sulphide in causing pyridine nucleotide reduction. Fluorescence increase due to pyridine nucleotide reduction in this and all subsequent figures corresponds to a downward deflexion of the trace. The calibration fluorescence, 20 per cent in the ileum in *A* and 10 per cent in *B*, is an arbitrary value, with 100 per cent corresponding to the initial level of fluorescence (dark current as zero) from the aerobic tissue, in this and the following illustrations

is less predictable, and unfortunately spleen, which has often been investigated for radiation effects, was found unsuitable due to the high concentration of haemoglobin in this tissue causing excessive absorption of the fluorescence signal. Therefore, *in vivo* irradiation investigations were confined to kidney, smooth muscle of the ileum and gastrointestinal mucosa. The dose-rate was usually 6–12 krads per min. Before irradiation one or two anoxic cycles were recorded by connecting the tracheal cannula to a nitrogen atmosphere. Immediately after irradiation another anoxic cycle was recorded, and such anoxic challenges were repeated every 15–20 min. Control experiments showed that most preparations gave reproducible responses for at least 1 h, especially if the tissue was kept moistened with warm saline and if the anoxic cycles were repeated at not over-frequent intervals. Some preparations remained apparently unchanged for up to 3 h, but sometimes fluorescence loss was observed in controls after 1 or 2 h, and thus it was considered that evaluation of the results should be restricted to 1 h after irradiation.

A typical record from the ileum is shown in Fig. 2. In this experiment cyclic activity was not seen. Nitrogen inhalation caused a large fluorescence increase, with a return to normal intensity preceded by a slight oscillatory response on ventilating the animal with oxygen, the oxygen being substituted by air as soon as spontaneous respiration was re-established (Fig. 2*A*). Irradiation with 12 krads produced no effect (Fig. 2*B*) and an anoxic cycle a few minutes after irradiation was essentially similar to the control responses prior to irradiation (Fig. 2*C*). Fig. 2*D* shows that even at 1 h following irradiation the fluorescence signal was similar to that before irradiation. This lack of effect of irradiation was seen in a total of fifteen experiments on this tissue with tissue exposures up to 100 krads. (High dose rates above 25 krads/min often produced a non-reversible decrease of the aerobic fluorescence amounting to 10 per cent–15 per cent when a total dose exceeding 50 krads was delivered. This decreased fluorescence with such large doses at high dose-rates does not, however, appear to be a biochemical oxidation of NADH since the magnitude of the fluorescence increase in the aerobic–anaerobic transition is unaltered, that is, the whole level of fluorescence has decreased. Similar effects were also observed in irradiation of a standard gelatine filter under the same conditions. This effect, therefore, appears to be a direct chemical phenomenon, such as depolymerization of macromolecules.) Experiments using ileal mucosa gave a similar result, and in preparations showing cyclic activity the latter was not abolished by doses up to 50 krads of X-radiation.

Experiments with kidney were performed in the same way, but in addition one or two flexible 50 mμ gold electrodes to record tissue pO_2 were inserted, to lie beneath the kidney capsule. The technique of measuring tissue pO_2 with such electrodes has been described previously[30,31].

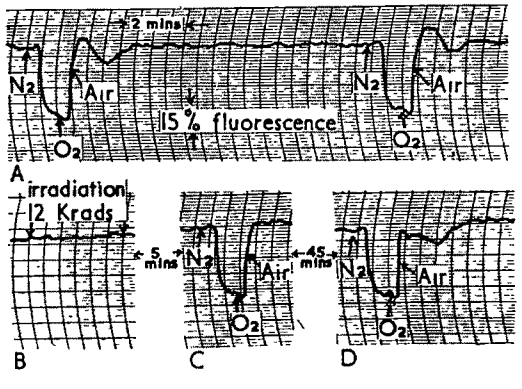


Fig 2. The effect of irradiation on the rat ileum (serosa) pyridine nucleotide fluorescence *in vivo*. Calibration figures as in Fig. 1

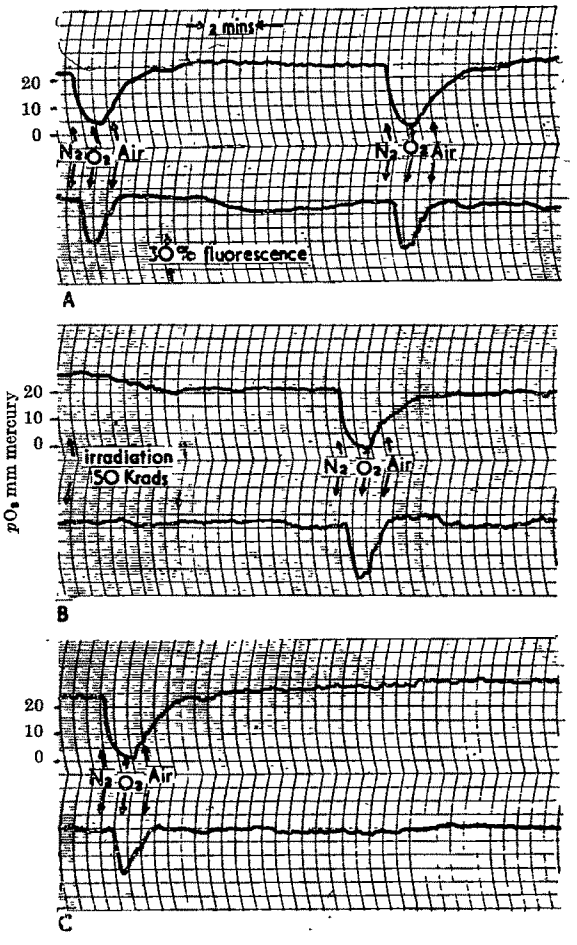


Fig. 3. The effect of irradiation on rat kidney pO_2 and fluorescence. The top trace in each section is the oxygen tension, the lower trace fluorescence. The figure is explained in the text; calibrations of fluorescence are as in Fig. 1. *A*, Before irradiation; *B*, during and immediately after irradiation; *C*, approximately 1 h after irradiation

The results obtained are illustrated by the example in Fig. 3. When nitrogen was inspired the pO_2 rapidly fell and when a level of only 1–2 mm mercury of oxygen was recorded, the fluorescence began to increase, to attain a maximum when the oxygen electrode recorded a near-zero pO_2 value. Chance[29] has calculated that the intracellular pO_2 for pyridine nucleotide reduction is of the order of 0·5 mm of mercury. On returning the animal to oxygen, followed by air, the fluorescence recovered more rapidly than the oxygen tension, and after some minutes equilibrium was restored. This control cycle was repeated and the tissue was then irradiated with 50 krads at a dose-rate of 12·5 krads/min. No effect was seen on the fluorescence trace but a slight decrease of oxygen tension occurred. Some minutes later nitrogen breathing resulted in a normal response, similar to the pre-irradiation effect, and 1 h later the responses still remain unaltered. The slight decrease in pO_2 during irradiation was seen consistently on doses of irradiation exceeding 50 krads. In some experiments with the kidney where pO_2 was recorded, 0·5 mm aluminium filtration was used to obtain better depth dose, but failed to affect the results. The magnitude of the radiation-induced pO_2 effect varied but never exceeded a 30 per cent decrease even with the highest doses of radiation used (100 krads). This decrease in tissue pO_2 could be due to local vascular effects, or to increased oxygen consumption through uncoupling of oxidative phosphorylation[10,11]. Partial uncoupling *in vivo* may occur without significant changes in pyridine nucleotide levels if the tissue is already in an active metabolic state (State 3) as discussed by Chance *et al.*[20]. It is also

possible that the decrease in pO_2 results directly from oxygen utilization due to the high radiation dose[32].

Ascites Lettrè hyperdiploid line tumour cells. For the irradiation of single-cell suspensions tumour cells were gathered from the intraperitoneal cavity of mice 5–6 days after inoculation with 10^6 cells, the cells from each mouse centrifuged gently, washed twice with buffered medium (*tris* buffer 0·005 M; sodium monohydrogen phosphate, 0·005 M; sodium chloride, 0·12 M; potassium chloride, 0·006 M; magnesium sulphate, 0·001 M; calcium chloride, 0·002 M) and then re-suspended in a volume of the same medium equal to the packed cell volume.

70 µl. of such a suspension was placed in a small cuvette as previously described[25]. All experiments were done at a room temperature of 26°–28° C. After establishing a control aerobic–anaerobic transition in the gassing chamber the sample was removed from the chamber for irradiation, as the quality of radiation was too soft for penetration through the thick-glass window of the gassing (pressure) chamber. A 0·5 mm aluminium filter was used and the dose was measured in 2 mm maximum depth of fluid with constant stirring during irradiation. The fluorometer was not moved, so that the sensitivity remained unaltered during the experiment. The dose in the vessel used was measured with a modification[33] of Fricke's chemical dosimetry technique[34]. Results of a typical experiment are shown in Fig. 4. Panel (*A*) shows the control response to anaerobiosis. About 30 sec after flushing with nitrogen an increase in fluorescence began, reaching a plateau in about 1·5 min. On flushing with oxygen re-oxidation of pyridine nucleotides immediately resulted and equilibrium was restored within 1 min. The cells in their container were removed from the chamber, and irradiated in air with 10 krads (dose-rate 2·5 krads min^{-1}). Rapid stirring was maintained during irradiation. Immediately after irradiation the cells were replaced in the gassing chamber and another anaerobic–aerobic transition recorded (Fig. 4*B*). The cycle recorded was similar to the pre-irradiation response. At room temperature, 1 h later, the response was still unaltered. Similar experiments were performed using three different samples of ascites tumour cells, and radiation doses of 1, 2, 5, 10 and 20 krads were delivered to aliquots of each sample. In addition, in separate experiments, the fluorometer was so positioned that the fluorescence signal could be recorded during irradiation. No definite effects of irradiation were observed in any experiment.

Thymocytes. Thymocytes prepared from Canberra black rats of 150–200 g weight were washed once in the medium already described and the packed cells re-suspended in an equivalent amount of medium. They were then subjected to the experimental methods already described for ascites cells. Thymocytes initially gave a somewhat weaker fluorescence signal than ascites cells, which is not unexpected in view of Scaife's finding that most of the pyridine nucleotide in these cells is present as NAD[17].

A small but definite oxidation consistently preceded the reduction of pyridine nucleotides in the aerobic–anaerobic transition of thymocytes (Fig. 5). The cause of this initial decrease in fluorescence is not known. Irradiation of thymocytes with 5 krads produced no change during irradiation (Fig. 5*B*), immediately after irradiation

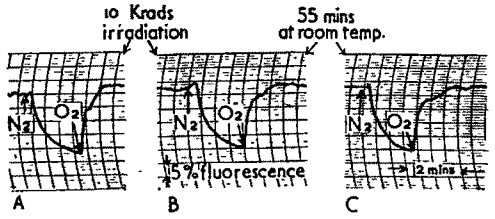


Fig. 4. Aerobic–anaerobic transitions in ascites tumour cells before (*A*), several minutes after (*B*) and 1 h (*C*) after 10 krads irradiation

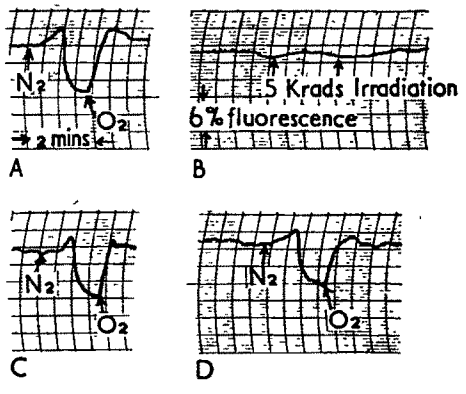


Fig. 5. Isolated thymocytes before (*A*), during (*B*), immediately after (*C*) and 1 h (*D*) after irradiation with 5 krads

(Fig. 5*C*), and little effect even 1 h later (Fig. 5*D*). Doses of 1, 2, 5 and 10 krads were given to each of three samples of cells, and the results were similar to those shown in Fig. 5. After doses of 20 krads there was again no immediate effect but the response to nitrogen, 1 h post-irradiation, showed some diminution. Nigrosin counts showed only 2–3 per cent non-viable cells in cells studied before or immediately after irradiation. The nigrosin dead cell count increased to 9–10 per cent after thymocytes were left at room temperature for 1 h, whether irradiated or not. Pyknotic counts showed only a slight increase between fresh cells and cells examined 1 h later (2–4 per cent respectively). Irradiation did not affect the pyknotic count at 1 h after irradiation.

The results reported here demonstrate the rather striking insensitivity of the electron transport chain, including dehydrogenases and associated coenzymes, to X-irradiation. The intracellular oxidation-reduction state was virtually unaltered even when massive doses of radiation were administered, and up to 1 h post-irradiation electron transport reactions appeared to function normally. Thus changes reported in respiratory enzyme-levels or coenzymes, assayed at later times after irradiation, appear to depend on other forms of cellular damage and are not a primary radiation lesion. Changes, also, in enzyme and coenzyme levels investigated by *in vitro* assay methods may not be a true reflexion of their functional activity in the cell. The present investigation includes several tissues of varying radiosensitivity—from kidney and smooth muscle, generally classed among 'radioresistant' tissues, to gastro-intestinal mucosa *in vivo*—a 'radiosensitivie' tissue and rapidly dividing ascites cells *in vitro* of known high 'radiosensitivity'. Non-dividing thymocytes are of special interest in that these cells have been shown to be destroyed by small radiation doses insufficient to cause the destruction of most other cells in the mammalian body[35–38]. This death is 'degenerative' rather than the result of mitotic aberrations induced by ionizing radiations, and pyknosis can be observed in thymocytes and small lymphocytes within a few hours of irradiation. The pyknotic death of these cells, which becomes evident about 3 h after irradiation, is a peculiar response, since high doses of radiation (> 5 krads) apparently inhibit the development of pyknosis[36,37]. Shrek[36] regards this high-dose effect as one of 'delayed fixation' of cells. However, irrespective of whether low doses or many times supra-lethal doses of radiation were received by these cells, their respiratory function remained unaltered.

Laser[39–41] has produced evidence for the inactivation of some electron transport components (namely, cytochrome *c* and riboflavin) by X-irradiation *in vitro*. Large radiation exposures, in excess of 10 krads, were used to produce these effects and the reaction recorded showed a reversed

'oxygen effect'; with greater damage resulting from irradiation under anaerobic conditions, where these components are in the reduced state. However, the observed effects would seem of little radiobiological significance in explaining irreversible radiation damage to cells, for with very few exceptions both *in vivo* and *in vitro* biological and chemical systems show a positive oxygen effect, and cellular cytochrome *c in vivo* would under normal physiological conditions be almost fully oxidized[42]. The lack of an oxygen effect and the radiation protection afforded by cyanide in the bacteria used by Laser[9] remains paradoxical, and is certainly not a universal phenomenon in radiation damage to micro-organisms[43].

The results of Barron *et al.*[44] and Scaife[17] are particularly relevant to the present investigation. Barron has shown that irradiation of NAD in solution has little effect and no conversion to NADH occurs even with doses of 100 krads, but NADH in solution is fairly sensitive to irradiation, with 1 krad producing measurable effects. This is in contrast to *in vivo* effects, where very high doses of irradiation fail to cause NADH oxidation, verifying that binding of substances and the presence of cellular components may greatly modify their radiosensitivity, as demonstrated by Dale[18].

Scaife[17] assayed NAD-, NADP- and NADH-levels in rat thymus and isolated thymocytes before and after irradiation. One h after irradiation a decrease of NAD content had occurred in the thymus and isolated thymocytes which increased with time, with no corresponding effect on NADH. Such NAD loss without reduction to NADH can occur without affecting the fluorescent signal recorded in these experiments, which is a measure of NADH and its changes during transitions between various metabolic states. Probably either the stored NAD from a different cellular compartment (nuclear ?) or defective NAD synthesis is responsible for the reduced NAD content of thymocytes assayed after irradiation. Indeed, Scaife concluded that the most probable explanation of his results was that NAD is released from bound NAD within the cell after irradiation, and metabolically destroyed—a view in keeping with the enzyme release theory of Bacq and Alexander[45].

A further aspect of this investigation concerns the theory that the effects of ionizing radiations and high-pressure oxygen are produced by similar mechanisms, a theory proposed by Gerschman *et al.*[46] and elaborated by Laborit and Brue[47]. Considerable evidence exists which fails to support this theory[48], which states that similar oxidizing free radicals may be produced by hyperbaric oxygen and ionizing radiations, and cause similar intracellular lesions. The present work fails to support this hypothesis. The lack of effect of irradiation on pyridine nucleotide fluorescence contrasts sharply with the immediate effect of hyperbaric oxygen on the intracellular oxidation reduction state, described previously for various systems, including rat kidney, brain, liver, ascites tumour cells, isolated mitochondria, and succinoxidase particles[25]. The same effect of high oxygen pressure was found also in rat thymocytes in this investigation and is illustrated in Fig. 6. If indeed 'oxidizing free radicals' are the primary cause of cellular lesions induced by radiation and high-pressure oxygen respectively, then their reaction with cell components must occur at different loci, with electron transport reactions being particularly susceptible to high-pressure oxygen but, as shown in the present investigation, particularly resistant to ionizing radiations.

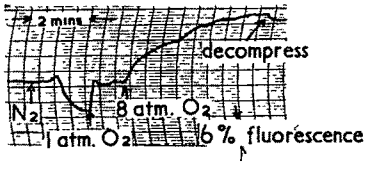


Fig. 6. The response of pyridine nucleotides to hyperbaric oxygen. After a typical fluorescence increase in anaerobiosis the cells are re-oxygenated, then when equilibrium is restored rapidly pressurized to 8 atm. absolute in oxygen. An immediate decrease in fluorescence occurs indicating oxidation of reduced pyridine nucleotide, and reaches a plateau in about 4 min

I thank Prof. Britton Chance for supplying me with the fluorometer, and the gift of the gassing chamber, used in this investigation. I also thank Prof. Chance and many members of the Johnson Foundation, Philadelphia, for their help with the techniques involved. Dr. H. A. S. van den Brenk is thanked for histological examination of the thymocytes, his advice and criticism of the manuscript.

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CRYSTALLIZATION OF PETROLEUM WAXES

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THE nature of petroleum wax crystals is often important in both the processing and the end usage of these waxes. Likewise, the modification of the normal crystal habits is practised in a number of applications. Typical of these are the use of pour point depressants in lubricants, the use of modifiers in refinery wax filtrations, the use of modifiers to control the physical properties of commercial waxes, and the use of 'paraffin inhibitors' in the production of crude oils. This article reviews the present knowledge of this topic as reflected by the literature.

Crystal Habits of Pure Hydrocarbons

X-ray investigations of pure hydrocarbons have revealed that *n*-paraffins crystallize in triclinic, monoclinic, hexagonal or orthorhombic crystal systems, depending on the particular hydrocarbon and the temperature[1–5]. The reasons for assumption of one of these symmetry relations by the crystals have been discussed by Kitaigorodskii[2] on the basis of even–odd carbon numbers and packing (or stacking) efficiencies.

The temperature relation concerns a solid-state transition that many (but not all) the *n*-paraffins exhibit[2,6–10], and involves symmetry and packing changes associated with rotation or non-rotation of the molecules about their chain axes.

In all cases, the *n*-paraffins crystallize with the chain axis parallel to the crystallographic *c*-axis. In the hexagonal and orthorhombic lattices, this implies that the chain ends will all lie in a plane joining one layer of the long molecules to the next layer. Hence, the bonds joining the layers will be different from those joining the members of each layer. Such an arrangement is typical of the platy minerals (such as mica and clays). It is not surprising, then, that pure *n*-paraffins exhibit a tabular or platy crystal habit, the most common forms of which are thin hexagonal or rhombic plates[7,11].

There is much less crystallographic information in the literature on the iso-paraffins than on the *n*-paraffins. No X-ray data for these hydrocarbons were located. Investigations using polarized light microscopy[7,12] indicate that pure branched chain paraffins also predominantly crystallize as plates with an orthorhombic lattice, with some apparently crystallizing in monoclinic or triclinic plate forms. In one case it has been reported that the pure naphthenic waxes crystallized primarily as orthorhombic plates, with one compound crystallizing in rectangular plates apparently belonging in the tetragonal system[7]. All naphthenes investigated, however, possessed long alkyl side-chains.

X-ray data suggest that most of the pure aromatic waxes crystallize in the monoclinic system with a few taking the orthorhombic forms[2,13]. Plates were observed as the predominant forms in a limited investigation[7].

Crystal Habits of Refined Waxes

The highly refined petroleum waxes that contain high proportions of *n*-paraffins exhibit crystal forms and sizes very similar to those characteristic of the pure *n*-paraffins[8,12,14–16]. This indicates that the *n*-paraffin waxes are fairly compatible with each other and even tolerate small amounts of other types of hydrocarbons without drastic modification of the crystal habit. Edwards[14,17] suggested that this is because the *n*-paraffins tend to form continuous series of solid solutions over a fairly wide range of chain-lengths. He also suggested that the crystal lattice is largely controlled by the more numerous *n*-paraffins, and the hexagonal and orthorhombic lattices seem to be able to tolerate solid solutions better than the other crystal systems. Others also maintain that *n*-paraffin mixtures crystallize as a series of solid solutions[3,14,17–19], although some believe that the refined microcrystalline waxes, containing considerable amounts of iso-paraffins and naphthenes (and possibly aromatic resins), form systems exhibiting eutectic compositions[8,16].

One group has reported that they could not use X-ray diffraction as a means of determining the iso-paraffin content of refined petroleum waxes, since the moderately high iso-paraffin content fractions investigated gave crystallographic data very near that of pure *n*-paraffins[11]. At very high iso-paraffin percentages, however, the crystal forms became almost exclusively ribbon or blade-like, rather than rhombic plates.

Less highly refined petroleum waxes, generally having low *n*-paraffin content and a wide variety of impurities, almost never crystallize as plates, but rather produce crystals described as 'needles'[6,8,12,20,21] and 'malcrystalline'[12,22,23] forms.

Mechanisms of Crystal Habit Modification

A review of several texts and a number of papers on the general topics of crystal nucleation, growth and habit modification revealed that these subjects are far from well understood.

Supersaturation is a basic requirement for nucleation. Some impurities permit only minute supersaturation before spontaneous nucleation occurs, and others necessitate quite large supersaturation before nucleation is possible; yet we can seldom predict how a given impurity will affect the crystallization of a particular solution.

Many theories of crystal growth have fallen into disfavour through the years; present-day theories, however, still cannot explain many crystallization phenomena, and continue to be controversial.

The control of crystal habit modification is desirable in many industries, and there is a wealth of reported information in the literature concerning specific instances; however, a general theory of habit modification has not been possible. Indeed, Wells[24] points out that, "The discussion of abnormal crystal growth implies that we know what is meant by normal growth, but this is far from being true".

The most controversial of the modified forms the petroleum waxes assume is that of 'needles'. Some of the earliest research workers in this field ascribed the crystallization of petroleum waxes in the needle form (rather than plates) to the effect of high viscosities of the solvent media[25,26]. Others considered that high rates of cooling caused the needle forms[7,21]. The temperature of crystallization and its relation to the transition temperature and melting-point of the wax was assigned importance by some; crystallization above the transition point was supposed to cause needle forms, and crystallization below the transition temperature was believed to create plates[6–8]. One group of investigators considered that there were simply different homologous series present in the waxes and that some crystallized as plates, some as needles, and some as

malcrystalline masses[12,27]. Others considered that hydrocarbons other than *n*-paraffins were indeed present, but that they caused needle formation through their action as impurities rather than imposing their form as 'needle waxes'[7,20,23,28]. Still others agreed that impurities were the cause (these impurities could be even other *n*-paraffins), but that the mechanism involved was curling of stressed plates to form 'needles' of oval or rounded cross-section[14,29–31].

Curling of the plates to form needles appears to be a real phenomenon; the origin of the stresses responsible for the curling is still controversial, however, and there are a number of reports of the formation of needles which cannot be explained by the curling mechanism.

It seems probable that the milder modifications of the plate forms, such as rounded and irregular corners, notched ends and truncations, are the result of hindered ordering in the development of the crystal. That is, the *n*-paraffin molecules of different chain-lengths do not have sufficient time during the crystallization to arrange themselves in the most efficient manner; the iso-paraffins and naphthenes similarly are caught up in the lattice or adsorb on the crystal faces before they can be displaced by the *n*-paraffins. As a result, many deformities and dislocations occur in the lattice structure, and the 'normal' geometric habit is not always completely obtained.

It would be expected, on the basis of this postulate, that modifications would occur increasingly with both (*a*) the concentration of components other than *n*-paraffins (or range of *n*-paraffins), and (*b*) the rate of crystallization. Increased malformation of plate crystals with increased rates of cooling has been reported even for pure hydrocarbons[7]; similarly, a gradually increasing degree of plate modification due to increased amounts of non-normal paraffin content at the same rate of cooling has been demonstrated[14].

The solvent itself can be considered an impurity. The ease with which the solvent molecules can diffuse away from the area immediately surrounding the growing crystal will affect diffusion of the wax molecules toward the wax crystal, and could thus cause disorder at high rates of crystallization. This probably explains the viscosity effects on wax crystal habit that have been reported[25,26]. That the chemical nature of the solvent will also effect mild modification also has been reported[23,27]. Further, adsorption of a solute is known to differ even from solvents of identical solubility for the particular solute[32], and the solvent itself is often appreciably adsorbed[33]; thus, it is to be expected that the solvent will play at least a minor part in the crystal modification mechanism.

The tendency toward disordering may offer an explanation for the dislocation spirals and rosette aggregates formed[34]. Indeed, the dislocation spirals themselves may be necessary for the crystals to grow at all in thickness, since two-dimensional nucleation on the lamellae surfaces would be expected to occur with difficulty[35]. The frequent occurrence of wax crystals with features that appear to be associated with twinning operations again suggests that lattice dislocations are an important mechanism.

Concentration of impurities alone does not explain all the crystal modifications, as equal concentrations of different impurities quite often produce strikingly different results. A plausible explanation for this difference would seem to lie in specificity of adsorption of the additives, although this effect surely would not be as im[illegible] as with the habit modification of [illegible] even those containing polar mol[illegible] of the *n*-paraffin plate crystals [illegible] packed CH_3 groups, while th[illegible] are composed of CH_2 g[illegible] surfaces of the plate edges), but gave contact angles of 25°–46° on the basal (001) faces of *n*-hexatriacontane plate crystals. Gutsalyuk and Yatsenko[43] have observed that mono- and bi-cyclic aromatic compounds selectively adsorb on to paraffin wax from cracked residues. Indeed, the adsorption of alkyl-aromatics on to paraffin wax crystals has been postulated for many years in connexion with the pour point depression of lubricating oils[44–49].

On the basis of this evidence, it would seem reasonable to presume that condensed ring aromatics, such as naphthalene and anthracene, would adsorb on to the 'edge' surfaces of the wax plates and provide sufficient bulk to retard the accretion of paraffin wax molecules on to these surfaces. Recently, however, experiments by one of us[34] have indicated that a number of aromatic compounds have virtually no effect on the particular wax crystals studied, and certainly did not 'stunt' the plates by inhibiting their growth on the (110) faces (plate edges).

It would appear, then, that the adsorption-type aromatic additives are of questionable value in modifying wax crystals, unless they possess the long alkyl side-chains characteristic of the well-known alkyl-aromatic pour point depressants. One should note, however, that Lorensen[48] found that a biphenyl–isopropyl ether reaction product (containing no long side-chains) exhibited mild pour depressant qualities, although he did not describe the crystal forms produced in the presence of this additive.

The most severe modifications observed on the wax crystals, commonly called malcrystalline forms, have received only minor comment in the wax refining literature. Ferris and Cowles[12] believed that these malcrystalline forms were simply due to the presence of 'mal-type waxes'. Clarke[7], however, showed that they could be obtained even in pure hydrocarbons of *n*-paraffin, iso-paraffin, naphthenic, and aromatic types under conditions of very rapid cooling. He suggested that these malcrystalline forms were associated with high supersaturation. Few other explanations are offered in the literature, although the malcrystalline forms are generally associated with the most impure wax fractions, for example, petrolatums or distillation residues. Some have postulated that these forms are clusters of ordinary wax crystals cemented together by a nucleus of naturally occurring aromatic resins or asphaltic particles[50,51]. This has also been implied by others, but such a mechanism is not compatible with that involved in Clarke's work with pure hydrocarbons.

Recent work by one of us[34] has shown that the presence of asphaltic and resinous impurities definitely promotes these malcrystalline forms. It is believed, however, that they promote these forms by causing the wax to grow dendritically, rather than serving as nucleating or cementing agents. Close examination of photomicrographs of malcrystalline forms in the literature indicates that almost all represent dendritic growth.

There have been many theories of dendritic crystal growth presented, but most of them agree that one of two causes are generally present: a rapid crystallization rate, or high-impurity concentration. The explanation for the characteristic branching is usually that the crystal must rapidly change growth directions to avoid the localized accumulation of (*a*) heat of crystallization under rapid, extreme cooling[36], or (*b*) impurity concentration under high-impurity conditions and/or high supersaturation[52].

It see[illegible]tically reasonable, then, that malcrystalline [illegible]duced on pure substances [illegible]y high rates of cooling, or [illegible]gh-impurity concentrations [illegible]ooling; hence the data in [illegible]onflicting.

[illegible]for dendritic growth

nucleate or promote growth in new directions. A second is the formation of foliated dendrites through a mechanism involving impurities and screw dislocation growth, as proposed by Gorodetsky and Saratovkin[53].

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WINE TANNINS—ISOLATION OF CONDENSED FLAVONOID PIGMENTS BY GEL-FILTRATION

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THE molecular size of polyphenols is generally considered to be the most important factor in determining tanning activity, astringency, and also the inhibition of enzymes by such compounds[1,2], the term 'tannin' being best reserved for those phenolics of sufficiently high molecular weight (> 500) to have such properties[3]. However, no data on molecular size of tannins are available except for that concerning several oligomers of leucofisetinidin which were isolated from black wattle heartwood by counter-current extraction and two-dimensional paper chromatography[4]. The lack of a satisfactory procedure for routine isolation of condensed tannins from plant materials has greatly hampered progress in this field.

Monomeric flavonoids are conveniently isolated and purified by cellulose and polyamide chromatography, but highly polymerized polyphenols are irreversibly adsorbed on these materials. Consequently, the analysis of tannins has depended on examini[illegible] reactions which have some[illegible] structural features, as in t[illegible] glucinol residues and the e[illegible] content by acid convers[illegible] reasons, little is kn[illegible]

This article reports some preliminary investigations which we have carried out using the technique of gel-filtration in both the preparation and analysis of wine tannins. By means of this technique we were able to investigate for the first time their chemical constitution.

The adsorptive capacity of 'Sephadex *G*-25' dextran-gels for wine tannins is almost completely eliminated by preparation of the gel in aqueous alcoholic media. The gel then functions as a molecular sieve for tannins when irrigated with the same aqueous alcoholic solvent, and enables separation of the high polymers in a narrow band. The procedure is rapid and gives quantitative recovery of extract. It has been applied on a preparative scale to the isolation of condensed tannins from dry red table wines. Residual anthocyanins present in the wine are also partially resolved, apparently by adsorptive partition.

Optimal separations were obtained in a medium con[illegible] of 60 parts ethanolic 0·1 per cent hydrochloric [illegible]ated hydrochloric to 1 l. ethanol) and a *p*H of about 2·0. Aqueous meth[illegible] of the same composition serves [illegible]t critical but, as all fractions [illegible]e colour was dependent on [illegible]

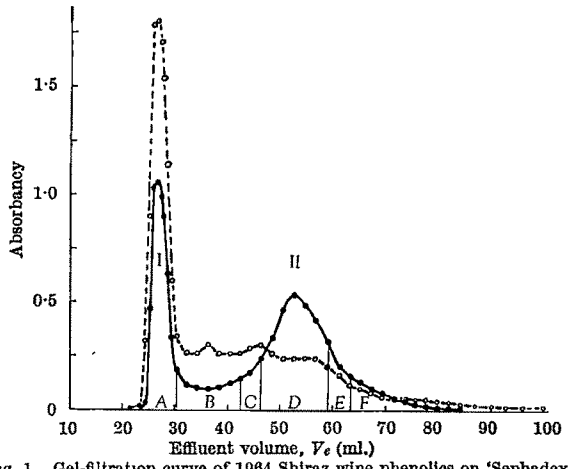


Fig. 1. Gel-filtration curve of 1964 Shiraz wine phenolics on 'Sephadex G-25' in 60 per cent aqueous alcohol (hydrochoric acid). Visible colour. —●—●—, absorbancy at 535–540 mμ (twenty-fold dilution); —○—○—, absorbancy at 278 mμ (fifty-fold dilution)

aforementioned solvent—a glass paper disk was used to prevent disturbance of the gel surface. Non-phenolic wine constituents were first removed from a 1964 dry red wine (*V. vinifera*, cv. Shiraz, 20 ml.) and the phenolics concentrated twenty-fold by precipitation of total phenols with basic lead acetate solution, regeneration and solution in the solvent (1·0 ml.), keeping temperatures below 35° C. The concentrate was applied to the column and the flow-rate adjusted to about 12 ml./h. The pigment quickly separated into two bands, the first (I, very dark red) being completely excluded from the gel ($K_d \neq 0$) and the second (II, bright red) becoming more diffuse with development, adsorption effects being finally evident ($K_d > 1$). Optical densities at the visible maxima (535–540 mμ) and 278 mμ were read after suitable dilution in the same solvent, and plotted against effluent volume, V_e (Fig. 1).

The nature of bands I and II was revealed by paper chromatography of all fractions in *n*-butanol–acetic acid–water (4 : 1 : 5, upper phase). Band I fractions *A* (Fig. 1), dark red in solution, were almost completely immobile, showing as brown stains at the origin with slight brown trailing to about R_F 0·5. Subsequent fractions *B* contained tannins of relatively greater mobility, and anthocyanins (Band II) of progressively decreasing R_F appeared from this point onwards. However, slight quantities of tannin, judged by chromatographic behaviour, still persisted through all fractions to V_e 80 ml. Recovery of total phenolic material was almost 100 per cent within 60 ml. of effluent, and overnight elution with the same solvent gave effluent of zero absorption at 278 mμ. Such columns have been used repeatedly for fractionation of wine pigments in this way.

By co-chromatography with anthocyanins previously identified in Shiraz grape pigment[6], the fractions *D* were shown to contain malvidin 3-glucoside with some petunidin 3-glucoside, the peak fraction having only the former; the content of fractions *E* was similar but with relatively more of the petunidin pigment, and the final eluates from V_e 63 ml. (*F*) contained petunidin 3-glucoside and delphinidin 3-glucoside. The early eluates of Band II, fractions *C*, contained unidentified anthocyanins, probably peonidin 3-glucoside with traces of acylated anthocyanins.

By precipitation with dry ether from solution in ethanol, the condensed tannins of Band I (*A*) were recovered quantitatively as a stable mauve red powder (14 mg), representing, with allowance for that used on optical density measurements, 900 mg/l. in this 1964 wine. The material precipitated gelatin from solution, and the tannin solution in 12 per cent v/v aqueous alcohol saturated with potassium bitartrate had both flavour and astringency.

Gel filtration on 'Sephadex G-25', modified for the polyphenols by use of aqueous alcohol, thus provides a solution to the problem of separate measurement and isolation of wine tannins. An elution curve of total phenols, as in Fig. 1, gives both qualitative and quantitative information regarding the polymerization state of these compounds, as the area under the peaks is a direct measure of contribution to colour at the visible maximum by condensed tannins and monomeric anthocyanins at this *p*H. The content of individual anthocyanins may also be estimated fairly accurately by use of control paper chromatograms.

The ultra-violet absorption of the condensed tannin fraction (Band I, Fig. 1) paralleled the visible absorption, and no phenolic material was eluted which was not highly coloured. The intensely red colour of the tannin band and the fact that the colour is dependent on *p*H suggested that anthocyanidin or anthocyanin pigments are incorporated in the tannin structure. This is quite contrary to present-day theories on wine tannins, which are supposed to be yellow-brown in colour rather like some wood tannins and similarly constituted, principally of leuco-anthocyanidin units[7]. Thus the red colour of wine has been attributed to the monomeric anthocyanin pigments only, with superimposed tannin effects producing the familiar brick-red tints of an aged wine. However, degradative and other data described below have shown that the tannins of red wines actually contain anthocyanin units in their structure, and must be regarded as condensed flavonoid pigments. The changing hues and tints of a red wine are then explicable in terms of the relative contributions of polymeric and monomeric pigments to total colour.

For experimental purposes 160 mg of wine tannin was used. This had been isolated as before by gel-filtration from a 1959 Shiraz red wine (200 ml.), in which the anthocyanin contribution to total colour (Band II) was quite low. The solution of this tannin in 12 per cent v/v aqueous alcohol saturated with potassium bitartrate (*p*H 3·8) had, like that of the 1964 wine tannin, a similar appearance and absorption spectrum to that of an aged red wine, with a maximum at 520 mμ and high absorbance in the 400–500-mμ region (Fig. 2)—an $E^{1\%}_{1\,cm}$ value of 25 was found for the 'simulated' wine solution using this tannin sample. The effect of *p*H on visible colour (Fig. 2), while much less marked than with anthocyanins, was similar to that which characterizes the anthocyanins, through the *p*H range 2–10. At lower *p*H the material was partially precipitated from aqueous or dilute alcoholic solutions.

Micro-analysis of the 1959 tannin gave the following percentages: C 55·4, H 4·7, O 36·5, alkoxyl (as OCH_3) 3·8, N < 0·5. A single extraction with *iso*-amyl alcohol of the products of mild acid hydrolysis removed all pigment

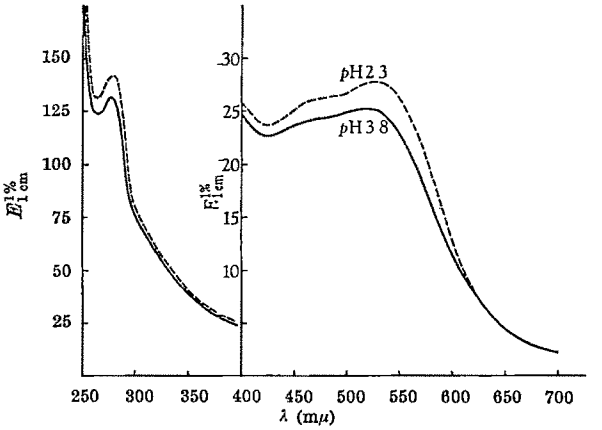


Fig. 2. Absorption spectrum of 1959 Shiraz wine tannin: ———, in 12 per cent v/v aqueous alcohol saturated with potassium bitartrate (*p*H 3·8), – – – –, in 12 per cent v/v aqueous alcohol citric phosphate buffer (*p*H 2·3)

from the reaction mixture, and chromatography of this extract in Forestal solvent or in formic acid–conc. hydrochloric acid–water (5 : 2 : 3) gave three anthocyanidin bands as obtained from Shiraz grape pigment. Multiple chromatography in *n*-butanol–2 N hydrochloric acid (1 : 1, upper layer) of eluted bands enabled identification, by co-chromatography with authentic pigments, of all of the anthocyanidins which occur as their 3-glucosides in Shiraz grapes[6], namely, delphinidin, petunidin, malvidin and peonidin. However, the major anthocyanidin found was cyanidin, derived from the high leuco-anthocyanidin content of the tannin. The aqueous fraction from the acid hydrolysis reaction contained glucose which, analysed by the method of Pridham[8], was found to constitute about 8 per cent of this wine tannin.

The content of 'hydrolysable tannins' in wine is known to be very low[9], so that wine tannins are regarded as 'condensed tannins', and the glucose found present must be still in its original state of combination with anthocyanidins. It is thus evident that the anthocyanin pigments of a new wine, which are known to disappear very rapidly during maturation and ageing, are actually incorporated into the polymeric complex of leuco-anthocyanidin and possibly other flavonoid units. A measure of the extent of this reaction is given by the gel-filtration elution curve, as in Fig. 1. The glucose and methoxyl contents of the wine tannin, together with the relative yield of cyanidin from acid hydrolysis, suggest a ratio of one anthocyanin molecule to about four leuco-anthocyanin moieties in the polymer.

That the mean molecular weight of the wine tannin is comparable with that of other condensed tannins was indicated by application of the concept of Goldstein and Swain[3]. Thus, analysis by the vanillin (V) and Folin Denis (F.D.) reagents for phenols, using catechin as standard, gave $E^{1\%}_{1\,cm}$ values of 116 and 531 respectively. The value of 22 for the ratio V/F.D. per cent, which they showed to be a measure of polymerization, is actually lower than any quoted by these workers for various plant tannins and catechin polymers, indicating a somewhat higher average molecular weight for the wine tannin. The use of the V/F.D. per cent concept is valid in this case as simple phenols, which otherwise give rise to meaningless results[3], have been removed by the gel-filtration procedure.

However, the characteristics of 'Sephadex' gels, once established for condensed polyphenols, may enable the assignment to tannins of accurate molecular weight ranges, as has been done with proteins[10]. In the absence of known standards, it is considered that, since 'Sephadex *G*-25', in the 60 per cent aqueous alcoholic solvent used, swells to about half its volume in aqueous medium, the minimum molecular weight for complete exclusion from the gel, as found with the wine tannins, would be about 2,000. Evidence that a wide range of molecular weights occurs in wine tannins and some idea of the relative distribution throughout this range were obtained by further gel-filtration of the 1959 tannin and of total wine phenols on 'Sephadex *G*-50' and '*G*-100' gels in 40 per cent and 33 per cent aqueous alcohol (hydrochloric acid) respectively. Both gels swelled to about half the normal aqueous value in these solvents. Neither separated tannins from anthocyanins but each gave a wide spread of tannin pigment up to the exclusion point, though no bands were observed. Recovery of tannin from each experiment was quantitative and again no phenolic material was eluted which was not pigmented. Use of '*G*-50' in 60 per cent aqueous alcohol (hydrochloric acid), in which the gel swells to one-quarter the aqueous value, gave complete exclusion of tannin as a narrow band and just separated tannins from smaller pigments in the total phenol extract, showing how the same gel may be modified to give different performances as a molecular sieve for tannins. From these observations it is concluded that most of the condensed polyphenols in this 1959 wine tannin were in the molecular weight range 2,000–5,000, but that some material having molecular weights up to 50,000 was present.

Tannin formation in wines probably proceeds initially by acid catalysed polymerization of leuco-anthocyanidins which are very acid sensitive, but no information is available on the mode of cross-linkage in such poly-leuco-anthocyanins[11]. The finding of anthocyanins in the wine tannin structure poses further problems—so far as we are aware, no 'polymeric anthocyanins' have previously been reported. The ease with which anthocyanidin pigments are released from the tannin structure by mild acid hydrolysis suggests that only weak chemical bands are involved between the main leuco-anthocyanidin matrix and anthocyanins, if indeed any at all. It does, in fact, seem reasonable to postulate that the pigment molecules are physically rather than chemically incorporated into the three-dimensional tannin structure of C_{15} leuco-anthocyanidin units. Between five and fifteen flavonoid units are apparently involved in the tannin structure, and physical inclusion in a coiled structure is a possible explanation of the development of tannin pigments in an ageing wine.

Although condensed tannins from different sources are of diverse chemical types, it seems likely that any extract of plant phenolics could be handled by gel-filtration in the manner described for wine phenolics, and the condensed tannin fractions thus isolated. Lignin degradation products of suitable solubility might also be purified in this way. Finally, the adsorptive effect observed with the anthocyanins suggests that 'Sephadex'-gel columns could be useful for preparative partition chromatography of these pigments.

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VARIATIONS IN AMINO-ACID SEQUENCE NEAR THE DISULPHIDE BRIDGES OF BENCE-JONES PROTEINS

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ONE of the key problems in investigations of the molecular basis of the immune response is the mechanism by which an indefinite number of protein molecules are synthesized to account for the large number of highly specific antibody activities. In spite of their functional heterogeneity, a surprising structural similarity of the antibody molecules is observed. This similarity is well illustrated by the common arrangement of two pairs

of chains—$A(H)$ and $B(L)$ chains—proposed for all 7S antibodies[1,2]. However, a high degree of heterogeneity has been shown on isolated L chains[3,4], and has also been suggested on isolated H chains[5]. It is now generally believed that this heterogeneity is the result of the functional heterogeneity already referred to. What appear to be single molecular species of chains have been isolated from myeloma proteins, which do not share the heterogeneity, possibly because they derive from a single clone of tumour cells[6]. The observation that the Bence-Jones proteins of myeloma patients represent in most cases isolated L chains[7,8] has helped considerably in a more detailed chemical investigation of the nature of the heterogeneity. So far two immunologically distinct types of Bence-Jones proteins have been established[9,10] and named, type K (I) and type L (II). Each of these has a characteristic C-terminal sequence, and studies of the yield of these sequences have indicated that most of or all the heterogeneous population of normal L chains contains them in the same proportions[11] as the proportion of type K and type L reacting material observed in normal serum[9,10]. Recent work has shown the sequences of two individual Bence-Jones proteins to be similar along a stretch near the C-terminus of the chain. With the exception of a single residue, identical peptides accounted for half the total protein, and overlapping of the peptides located them at the C-terminal half. The peptides of the other half showed no clear similarities[12]. Analysis of a third Bence-Jones protein indicated in some detail that the sequence in the C-terminal half of the chain was the same as in the other two, with the exception of the single residue already mentioned. However, in this case the N-terminal peptide, which comprises eighteen residues, was probably also identical with that of one of the two other proteins[13]. There is an obvious need for analysing a larger number of proteins, which is difficult, but a wider survey of selected stretches of chain, such as those adjacent to the disulphide bridges, may give useful information.

Two disulphide bridges may be common to many or all individual type K Bence-Jones proteins[14]. The corresponding sequences have also been found in the three Bence-Jones proteins already mentioned, suggesting that the common C-terminal half is folded so as to link cysteines in positions 192 and 132. (Numbering of residues throughout is taken from ref. 12.) The C-terminal cysteine provides the interchain link[13]. Another two cysteine residues have been observed in at least six proteins—the protein of Roy[12] and five individual Bence-Jones proteins in which the cysteine peptides were investigated (see later). These two cysteine residues were bridged to form the third disulphide in the protein B.J.[13]. It remains to be seen if all proteins of the K type have five cysteine residues linked in two intra-chain and one inter-chain bridge, or whether there are exceptions to this rule.

The variation of the sequence of the L chains has now been investigated with the help of a radioactive technique. The disulphide bridges of Bence-Jones proteins were quantitatively reduced and alkylated with ^{14}C-iodoacetate; the radioactive protein was then digested with trypsin or chymotrypsin, and the radioactive peptides purified by 'Sephadex' fractionation and paper electrophoresis. This technique is suitable for the analysis of longer stretches of sequences in a comparatively large number of proteins. I shall discuss the results obtained in a preliminary study of five of these proteins and compare them with the information obtained from other proteins.

The C-terminal stretch. Cysteines in positions 212 and 192 have facilitated the analysis of the last twenty-four amino-acid residues of the five proteins. The results summarized in Table 1 support the conclusion that in the eight proteins listed the only variation is that at position 189, where either Val or Leu is found. So far Leu has always been associated with the Inv factor (a) and Val with Inv (b). The detailed evidence for the new sequences reported here will be published elsewhere. The order of the residues in parenthesis has been decided only on comparative grounds. The next cysteine residue going towards the N-terminus has provided evidence for the similarity in a stretch comprising residues 112–133; that is, a stretch of 22 residues just after the first half of the molecule. The results listed in Table 2 show no detectable difference.

The 'variable' region. The peptides containing the two cysteine residues which occur next in protein Roy (going towards the N-terminus) can be identified in several Bence-Jones proteins from their amino-acid analysis and partial sequences. Table 3 shows a comparison of the sequences around the cysteine which, on the basis of these results, is probably residue No. 86. There are some interesting differences, some of them based on established sequences and others based only on amino-acid analysis and comparison with the established sequences. In

Table 1. A COMPARISON OF THE PEPTIDES CONTAINING THE CYSTEINE RESIDUES 192 (ROY NUMBERING) AND C TERMINUS OF SEVERAL TYPE $K(I)$ BENCE-JONES PROTEINS

Protein		Ref.
B J.	Leu Tyr.Ala.Cys.Glu(Val,Thr,His,Gln,Gly,Leu)(Ser,Ser,Pro,Val,Thr)Lys. Ser Phe Asn Arg. Gly.Glu Cys	
	190 200 210	
Roy	Leu Tyr Ala.Cys.Glx Val(Thr,His,Glx Gly)Leu. Ser(Ser,Pro,Val,Thr)Lys Ser.Phe. Asx Arg. Gly.Glx Cys	12
Cum	Val(Tyr,Ala,Cys,Glx,Val,Thr,His,Glx,Gly,Leu, Ser,Ser,Pro,Val,Thr)Lys Ser.Phe. Asx Arg. Gly(Glx Cys)	12
Ag	Val Tyr Ala Cys Glu Val.Thr His Gln.Gly.Leu. Ser Ser Pro.Val.Thr.Lys Ser.Phe. Asn Arg Gly.Glu Cys	13
Rad	Val Tyr.Ala Cys Glu(Val,Thr,His,Gln,Gly,Leu)(Ser,Ser,Pro,Val,Thr Lys)(Ser,Phe)(Asn,Arg) Gly.Glu Cys	
Day	(Val, Tyr,Ala.Cys,Glx Val,Thr,His,Glx Gly,Leu, Ser,Ser,Pro,Val,Thr,Lys)(Ser,Phe, Asn,Arg)(Gly Glu,Cys)	
Man	(Val Tyr,Ala,Cys,Glx Val,Thr,His,Glx Gly,Leu, Ser,Ser,Pro,Val,Thr,Lys)(Ser,Phe, Asn,Arg)(Gly,Glu,Cys)	
Ker	Leu.Tyr.Ala.Cys Glu(Val,Thr,His,Gln,Gly,Leu)(Ser,Ser,Pro,Val,Thr)Lys. Ser(Phe, Asn,Arg)(Gly,Glu,Cys)	

Glx and Asx mean that no distinction between glutamine and glutamic acid and between asparagine and aspartic acid is available
Proteins B J and Man were kindly provided by Prof. S Cohen and proteins Rad, Ker and Day by Dr. A. Feinstein. Rad, Day and Man are Inv (b+) and Ker is Inv (a+) (A. G Steinberg, personal communication) Roy is Inv (a+)[12].

Table 2. A COMPARISON OF THE PEPTIDES CONTAINING THE CYSTEINE RESIDUE 132 (ROY NUMBERING) OF SEVERAL TYPE $K(I)$ BENCE-JONES PROTEINS

Protein		Ref.
Ag	. . Ile Phe Pro Pro Ser Asn Glu Gln Leu.Lys Ser.Gly Thr Ala Ser.Val.Val Cys Leu . .	12
Rad	. . Ile(Phe,Pro,Pro,Ser)Asp Glx Glx Leu(Lys,Ser,Gly,Thr,Ala,Ser)Val.Val Cys Leu .	
B J.	(Ile,Phe,Pro,Pro,Ser,Asx)(Glx Glx Leu)Lys(Ser,Gly,Thr,Ala,Ser)Val.Val.Cys.Leu . .	
Ker	(Lys,Ser,Gly,Thr,Ala,Ser)Val(Val,Cys,Leu) . .	
	120 130	
Roy	. . Ile(Phe,Pro,Pro,Ser, Asx Glx Glx)Leu Lys.Ser(Gly,Thr,Ala,Ser)Val(Val,Cys)Leu . .	13

The corresponding peptides from the proteins of Day and Man have not been prepared.

several cases the peptides are extremely similar. A reasonable guess about the sequence of a variable peptide of which only the amino-acid composition is known seems to be possible, and this in itself is a very interesting observation. Table 4 shows the comparative sequences around the cysteine residue, which is probably No. 23 (Roy numbering). Again great similarities are observed, but there are also well-established differences in some residues. Investigations of disulphide bridges have been carried out on three of these proteins (B.J., Rad, and Ker). The results indicate that the two variable cysteine residues form a disulphide bridge.

The detailed analysis of the variable region is particularly interesting because it makes it possible to test hypothetical mechanisms by which variability in protein sequences could be achieved starting from a much simpler set of genes. There are several assumptions one is forced to make for such a test, the most important being that the proposed genetic code[15] is applicable to the system under investigation.

The simplest question one can ask is the following: Is it possible to explain all the differences by using only two different sets of nucleic acid sequences with multiple crossing-over and strict preservation of phase? Let us assume, as an example, the following two sequences of nucleotides:

(1) GAA ACG UCU
(2) CUU GAU CUU

Sequence (1) may code for—Glu.Thr.Ser.
Sequence (2) may code for—Leu.Asp.Leu.

Sequence (1) corresponds to the sequence of Rad 90–92 (see Table 3). All variations found in those three positions of Table 3 can be explained by interchanging nucleotides of these two sequences as follows:

GAU GAU CUU (crossing at second base)
Asp.Asp.Leu as Ker
GAA AAU CUU (crossing at fourth base)
Glu.Asn.Leu as B.J.

The only other established position in this region is Cu 90 which is a Leu, which is also in agreement with the nucleotide sequence (2). Unfortunately, this hypothesis does not explain the three residues at position 94 (Thr, Pro and Tyr) which require as first code letter A, C and U. The position is worse if either Leu or Phe is confirmed in position 94, if Gly is confirmed at position 98, and if either Phe or Leu is confirmed at position 89 (all in Roy protein).

The variations observed in Tables 3 and 4 do not seem to fit with the intra-chromatid inversion hypothesis[16]. A direct test can be made if only one inverted loop is allowed. The model predicts in that case that there should not be two variant regions separated by a common stretch. Protein B.J. and Rad (Table 3) have a region of variation between positions 90 and 94 and the five residues preceding it are identical. Yet the peptide with cysteine in position 23 of B.J. was not found in Rad, but a completely different peptide around a cysteine residue was found instead, indicating that at least two variable regions were separated by a common stretch which involves not less than five residues. Other examples of this sort may be inferred from the available data.

Another hypothesis which has been tested is the one proposed by Potter, Appella and Geisser[17]. According to this hypothesis, some unusual triplets coding only for the variable region will bind specific *s*RNA charged with amino-acids which will differ from clone to clone due to specificity changes of the activating enzymes. This would suggest that replacements of a given residue in a new sequence should be done with only one amino-acid. That is, the *s*RNA involved in, say, two sites containing aspartic acid, instead of being charged with Asp in a different clone will be charged with some other residue; it would then be likely that both sites will contain the same residue, whichever it may be. In Table 3 protein Ker shows Asp in positions 90 and 91 and the corresponding residues in B.J. and in Rad are Glu. Asn and Glu. Thr, respectively.

Investigations of the sequences around the cysteine residues of several individual Bence-Jones proteins, combined with previous sequence studies, indicate that the heterogeneity of the *L* chains of γ-globulin is the result of the heterogeneity of restricted stretches of the polypeptide chain. So far, and with the exception of a Val/Leu substitution, which seems to be related to the Inv factors, no variation in the C-terminal half of the molecule has been found. This half includes one common intra-chain disulphide bridge and the inter-chain disulphide bridge. Possibly the invariance of this region is due to the strict requirements for inter-chain binding, essential to the

Table 3. A Comparison of the Peptides containing the Cysteine Residue 86 (Roy Numbering) of Several Type *K* Bence-Jones Proteins

Protein		Ref.
B.J.	———Tyr.Tyr.Cys.Gln Gln.Tyr Glu Asn. Leu(Pro,Tyr)———	
Ker	———Tyr.Tyr.Cys.Gln.Gln.Tyr.Asp.Asp.Leu.Pro.Pro.Thr.Phe.Gly.Pro.Gly.Thr.Lys ———	
Rad	———Tyr Cys Gln Gln.Tyr Glu Thr Ser. Pro.Thr.Thr.Phe.———	
Roy	.....Tyr(Tyr,Cys,Glx Glx Phe,Asx Asx Leu,Pro,Leu,Thr,Phe)Gly(Gly,Gly,Thr)Lys. (90–100)	12
Cu	(....Tyr,Tyr,Cys,Glx Glx)Arg Leu(Glx Ile, Pro,Tyr,Thr,Phe,Gly,Glx Gly,Thr)Lys.	12
Ag	———Thr.Phe.Gly.Gln.Gly.Thr.Lys	13

The position of the residues in parenthesis is suggested by comparison with the other specimens. When there were no grounds for comparison the residues were placed in random order and underlined. From Day's protein a peptide was isolated with an amino-acid composition, His,Cys,Thr,Ser,Glu,Ala,Phe. This is presumed to be a large variation of the sequence around the corresponding half cystine. Similarly the protein from Man gave two Cys peptides which could not be related to either the common sequences of this Table or those of Table 4.

Table 4 A Comparison of the Peptides containing the Cysteine Residue 23 (Roy Numbering) of Several Type *K* Bence-Jones Proteins

Protein		Ref
B J.	Arg Val.Thr Ile Thr Cys Gln Ala.Ser Gln Asp Ile Asn Lys.	
Ker	Asp Arg Ile Thr Ile Thr.Cys Gln.Ala(Ser,Gln,Asp,Ile,Lys)	
Day	Val(Thr,Ile, Thr,Cys,Gln,Ala,Ser,Gln,Asp,Ile,Ser, Asx Phe,Leu,Asx Try,Tyr)	
Roy	Asx.Arg($\frac{\text{Val}}{\text{Iso}}$,Thr,Ile, Thr,Cys,Glx Ala,Ser,Glx,Asx,Ile,Ser, $\frac{\text{Iso}}{\text{Val}}$,Phe)Leu(Asx,Try,Tyr)	12
Ag	Leu Asn.Try Tyr	13
Rad	Ala Thr Leu Ser Cys Arg Ala Ser Gln	

The conventions are the same as those used in Table 3

formation of the complete antibody, and perhaps other common—but so far unestablished—functions.

The variations of the other half of the molecule present varying characteristics. Some proteins may be very similar indeed. In those cases (as Ker, B.J., Roy and Ag) all that has been observed so far are variations of individual residues, which may include stretches of a small number of residues. In other examples much larger variations around a cysteine residue are found, which may reflect very large variations in a given stretch. Some attempts have been made to see whether these sequences could be related by phase shifts and/or inversions, but without success. We cannot exclude the possibility of some cysteine residues being located differently in individual Bence-Jones proteins, and this is in fact suggested by the available data on Cummings's protein[12].

Several simple hypotheses which could explain the variability of certain regions of the protein starting from a small number of parent genes have been tested but none of them accounts for all the variations observed. If further assumptions are made, such as, for example, the operation of more than one of these hypothetical mechanisms, it becomes progressively more difficult to exclude them on the sole grounds of comparative sequences.

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SPECIFICITY AND PREVENTION OF ANTIBIOTIC RESISTANCE IN *Staphylococcus aureus*

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In previous communications[1–6] it was reported that the emergence of antibiotic resistance in *Staphylococcus aureus* and *Escherichia coli* is prevented by incorporating into the medium the combination of an antibiotic, to which the organisms have never previously been exposed, together with atabrine or spermine. It must be noted that, conversely, prior exposure to an antibiotic enabled these organisms to grow in the presence of the homologous antibiotic–atabrine combination. Thus, it is possible to differentiate the *de facto* antibiotic-resistant bacteria from those which have the potentiality but fail to give rise to demonstrable resistance. Streptomycin resistance can develop in penicillin-resistant staphylococci following their exposure to streptomycin, but our experimental results show that this resistance failed to appear when the penicillin-resistant organisms were exposed to the combination of atabrine plus streptomycin. We decided to carry out a more comprehensive investigation in order to determine whether or not these findings applied to other antibiotics of diverse chemical structure and mechanism of action.

To evaluate our results on a comparative basis, the inocula in a prototype experiment were prepared from single clones and replicated according to the Lederberg technique[7], on antibiotic-free and corresponding antibiotic-supplemented agar plates. It was necessary to determine the optimal concentration of antibiotic in these plates by prior titration. Under the conditions used, the same number of colonies grew in both antibiotic-free and antibiotic-supplemented replica agar plates.

The sister colonies obtained by this method were individually suspended in M/30 phosphate buffer, *p*H 7·35, and used as the inocula into growth systems. All the growth systems contained the same liquid medium (see footnote to Table 1) and included the specific antibiotic in the presence of which the colony had grown on an agar plate. At the same time, similar growth systems, each containing other individual antibiotics, with and without atabrine, were inoculated with the same number of cells from the corresponding bacterial suspensions of replicated colonies. In this report only the results with streptomycin and erythromycin are presented; these are considered to be representative of the results which we obtained.

The data presented in Tables 1 and 2 reveal that, whereas the clone exposed to a given antibiotic can survive the combination of that antibiotic plus atabrine, the same clone is incapable of surviving the combination of other antibiotics plus atabrine. This would seem to emphasize the fact that each drug elicits the development of specific resistance.

The results presented in Table 3 are from a systematic investigation undertaken to determine the stability of drugs incubated for a prolonged period in a sterile medium. This was made necessary by the fact that aureomycin deviated from the common pattern of results (see under "Freshly prepared systems", Table 3) by growth during a period of 221 h. It can be seen that the potency of aureomycin, on incubation in a sterile medium, with or without atabrine, decreases, and thus this antibiotic was found to be incapable of preventing the emergence of even normal strains. It can also be seen that, with the exception of chloromycetin, all other drugs deteriorate to varying extents (see the results given under "Aged systems", Table 3). It should be emphasized, therefore, that reliable conclusions can be made from results observed in experiments with antibiotics plus atabrine, which are confined to a short period of time (that is, 48–96 h) during which a complete bactericidal action must occur. The results presented in Table 4 show that the two different clones, when exposed to streptomycin and 'Novobiocin', respectively, may reveal differences as regards their ability to survive in 150 μg of streptomycin alone (compare system *A*-3 with system *C*-3, Table 4). Furthermore, the clone exposed to streptomycin and later grown in 'Novobiocin' plus atabrine (system *B*-6), and the clone exposed to 1·5 μg of 'Novobiocin' and later inoculated into 5 μg of 'Novobiocin' plus atabrine (system *D*-6), failed to produce a 'Novobiocin'-resistant strain, thus indicating the possible need for added exposures to acquire a capacity for survival in 5 μg of 'Novobiocin'.

Bacteria inoculated into series of identical growth systems, which individually contain various antibacterial agents, multiply following a period of suppression of division, or following the elimination of certain numbers of bacteria by death, or when both actions occur simultaneously. Those which can survive the drug action and multiply are considered to be drug-resistant. Bacteria that are already resistant to one drug but can also multiply in the presence of unrelated drugs are considered to possess cross-resistance. These facts have been explained by the supposition that a normal population of bacteria contains pre-existent drug-resistant individuals which survive the drug-action and multiply. Since strains capable of surviving various other drugs can emerge from a given normal population, it has been surmised that the normal population may contain as many pre-existent drug-resistant individuals as there are drugs. On this basis, one could postulate that a normal population of bacteria should manifest a cross-resistance behaviour.

The concept outlined here has derived additional support from the results of experiments by Lederberg, using a replicating procedure. Originating from a single clone or a 'master' colony, the growth of replicated colonies on geometrically superimposable loci in drug-supplemented plates has been interpreted to show that the corresponding sister colonies on drug-free plates, and the master colony as well, possess the same drug-resistant character as that which grows on the drug-supplemented agar plate. In other words, the master colony and the sister colony on the drug-free plate are interpreted to be resistant without prior exposure to the drug used.

In reality, however, and despite the refined procedure used by Lederberg, the fundamental question remains unanswered of whether or not the cells in the parent colony and the sister colonies derived therefrom (with and without drug) are in all cases resistant to start with, or prior to multiplication in the presence of drug. To answer this question, a more sensitive procedure was required which made allowances for the differentiation of the actually resistant bacteria and those which were disposed to, or possess the potentiality for, the acquisition of a resistance character triggered by drug action. In addition, it was most important to set up a critical growth system which made allowances for the free growth of bacteria with

Table 1. Specificity of the Resistance Reaction to the Combined Action of Streptomycin and Atabrine of Clones (that is, Respective Inocula) from Replicated Sister Colonies grown without and with Prior Exposure only to Streptomycin and Absence of Cross-resistance Reaction to Other Antibiotics in Combinations with Atabrine

Growth systems	Exp.	A Inoculum from a sister colony grown on drug-free agar							B Inoculum from a sister colony grown on agar with 10 μg of streptomycin/ml.						
		Growth turbidity readings at hours of:							Growth turbidity readings at hours of:						
Additions (μg/ml.)		0	24	48	72	96	132–168	204–240	0	24	48	72	96	132–168	204–240
(1) None	IX *A*	3	320						5	320					
(2) Atabrine 150	IX *A*	3	93	250					0	120	244				
(3) Streptomycin 150	IX *A*	0	—	92	272				0	0	71	250			
(4) Streptomycin 150 + atabrine 150	IX *A*	0	0	0	0	—	—	0	0	0	5	50	80	222	
(5) Tetracycline 1	IX *A*	8	13	—	25	220			6	10	14	41	226		
(6) Tetracycline 1 + atabrine 150	IX *A*	2	0	0	14	14	7	14	1	6	9	6	8	11	2
(7) Penicillin 0·5 u.	IX *A*	3	0	15	141	—	—	—	7	9	14	135	—	—	—
(8) Penicillin 0 5 u. + atabrine 150	IX *A*	1	2	5	3	—	—	11	0	2	7	6	—	—	0
(9) Erythromycin 0·5	IX *Ar*	10	10	14	—	15	300	—	9	14	10	96	179	280	—
(10) Erythromycin 0 5 + atabrine 150	IX *Ar*	12	13	14	—	—	15	21	13	13	12	—	—	—	11
(11) Chloromycetin 10	IX *Ar*	3	39	76	123	153	—	—	3	25	47	71	155	—	—
(12) Chloromycetin 10 + atabrine 150	IX *Ar*	5	7	9	—	10	—	15	7	5	9	6	—	—	7

Inoculum size for experiment IX *A* = 1·8 × 10⁷ cells and for experiment IX *Ar* = 3·1 × 10⁷ cells/5 ml. medium.

Inoculum size for experiment IX *A* = 2·4 × 10⁷ cells and for experiment IX *Ar* = 2·6 × 10⁷ cells/5 ml. medium

Medium: 8 g of nutrient broth (Difco) per litre were supplemented by sodium chloride 8·5 g, sodium monohydrogen phosphate 7·58 g, potassium dihydrogen phosphate 1·82 g and, following sterilization, sterile glucose solution added to render 0·5 per cent. Final *p*H was 7·34.

Table 2. Specificity of the Resistance Reaction to the Combined Action of Erythromycin and Atabrine of Clones (that is, Respective Inocula) from Replicated Sister Colonies grown without and with Prior Exposure only to Erythromycin and Absence of Cross-resistance Reaction to Other Antibiotics in Combinations with Atabrine

Growth systems	Exp.	A Inoculum from a sister colony grown on drug-free agar							B Inoculum from a sister colony grown on agar with 0·1 μg erythromycin/ml.							
		Growth turbidity readings at hours of.							Growth turbidity readings at hours of.							
Additions (μg/ml.)		0	24	48	72	96	168–204	204–240	0	24	48	72	96	132–168	168–204	204–240
(1) None	IX *Er*	2	305						2	225	—	270				
(2) Atabrine	IX *Er*	12	12	46	265				17	15	16	17	32	252	—	288
(3) Erythromycin 0 5	IX *Er*	14	17	18	40	128	300	—	17	155	310	—	—	—	—	—
(4) Erythromycin 0 5 + atabrine 150	IX *Er*	19	25	22	20	—	—	18	22	20	20	17	15	15	232	305
(5) Tetracycline 1 0	IX *Er*	14	40	85	147	290	—	—	15	17	29	286	305	—	—	—
(6) Tetracycline 1 0 + atabrine 150	IX *Er*	15	—	—	—	—	15	15	15	—	—	—	—	—	—	10
(7) Chloromycetin 10	IX *Er*	12	32	100	—	219	—	—	13	25	115	230	—	—	—	—
(8) Chloromycetin 10 + atabrine 150	IX *Er*	9	9	—	—	—	—	11	11	11	—	—	—	—	—	5
(9) Penicillin 0 5 u.	IX *Er*	13	11	15	125	—	—	—	15	19	110	125	—	—	—	—
(10) Penicillin 0·5 u. + atabrine 150	IX *Er*	12	12	—	—	—	—	8	13	12	—	—	—	—	—	7
(11) Streptomycin 150	IX *E*	4	3	0	125	260	—	—	9	8	55	180	232	—	—	—
(12) Streptomycin 150 + atabrine 150	IX *E*	4	2	—	—	—	—	10	5	4	—	—	—	—	—	10
(13) 'Novobiocin' 5	IX *E*	11	160	286	300	—	—	—	5	125	226	260	—	—	—	—
(14) 'Novobiocin' 5 + atabrine 150	IX *E*	11	11	—	—	—	—	16	9	9	—	—	—	—	—	15

Inoculum size for experiment IX *Er* = 3·2 × 10⁷ cells and for experiment IX *E* = 3·3 × 10⁷ cells/5 ml. medium.

Inoculum size for experiment IX *Er* = 6 × 10⁶ cells and for experiment IX *E* = 2·6 × 10⁷ cells/5 ml. medium

established resistance character, but which at the same time eliminated, by death, those which possessed potential, but not actual, resistance. Such requirements for the absolute characterization of these two types of bacteria were met by the findings[2,3] that a strain of bacteria which has been exposed only once to a given drug can readily grow in a combination of that drug plus spermine or atabrine. In contrast, the strain of bacteria which has never before been exposed to that drug is completely eliminated by death under identical conditions. Furthermore, the specificity of these reactions, or the absence of a cross-resistance character in a bacterium resistant to a single drug, was demonstrated by the finding that, where penicillin-resistant staphylococci can survive the action of streptomycin *per se*—giving the appearance of the presence of a cross-resistance character—the same penicillin-resistant strain is annihilated in a combination of streptomycin plus spermine or atabrine. This shows the absence of streptomycin-resistance character in the penicillin-resistant strain, and that the survival in the absence of spermine or atabrine is due to an acquisition of a secondary resistance to streptomycin.

Expanding the foregoing findings in the present study, replicated colonies grown both in the presence and absence of an antibiotic were subjected to a systematic investigation to elucidate, in a broad sense, the question of the specificity of resistance to a drug and of cross-resistance to other drugs (such as streptomycin, tetracycline, penicillin, erythromycin, chloromycetin, 'Novobiocin' and aureomycin). For example, the sister colonies which were grown with, and without, streptomycin were challenged by the afore-named antibiotics with and without atabrine.

The findings from all freshly prepared systems, as presented in Tables 1, 2 and 4, clearly show that each antibiotic produces a certain degree of its own specific effect of resistance. In other words, the colonies grown in the presence of a given antibiotic are capable of surviving a combination of that antibiotic plus atabrine, but are incapable of surviving similar combinations with other antibiotics.

In conclusion, we believe that our previous findings[1-6,8-17] and those presented here differentiate the resistance character established in bacteria following exposure to a drug from those of a potential nature. It would seem that the commonly supposed selection of resistant individuals in a bacterial population deals primarily with the selection of those bacteria which possess the potentiality for resistance. This potentiality is specifically triggered into an actual resistance by a

Table 3. Effect of Ageing, with and without Atabrine, on the Potency of Antibiotics as compared with Freshly Prepared Corresponding Systems
(Ageing for 300 h in sterile growth media)

Growth systems	Aged systems*								Freshly prepared systems*							
	Growth turbidity readings at hours of.								Growth turbidity readings at hours of:							
Additions μg/ml.	0	13	33	53	77	103	125	221	0	13	33	53	77	103	125	221
None	0	270	—	—	305	—	—	—	3	170	280	—	—	245	—	—
Atabrine 150	0	190	—	—	246	—	—	—	5	6	40	—	230	—	—	—
Streptomycin 150	0	0	136	260	—	—	—	—	7	7	252	272	—	250	—	—
Streptomycin 150 + atabrine 150	4	5	—	—	—	8	240	290	6	8	9	—	—	—	15	17
Chloromycetin 10	0	5	85	—	185	—	—	—	10	28	99	—	240	—	—	—
Chloromycetin 10 + atabrine 150	3	3	—	—	—	—	11	17	7	5	9	—	—	—	—	7
Tetracycline 1	1	188	—	288	315	—	—	—	11	18	—	34	78	117	270	290
Tetracycline 1 + atabrine 150	4	25	185	—	197	—	—	—	3	5	—	—	—	—	11	16
Erythromycin 0 5	0	3	105	—	—	280	—	—	5	8	7	27	103	158	190	208
Erythromycin 0 5 + atabrine 150	3	6	3	85	247	—	—	—	6	10	—	—	10	—	10	17
Aureomycin 3	1	252	—	—	—	315	—	—	7	10	83	197	267	—	—	—
Aureomycin 3 + atabrine 150	7	10	31	128	228	—	—	—	7	8	10	8	—	11	13	140
Penicillin 0·5 U.	0	214	—	205	—	188	—	—	0	0	—	—	—	0	31	38
Penicillin 0 5 U. + atabrine 150	3	27	160	—	141	125	—	—	10	10	—	—	—	—	10	17
'Novobiocin 5'	0	23	190	—	207	—	—	—	3	22	248	250	—	—	—	—
'Novobiocin 5' + atabrine 150	20	0	0	2	152	—	190	155	0	3	3	—	—	—	3	8

* Inoculum size $3{\cdot}5 \times 10^7$ cells/5 ml. medium for all systems.

Table 4 Reciprocal Effects of Streptomycin and 'Novobiocin' on the Behaviour of Inoculum from Colonies grown on Plates containing One or the Other Antibiotic

Growth systems	*A* Inoculum from a sister colony grown on drug-free agar								*B* Inoculum from a sister colony grown on agar with 15 μg of streptomycin/ml							
	Growth turbidity readings at hours of:								Growth turbidity readings at hours of.							
Additions (μg/ml)	0	18	44	66	90	162	210	234	0	18	44	66	90	162	210	234
(1) None	3	300	320						0	285	320					
(2) Atabrine 150	0	33	110	185	210				4	10	90	230	270			
(3) Streptomycin 150	3	8	7	8				6	0	5	140	260	295			
(4) 'Novobiocin' 5	0	87	262	278					0	50	278	290				
(5) Streptomycin 150 + atabrine 150	7	11	11	13				11	5	10	7	11	55	240		
(6) 'Novobiocin' 5 + atabrine 150	5	13	12	12				15	4	12	11	12	15			

Additions (μg/ml.)	*C* Inoculum from a sister colony grown on drug-free agar								*D* Inoculum from a sister colony grown on agar with 1 5 μg of 'Novobiocin'/ml.							
(1) None	4	295	320						2	296	315					
(2) Atabrine 150	6	12	80	211	259				5	12	66	200	260			
(3) Streptomycin 150	3	6	183	290	320				4	32	305	330				
(4) 'Novobiocin' 5	1	100	280	295					2	195	280					
(5) Streptomycin 150 + atabrine 150	3	6	6	6				6	0	2	0	1				0
(6) 'Novobiocin' 5 + atabrine 150	6	15	12	13				16	4	8	8	8				6

Inoculum size for system $A = 2{\cdot}3 \times 10^7$ cells; system $B = 1{\cdot}5 \times 10^7$ cells, system $C = 1{\cdot}3 \times 10^7$ cells; system $D = 8{\cdot}0 \times 10^6$ cells/5 ml medium.

drug, but is blocked by a combination of any number of antibiotics plus atabrine or structurally related phenothiazine tranquillizers or antidepressants[6]. Conversely, the pre-existent drug-specific resistance character would seem to be insusceptible to this blockage.

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FUNCTIONAL SITES IN TRANSFER RIBONUCLEIC ACID

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ACCORDING to present concepts of protein biosynthesis[1,2] the raw materials of a protein molecule, amino-acids, are arranged on a template by transfer ribonucleic acids (*t*RNAs). Each *t*RNA molecule, which is specific for each of the twenty common amino-acids, combines with a definite amino-acid with the aid of a specific aminoacyl RNA synthetase. An aminoacyl RNA thus synthesized is transferred by a common transfer enzyme on to a definite position on the messenger RNA (*m*RNA). The *m*RNA serves as a template for a polypeptide and forms a polysome with a few ribosome particles. A *t*RNA molecule selects the correct position among nucleotide sequences of the *m*RNA by virtue of its adaptor site (or coding site), which is considered to be a complementary sequence to the codeword of the *m*RNA. However, little is known about the sites of specific interaction between *t*RNA molecules and aminoacyl RNA synthetases. A *t*RNA molecule must 'recognize' a specific aminoacyl RNA synthetase in order to pick up a specific amino-acid from the amino-acid reservoir. In order to determine the site in *t*RNA which 'recognizes' the aminoacyl RNA synthetase, an investigation was undertaken of the inhibitory effect of various kinds of polynucleotides on the aminoacylation of *t*RNA[3]. It was assumed that inhibition would be observed when the polynucleotide contained a specific structure corresponding to the site in *t*RNA which 'recognized' the aminoacyl RNA synthetase.

Modified *t*RNA, which was oxidized by periodate to destroy the acceptability of amino-acid, inhibited the aminoacyl RNA synthetase. Similar results have already been reported by other research workers[4,5]. Ribosomal RNA showed no inhibitory effect, but the ribosomal RNA partially degraded by mild alkaline treatment had an inhibitory effect. A mild alkaline digest of *t*RNA was also found to be inhibitory. The inhibition by these poly- (or oligo-) nucleotides was competitive with the aminoacylation of *t*RNA, and this did not depend on the terminal phosphate of the oligonucleotide. For valine incorporation into *t*RNA, oligonucleotides longer than tetranucleotides in the ribonuclease digest of RNA showed an inhibitory effect, whereas dinucleotides showed no effect and trinucleotides showed little effect. This article reports on the inhibitory effect of several kinds of synthetic oligonucleotides on the incorporation of amino-acid into *t*RNA, and discusses a possible structure for the site in *t*RNA responsible for 'recognizing' the aminoacyl RNA synthetase.

Aminoacyl RNA synthetase, which was partially purified from brewer's yeast, was incubated with *Torula* yeast *t*RNA, amino-acids and other requirements. (Details of the reaction mixture were given in an earlier report[3].) The amino-acid to be tested was labelled with carbon-14 and added to a mixture containing the other nineteen unlabelled amino-acids. The incubation was carried out at 37° C. At various times the radioactivity incorporated into the nucleotide (insoluble in 5 per cent cold trichloroacetic acid (TCA)) was counted. The polypeptide was insoluble both in cold TCA and in hot TCA, whereas aminoacyl *t*RNA was soluble in hot TCA. In this experiment there was a small, but negligible, radioactivity in the hot TCA insoluble fraction of the reaction product. Therefore, it would seem that amino-acids were only incorporated into *t*RNA. The incorporation of amino-acid into *t*RNA increased linearly during the initial 10 min of the reaction. We measured the incorporation 3 min after the commencement of incubation. It was felt that this might give an indication of the initial rate of the reaction. In order to observe an inhibitory effect for this reaction, oligonucleotide was added to the reaction mixture at various concentrations. The oligonucleotides were synthesized chemically according to Michelson[6].

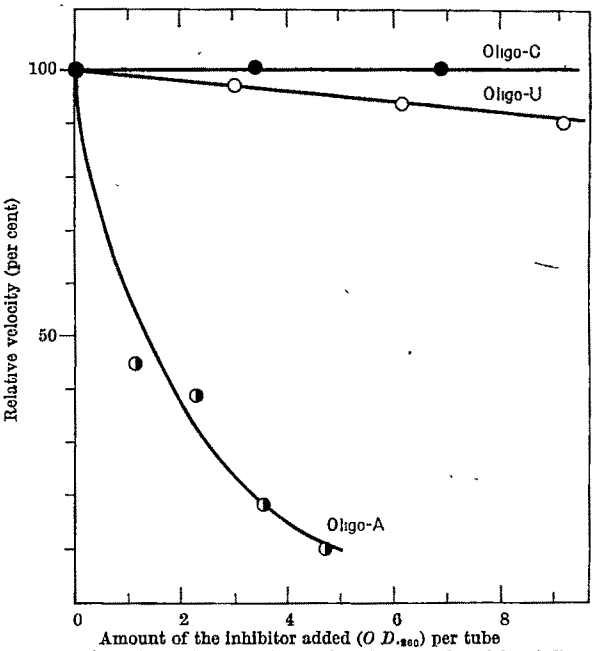


Fig. 1. Inhibitory effect of oligonucleotides for phenylalanyl RNA synthetase. Reaction medium contains: 6 μmoles of ATP, 60 mμmoles of 19 amino-acids, 60 mμmoles of ^{14}C-phenylalanine (10⁶ c.p.m.), 50 μg of partially purified aminoacyl RNA synthetase, 12 mμmoles of transfer RNA, and various amounts of inhibitor in 0·6 ml. of 0·01 M Mg^{++}–0·05 M *tris* buffer, *p*H 7·5. Incubation at 37° C for 3 min gives an initial velocity

(Oligonucleotide samples were gifts from Dr. A. M. Michelson, whom we thank.) The average chain-lengths (estimated by terminal and total phosphate analysis[7]) of oligouridylic acid (oligo-U), oligoadenylic acid (oligo-A), oligocytidylic acid (oligo-C), and oligoguanylic acid (olgio-G) were 4·8, 5·6, 5·2 and 5·6, respectively. These synthetic polymers contained 2′-,5′-phosphodiester linkages as well as 3′-,5′- phosphodiester bonds. A mild alkaline digest of poly-A synthesized by polynucleotide phosphorylase from *Micrococcus lysodeikticus*[8] was also used. The free phosphate ends of these oligonucleotides were eliminated by *Escherichia coli* phosphomonoesterase[9].

The rate of the formation of phenylalanyl *t*RNA was strongly inhibited by the addition of oligo-A, but only slightly by oligo-U, while oligo-C showed no inhibition (Fig. 1). The inhibition of the phenylalanyl RNA formation by oligo-A was a typical competitive inhibition as seen in the Lineweaver–Burk plots of $1/v$ against $1/s$ for various concentrations of the 'inhibitor' (Fig. 2). The rate of lysine incorporation into *t*RNA was strongly inhibited by oligo-U in a competitive manner, only slightly inhibited by oligo-A, and not inhibited by oligo-C (Fig. 3 and Fig. 4). The incorporation of proline into *t*RNA was inhibited

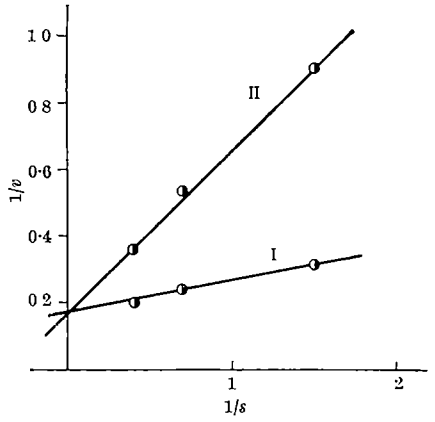


Fig. 2 Competition between *t*RNA and oligo-A for yeast phenylalanyl RNA synthetase. I, Reaction medium contains 10–40 mμmoles of *t*RNA, others are same as Fig. 1, without inhibitor. II, Oligo-A is added as an inhibitor at the concentration of 4·8 *O.D.* units per 0·6 ml. Units of the substrate concentration are μmoles of phenylalanyl *t*RNA/l , and velocity is mμmoles *t*RNA acylated per hour per tube

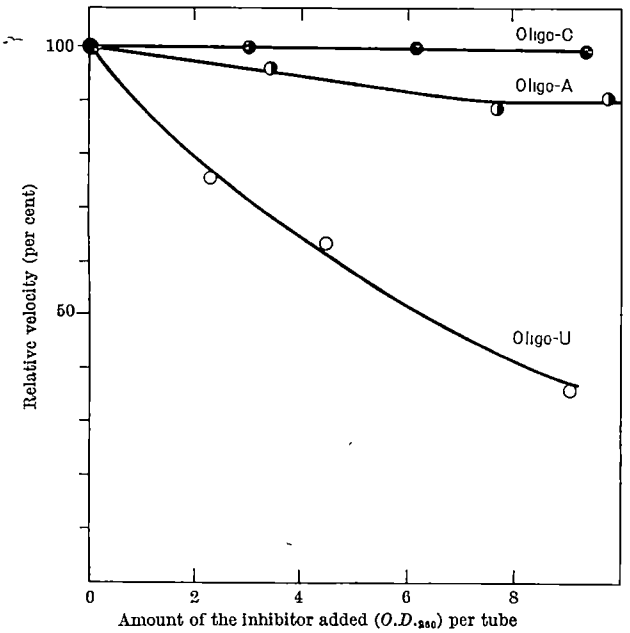


Fig. 3. Inhibition effect of oligonucleotides for lysyl RNA synthetase. Reaction mixture is the same composition to Fig. 1, except ^{14}C-lysine ($1·7 \times 10^4$ c.p.m./mμmole) has been used instead of ^{14}C-phenylalanine

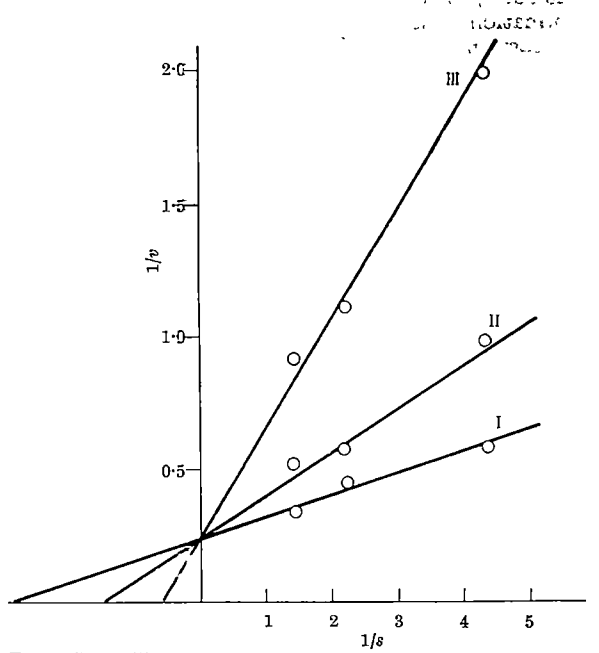


Fig. 4. Competition between *t*RNA and oligo-U for lysyl RNA synthetase. I, without inhibitor. II, Inhibited by oligo-U (4·5 *O.D.* units/0·6 ml.). III, Inhibited by oligo-U (9 0 *O.D.* units/0·6 ml.). (Units of *S* are mμmoles of lysyl *t*RNA per 0·6 ml. Units of *V* are mμmoles of *t*RNA acylated per hour per tube)

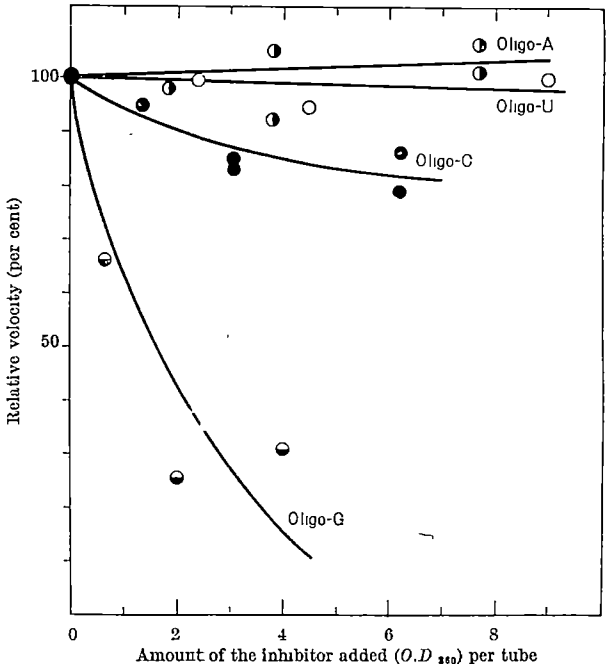


Fig. 5. Inhibition effect of oligonucleotides for prolyl RNA synthetase Reaction mixture is the same composition to Fig. 1, except ^{14}C-proline ($1·7 \times 10^4$ c.p.m./mμmoles) has been used instead of ^{14}C-phenylalanine

strongly by oligo-G, slightly by oligo-C, but not inhibited by either oligo-A or oligo-U (Fig. 5).

If a *t*RNA molecule contains a complementary sequence for a codeword of *m*RNA as suggested in the adaptor concept of Crick[1], phenylalanine RNA may contain ApApAp as a coding site for *m*RNA, since the codeword of *m*RNA for phenylalanine is UpUpUp[10,11]. A reasonable amount of triadenylic acid cluster was observed in unfractionated *t*RNA molecules[12]. The fact that the incorporation of phenylalanine into *t*RNA is inhibited by oligoadenylic acid clusters might be considered as follows: the enzyme recognition site in phenylalanine *t*RNA would also be an adenylic acid cluster. Slight inhibition of phenylalanine incorporation into *t*RNA by oligo-U would be caused by the formation of complementary complexes

for the adenylic acid clusters in *t*RNA. It is possible that the formation of such complexes may reduce the velocity of the reaction. The inhibition by oligo-U to phenylalanine incorporation did not extend beyond 15 per cent with increasing quantities of oligo-U.

For the incorporation of lysine and proline into *t*RNA also, the complementary oligonucleotide to the *m*RNA codeword showed competitive inhibition and the same sort of oligonucleotide as the *m*RNA codeword caused slight inhibition, since the *m*RNA codewords for lysine and proline are ApApAp and CpCpCp, respectively[13,14]. If the anti-codon part (that is, the coding site in *t*RNA) were to consist of complementary nucleotides to the *m*RNA code and the codeword were to be universal for any species, we might assume from the foregoing results that the coding site also (or the same nucleotide sequence as the coding site) acts as an enzyme-recognizing site when a specific amino-acid is incorporated into the terminal site of the specific *t*RNA by the specific aminoacyl RNA synthetase.

Usually a cross-reaction between *t*RNA and amino-acyl RNA synthetase from different sources shows species specificity, which suggests that the enzyme recognizing site in *t*RNA would vary from species to species. Loftfield and Eigner[15], however, found that the Michaelis constants were quite similar for the amino-acylation of *t*RNA by heterologous enzyme when compared with the constants obtained for amino-acylation by the homologous enzyme. They concluded that the enzyme-recognizing site may be identical in all species and that it may be unnecessary to distinguish it from the coding site in *t*RNA. Modification of *t*RNA from different species by bromination led to inactivation of aminoacylation at different rates[16]. Yu and Zamecnik[16] concluded that the enzyme-recognition site in *t*RNA varied from species to species and that it differed from the coding site in the same molecule. Other workers came to similar conclusions after studying the effect of modifications of *t*RNA by treatment with nitrous acid[17], by methylation and bromination[18], or by irradiation with ultra-violet light[19]. These treatments, however, might conceivably inactivate *t*RNA not only by a direct modification of the enzyme-recognition site, but also through changes in the tertiary structure essential for the *t*RNA activity arising from modifications of parts other than the enzyme-recognizing site. Such a phenomenon is suggested by the various inactivation experiments of the aminoacylation of *t*RNA by semicarbazide modification of cytosine residues[20], or by ribonuclease treatment[21]; this possibility is discussed even in those reports which concluded that the enzyme-recognizing site and the coding site were different.

Structural differences in the *t*RNAs specific for an amino-acid among different species have, in fact, been observed[22–24]. If the enzyme-recognizing site were identical throughout all species, the structural differences would be located at sites other than these functional sites.

That the enzyme-recognizing site and the coding site in *t*RNA were identical was also suggested by a few modification experiments. Penniston *et al.*[25] compared the inactivation rates of the aminoacylation activity and of the transfer activity to polysome for the formaldehyde-modified *t*RNA and found that they (the rates) were similar. Zachau[26] modified uracil residues in *t*RNA with ultra-violet light, which makes uracil dimerize intramolecularly. The incorporation of lysine into *t*RNA decreased much more than did phenylalanine. He considered that the anti-codons of lysine *t*RNA and of phenylalanine *t*RNA might be UpUpUp and ApApAp, respectively, and that they might also act as the enzyme-recognition site, so that the ultra-violet inactivation was more effective for the inhibition of lysine incorporation. Similar experimental results[27] were also obtained by a specific modification of uracil residues in *t*RNA with treatment by hydroxylamine at *p*H 9. These data are in agreement with our present results and their interpretation.

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QUANTITATION OF PERIPHERAL BLOOD LYMPHOCYTE CULTURES

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THE tissue culture of human lymphocytes separated from peripheral blood has become widely used to investigate the responses of lymphocytes to antigens *in vitro*. This technique provides a possible method for investigating the immunological status of the lymphocyte donor.

The lymphocytes obtained from human blood are mostly small. If these cells are kept in culture without any apparent stimulus a small number of them spontaneously transform into 'blast' cells (Fig. 2). If the initial population of small lymphocytes is more than 90 per cent pure then after 80–90 h in culture the majority of leucocytes present are lymphocytes or 'blast' cells.

There are many reports that antigens stimulate the transformation of lymphocytes into 'blast' cells *in vitro*[1]. Our experiments with cultures derived from almost pure suspensions of lymphocytes suggest that the lymphocyte is the source of these dividing 'blast' cells. Experiments with lymphocytes from different donors mixed together confirm that in the presence of foreign cells the transformation into 'blast' cells is greatly increased over simultaneous unstimulated control cultures.

The problem is to quantitate this phenomenon of lymphocyte transformation *in vitro* in a meaningful way. Some workers have cited the percentages of blast cells found after a certain period as an indication of the responsiveness of the ... the antigen used *in vitro*. However, using tet... d as the antigen, a number of differing percentages has been found; these are listed in Table 1.

Factors affecting percentage. There are at least three separate events occurring in the cell suspension which have a direct bearing on the final percentage of 'blast' cells. First, part of the original small lymphocyte population may die and disintegrate, if not naturally as a result of manipulation procedures, then by the lethal effect of the antigen on sensitized cells. There are reports of *in vitro* lysis of leucocytes by tuberculin and by a soluble extract from an 8-h *Salmonella enteritidis* broth culture[5,6]. In addition, certain stimulants or the preservatives they contain may be directly toxic to some of the small lymphocytes.

The decline in total cell counts during short-term culture in the presence of tetanus toxoid is shown in Fig. 1. The scatter of the points does not permit a precise determination of the shape of this curve so the best straight line was fitted to the points as a first approximation. Infinite weight was attached to the point at zero time since all experiments had been standardized at the beginning, and a line was drawn through the centre of gravity of the points. The error was estimated by calculating the mean deviation from this best straight line of the y co-ordinates for those points above the line, and for those below.

Secondly, the 'blast' cells are actively dividing in culture. Sampling shows that over a 20-h period in the same culture there is an increase in the absolute number of 'blast' cells per mm³ of culture. This point is illustrated by results in Table 2.

Finally, the 'blast' cells may die and disintegrate. Thus it is more meaningful to refer to the absolute number of 'blast' cells per mm³ in a culture at any given time rather than to the percentage.

Index of response. Accurate quantitation of 'blast' cell production in culture is promoted by allowing cultures only a standard time for growth and development. In particular this time period should be such that the small lymphocytes have sufficient time to transform into large 'blast' cells, but not so long that the 'blast' cells have themselves proliferated by division to any great extent. Once the absolute number of 'blast' cells per mm³ has been determined it is possible to derive an index of response.

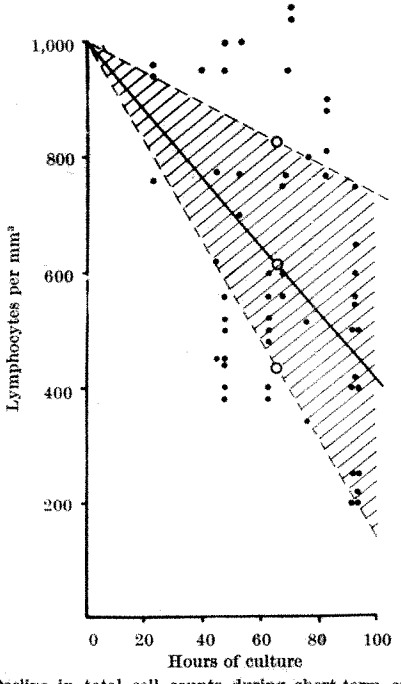


Fig. 1. Decline in total cell counts during short-term culture with tetanus toxoid. The points are readings taken from 25 cultures

Table 1. PERCENTAGES OF 'BLAST' CELLS FOUND IN LYMPHOCYTE CULTURES AFTER TETANUS TOXOID STIMULATION

Investigators	Time of sampling	Percentage
Ling and Husband (ref. 2)	4 days	4
Cowling and Quaglino (ref. 3)	72 h	7
Elves *et al.* (ref. 4)	72 h	> 30

Table 2. INCREASE OF 'BLAST' CELLS IN MIXED LYMPHOCYTE CULTURES WHICH INITIALLY CONTAINED 2,000 ± 90 LYMPHOCYTES PER MM³

Culture reference	Absolute number of 'blast' cells per mm³ At 70 h	At 90 h
EVA/COO	72 ± 4	173 ± 9
LAY/GOU	110 ± 6	297 ± 15

Let T_0 = time when culture starts,
T_s = time of sampling,
N = original absolute number of small lymphocytes per mm³ at T_0,
n = absolute number of 'blast' cells per mm³ present at T_s.

Assuming all 'blast' cells in culture are derived from small lymphocytes, and assuming that the time interval $T_s - T_0$ is sufficient for all the small lymphocytes that have been stimulated to assume a 'blast' cell morphology, the index of response is given by:

$$\text{Index of response} = \frac{1{,}000\,n}{N}$$

For example, using the data in Table 2, culture *EVA/COO* has an index of response given by

$$\frac{173 \pm 5 \text{ per cent}}{2{,}000 \pm 4{\cdot}5 \text{ per cent}} \times 1{,}000 = 87 \pm 7 \text{ per cent}$$

when $T_s - T_0 = 90$ h. Similarly for *LAY/GOU* the index of response in this case is

$$\frac{297 \pm 5 \text{ per cent}}{2{,}000 \pm 4{\cdot}5 \text{ per cent}} \times 1{,}000 = 150 \pm 7 \text{ per cent.}$$

The index of response is a measure of the actual number of original small lymphocytes which undergo transformation in culture.

Allowance must be made for the lymphocytes which transform into 'blast' cells in the control cultures with no apparent stimulus. We propose that a 'corrected index of response' may be calculated by subtracting the 'blast' cell count in the control culture from the 'blast' cell count in the antigen-stimulated culture. The resulting corrected index of response indicates more clearly the responsiveness of the lymphocytes to the *in vitro* stimulus. For example, in mixed lymphocyte cultures:

If n_a is the absolute number of 'blast' cells per mm³ present at T_s in a control culture of donor a; n_b, the absolute number of 'blast' cells per mm³ present at T_s in a control culture of donor b; n_{ab}, the absolute number of 'blast' cells per mm³ present at T_s in a mixed lymphocyte culture of donors a and b; N, the original absolute number of small lymphocytes at T_0 in all three cultures, then the corrected index of response is given by:

$$\text{corrected index of response} = \frac{1{,}000\left(n_{ab} - \frac{n_a + n_b}{2}\right)}{N}$$

The number of 'blast' cells, n, is obtained by multiplying the total white cell count at time T_s by the percentage of 'blast' cells seen in the smear made from the culture. Usually the smear is made from a cell concentrate obtained by centrifuging a sample taken from the culture to increase the cellularity of the smear. It is important that centrifugation is fast enough to ensure that all cells in

the sample are spun down, otherwise there might appear to be a falsely high percentage in the smear of the denser cells from the sample. There are three possible sources of error in determining the absolute number of 'blast' cells. The first lies in the error inherent in visual cell counting technique[7]. We have also found that electronic cell counting can give false results on samples taken from cultures due to the debris present.

The second possible source of error lies in performing the differential count. Rumke[8] has shown that counts of less than 1,000 cells are only reliable to within about ±2 per cent when the minor cell population lies between 1 per cent and 10 per cent. It may be necessary to examine as many as 10,000 cells from any one sample to achieve a significant result especially as there is a tendency for blast cells to be distributed at the tail and edge of the smear.

The third and most serious problem lies in the identification of 'blast' cells in the stained smear. The fully differentiated 'blast' cell is easily recognized (Fig. 2). In wet preparations the cell has an over-all diameter of about 16μ and the nucleus is central. After spinning, smearing and staining with Romanowsky stains the 'blast' cell has basophilic cytoplasm, the nucleus may appear eccentric, and the chromatin of the nucleus in interphase appears to be a combination of a stippled and a fine reticular pattern. The nucleus may contain up to three small nucleoli or one large nucleolus. In the cytoplasm there may be azurophilic granules and small, well-defined vacuoles. 'Blast' cells in mitosis are readily recognized (Fig. 4); indeed, some workers rely on the percentage of mitotic figures in a preparation to indicate the extent of the *in vitro* response[9]. The error in determining the mitotic index is ± 30 per cent (ref. 10).

The daughter 'blast' cells shown forming in Figs. 3–7 are a source of difficulty, as with a Romanowsky stain the main detectable difference between a medium lymphocyte and a daughter 'blast' cell is the depth of cytoplasmic basophilia. With experience, however, it is possible to distinguish these forms in well-stained preparations. The use of a size threshold as a criterion for transformation can be misleading as Winter *et al.*[11] have pointed out, because it is arbitrary and may exclude transitional forms. The size of a 'blast' cell can also vary depending on its position in the smear. It has also been shown that the size of a lymphocyte smeared on a slide often bears no relation to its size in the wet state[12].

Uptake of tritiated thymidine. At present 'blast' cell production is judged visually by many workers and the index of response is an attempt to make the measurement of the 'blast' cell response meaningful, but it would undoubtedly be of value to have a parameter by which to measure 'blast' cell activity other than a purely morphological one.

Pulse labelling of lymphocyte cultures with tritiated thymidine can later be quantitated by autoradiography or liquid scintillation counting. Autoradiography is, of necessity, followed by differential counting and is thus

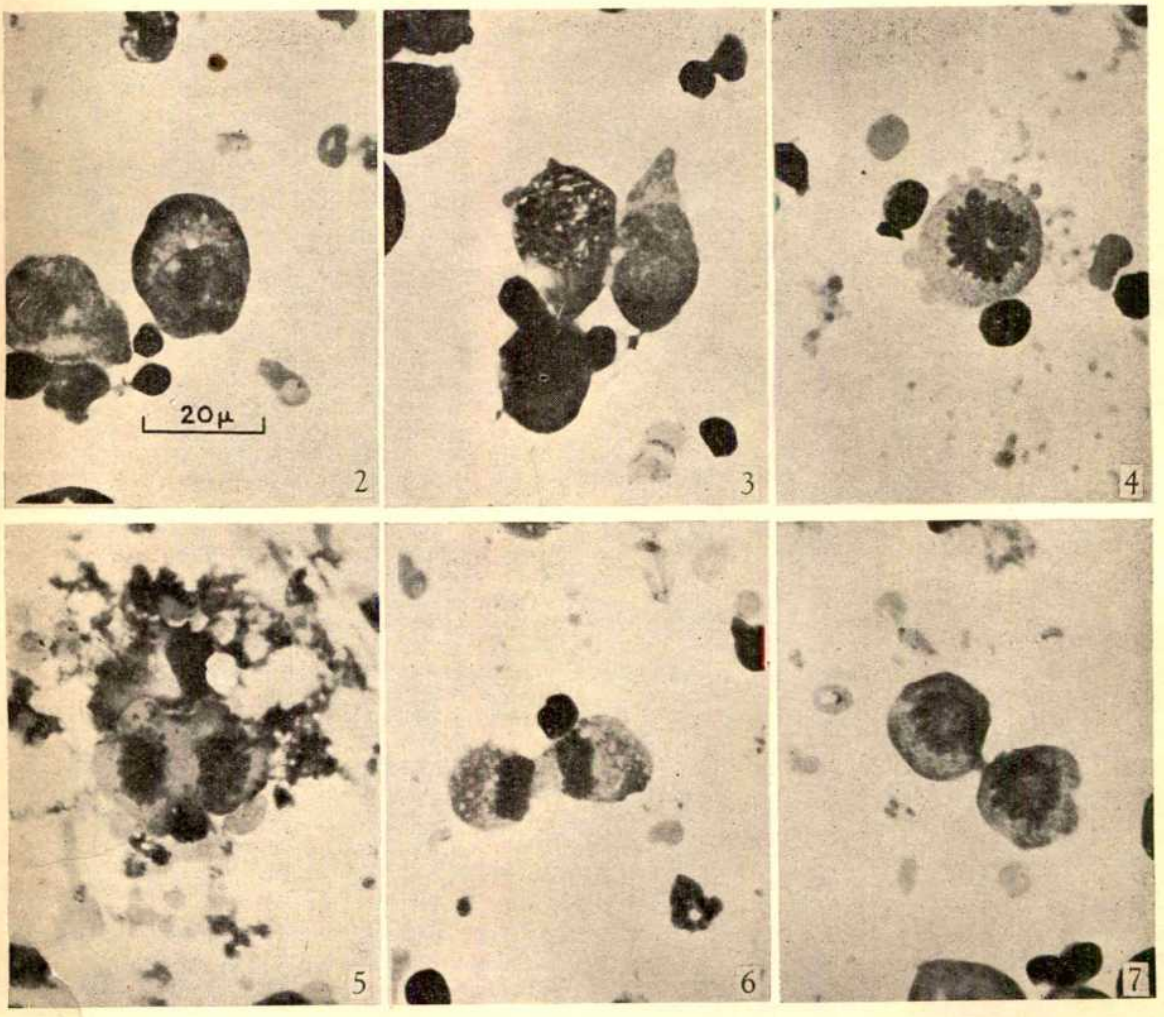


Fig. 2. 'Blast' cells. Figs. 3–7. Various stages of mitosis of 'blast' cell encountered in Romanowsky-stained smears made from lymphocyte cultures. The scale of magnification is the same for all the figures

subject to many of the errors encountered in the morphological techniques already discussed. Furthermore, autoradiography only detects those cells in the *S* compartment of the cell cycle. This information would be of greater value if it could be shown that the number of 'blast' cells in the *S* compartment bore a constant relationship to the total number of 'blast' cells present produced at the time of sampling. Our experience indicates that differential counting on Romanowsky-stained smears by eye is sensitive enough not to miss 'blast' cells that can later be identified autoradiographically.

Liquid scintillation counting detects the amount of label incorporated into the DNA of the cultured cells *en masse*. Besides the need to show a constancy of relationship between 'blast' cells in the *S* compartment and the total 'blast' cell population there is an added complication. Pelc[13] has shown that in many tissues DNA is not perfectly stable and that labelled precursors of DNA can be incorporated into non-dividing cells. It is possible that the conditions prevailing in culture may induce or exacerbate instability in the DNA of those lymphocytes which are not responding by 'blast' cell transformation. The subsequent incorporation of labelled precursors into the total DNA of the culture would thus not be correlated directly with DNA synthesis in the 'blast' cells. This would interfere with accurate quantitation.

The whole problem of quantitating a 'blast' cell response is a reflexion of the ability of the 'blast' cell to grow and divide and allowance must be made for the dynamic nature of the cell population in any attempt at quantitation. Certainly the citing of percentages of 'blast' cells at the time of sampling without simultaneous total counts is open to criticism, and in our experience may be misleading. The index of response is suggested as a method of quantitation for short-term culture and the errors in this approach have been discussed. It is to be hoped that it will prove possible to correlate the different methods used for measuring the cellular response in lymphocyte cultures, that is, labelled precursor uptake, mitotic index and the index of response.

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NUCLEAR STRUCTURE IN LYMPHOID PRIMORDIA

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THE first lymphoid cells appear in the thymus of vertebrates and in the thymus and the bursa of Fabricius of avian vertebrates and emigrate to lymphoid tissues which differentiate later[1]. The recognition and interpretation of this phenomenon differ sharply from earlier views. Most importantly, it differs from John Beard's view that all leucocytes, including the bursa lymphocytes, are derived from the thymus[2,3]. Beard's view, brilliantly in advance of those held by haematologists, cannot account for the proliferation and differentiation of polymorphonuclear granulocytes which occur before the formation of the thymus[4]. These granulocytes cannot originate in the thymus or from emigrant thymus cells. The thymus does not exist at the time when the granulocytes appear[5]. Nor can Beard's statements be reconciled with the histological and ultrastructural evidence that the bursa lymphocytes develop from cells of the bursa epithelium[4]. These two defects in Beard's hypothesis are not generally recognized. This may be due to frequent representation of the term 'leucocyte' to mean 'lymphocyte' and fortuitous inattention to his view of the bursa of Fabricius. Beard's 'thymus' hypothesis was his final contribution to a distinguished investigation of the pharynx and its derivatives. His hypothesis was derived, in major part, from an investigation of elasmobranchii, which would seem to account for its inadequacies when applied to all vertebrates.

Beard emphasized the origin of the thymus. In *Raja batis*, and other fish[6], the thymus appears as placodes of epithelial cells at the junctions of branchial ectoderm and pharyngeal endoderm. This peculiarity would, in Beard's time, serve to distinguish the thymus from the bursa of Fabricius of birds. It was commonly believed that the bursa originates from cloacal or caudal endoderm which is relatively distant from the proctodeal ectoderm[7–9]. We now know that the bursa, like the thymus, originates at or near a point of contact between ectoderm and endoderm[10]. The present realization might well have been derived from two classical studies[11,12]. Neither study, however, attributed importance to the contact of ectoderm and endoderm. In this respect, these investigations parallel the views of most students of the mammalian thymus[13]. The principal proponent of the significance of ectodermal–endodermal contact was Edgar Norris, whose views were derived from a careful investigation of the human pharynx and its derivatives[14]. The importance of the ectoderm was confirmed experimentally in 1954 (ref. 15). If the branchial ectoderm is removed before it contacts the pharyngeal endoderm the thymus does not develop normally. The present report describes nuclear peculiarities in the region of contact between ectoderm and endoderm.

Fig. 1 represents a sagittal section through the cloaca of a 5-day-old chicken embryo. For our purpose, it is impossible to describe any one section as a true mid-sagittal section because the most important 'mid-sagittal' structure is a curved membrane which does not lie entirely within one plane. This is the urodeal membrane (*UM*) seen between the cloaca (*CL*) and the proctodeum (*PR*). The urodeal membrane is a thin, median partition which separates the right and left halves of the body. The membrane is continuous from front to rear and top to bottom except for the vesicles (Fig. 2, *BV*), which will multiply, enlarge and fuse to form the cavity of the bursa of Fabricius.

Fig. 3 is an electron micrograph of an area adjacent to a bursa vesicle (*BV*). The vesicle contains material which is precipitated by routine preparation for electron microscopy. The vesicle is bound by elongated epithelial cells linked by desmosomes and interdigitations of the

cell membranes. The nucleus (*N*) of a typical cell lies at the bottom and its nuclear membrane (*NM*) appears as a double membrane in the centre. At the arrows (→←), the outer membrane of the nuclear membrane is directly continuous with the cell membrane (*CM*). The cell membrane parallels the nuclear membrane and is closely applied to the cell membrane of an adjacent cell. Continuity between the nuclear membrane and the cell membrane has been described in the fusiform cells of Rous sarcomata[16], but we are not aware of previous reports of direct connexions in normal cells of vertebrates. We attribute the direct connexion to the elongation of the cells which surround the vesicles. This brings the nuclear and cytoplasmic membranes into proximity, which may be a necessary condition for the visualization of the connexion in one electron micrograph. The resolution obtained is at the practical limit of our equipment. Fig. 3 includes a part of a bursa vesicle (*BV*) which obviously contains precipitated materials. The formation and growth of these vesicles will be discussed elsewhere.

Fig. 4 represents a frequent feature of the nucleoli of the urodeal cells. The nucleoli contain electron-dense material which forms serpentine threads or meandering

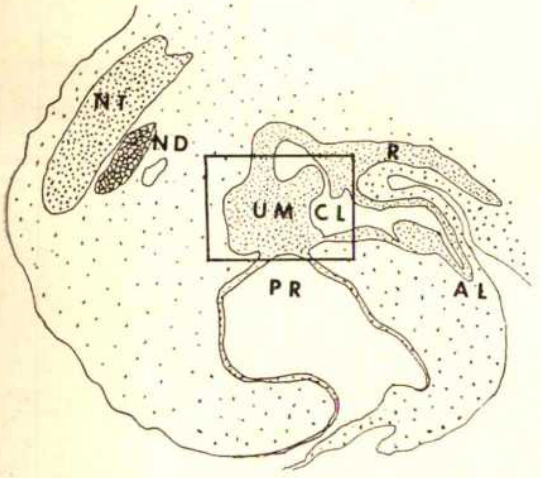


Fig. 1. A diagram of a sagittal section through a 5-day-old chicken embryo. The head lies to the right, outside the figure, and the tail curves to the left below the proctodeum (*PR*). The proctodeum is separated from the cloaca (*CL*) by the urodeal membrane (*UM*). *AL*, allantois; *R*, rectum; *NT*, neural tube; *ND*, notochord. The framed area corresponds to Fig. 2. (× *c.* 28)

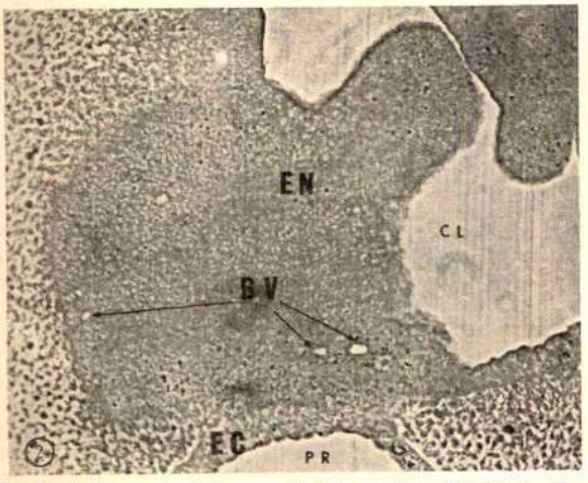


Fig. 2. A phase-contrast photograph of the area framed in Fig. 1. The vesicles (*BV*) which will fuse to form the lumen of the bursa of Fabricius are present in the urodeal membrane. The ectodermal epithelium (*EC*) of the proctodeum abuts against the endoderm (*EN*) which occupies most of the urodeal membrane. The tissue was fixed in 1 per cent osmium tetroxide, embedded in 'Araldite', and sectioned at 10μ. The photograph was taken with 'Agepe' *FF* 35-mm film, a Leitz '*n*' dark phase contrast 10× objective, and a Leitz Heine condenser. (× *c.* 120)

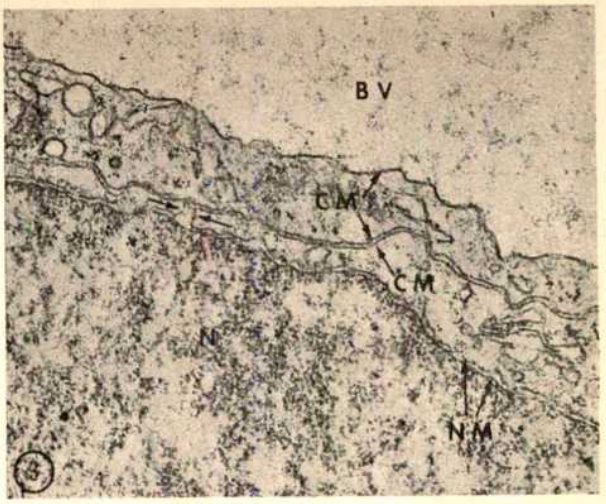


Fig. 3. An electron micrograph of part of the urodeal membrane of a 5-day-old chicken embryo. Part of a bursa vesicle (*BV*) is included. Parts of two typical, elongated cells are seen adjacent to the vesicle. Small vesicles may be seen in the cytoplasm of both cells. One of these appears as a continuation (→←) of the outer nuclear membrane (*NM*) with the cytoplasmic membrane (*CM*). *N*, Nucleus. The tissue was prepared in the same way as that represented in Fig. 2, except that the plastic sections were stained with uranyl acetate. A Phillips *EM*100*B* microscope was used with an objective aperture of 25μ. (×18,400)

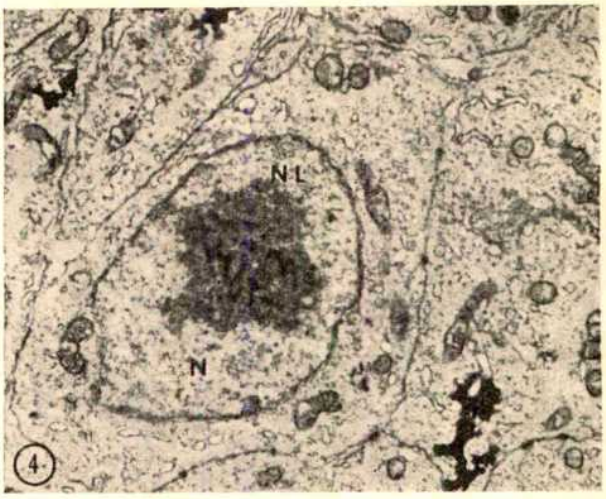


Fig. 4. An electron micrograph of the urodeal membrane of a 5-day-old chicken embryo. The dark, thread-like or membranous mass lies within the nucleolus (*NL*) of a typical nucleus (*N*). The figure was prepared to emphasize the contrast between this mass and the remainder of the nucleolus. The section was not stained with uranyl acetate. Otherwise the preparation and conditions were the same as for Fig. 3. (× *c.* 8,935)

membranes. It is difficult to imagine that these represent random aggregates of granules. The density is equivalent to that of desmosomes. The threads are found in large, dense nucleoli and do not reach the edges of the nucleoli. The only other structures in these nucleoli are the granules which are distributed uniformly. We advance no interpretation of the dense threads.

Fig. 5 represents the intimate contact (→←) of ectoderm and endoderm in the region of the thymus (←). The thymus primordium appears in the area marked by one arrow, but is in an adjacent section. Fig. 5 is selected because it shows the point of contact particularly well. Although the thymus primordium proper shows signs of active proliferation, both it and the adjacent epithelia undergo severe degenerative changes.

Fig. 6 represents a form of degeneration common in the primordium of the thymus. Normal nuclear pores (*NP*) are present in a nuclear membrane which has begun to form myelin figures. If we extend the term introduced by Hruban *et al.*[17] we might term this 'focal nuclear degradation'. This would be misleading because the

cytoplasm also forms myelin figures. Myelin figures sprout up everywhere. This is just one characteristic of a degeneration which involves the nucleus and cytoplasm of every cell in some parts of the epithelial complex. The degradation of the nucleus is a prominent feature in the formation of the thymus, but is less evident in the formation of the bursa. In the region of the bursa, the cytoplasm may undergo focal cytoplasmic degradation[17] while the nucleus remains normal. In the region of the thymus, the nucleus and the cytoplasm change and deteriorate simultaneously. The cytoplasmic changes and their relation to nuclear changes are the subject of a more detailed report.

The present report confirms the intimate contact of ectoderm and endoderm at the points of origin of the thymus and the bursa of Fabricius. This contact is associated with contrasts in cell behaviour. Many of the cells degenerate and die. Others persist to form the bursa and the thymus. The degeneration in the region of the thymus is characterized by a simultaneous degradation of the nucleus and the cytoplasm. The degeneration in the region of the bursa is characterized by degradation of the cytoplasm which precedes or is unaccompanied by degradation of the nucleus. The degeneration precedes

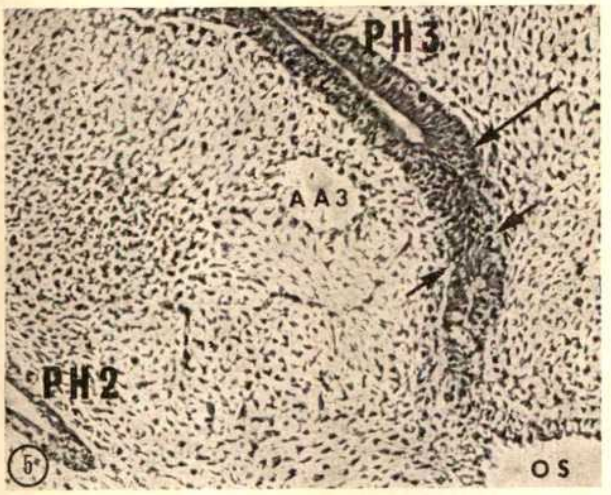


Fig. 5. A phase-contrast photograph of a frontal section through the pharynx of a 5-day-old chicken embryo. The branchial ectoderm extends inward from the outside surface (*OS*) of the body to a point (→←) adjacent to the primordium of the thymus of the third pharyngeal pouch (←). The endoderm of the third pharyngeal pouch (*PH3*) extends outward to the primordium of the thymus. The second pharyngeal pouch (*PH2*) lies to the left and the third aortic arch (*AA3*) lies between the pouches. The preparation of the tissue and photographic conditions were the same as for Fig. 2, except that the fixation with osmium tetroxide was preceded by fixation in glutaraldehyde. (× *c.* 135)

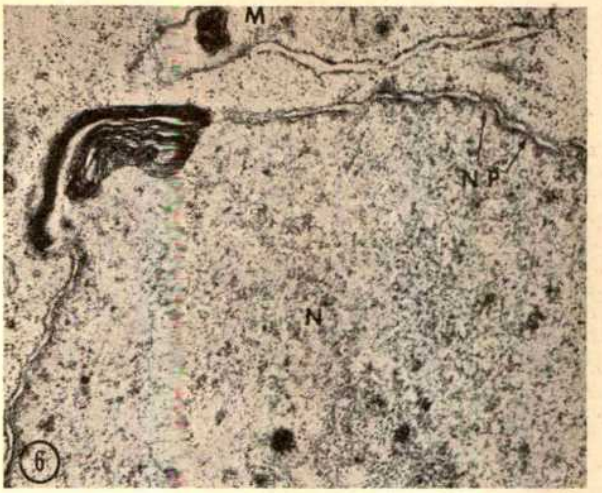


Fig. 6. An electron micrograph of the primordium of the thymus of a 5-day-old chicken embryo. Nuclear pores (*NP*) are seen in a nuclear membrane which is forming myelin figures at two widely spaced points, one of which is shown. Degradation of this type accompanies degradation of the mitochondria (*M*) in the thymus, but has not been seen in the bursa. The preparation of the tissue and the photographic conditions are the same as those for Fig. 3. (× 20,000)

the formation of the thymus and the bursa; the intimate contact between the persisting and degenerating cells suggests that the degeneration is a stimulus to the formation of the lymphoid primordia and an early step in the acquisition of specific immunological reactivity.

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INCREASED AORTIC BETA-GLUCURONIDASE ACTIVITY WITH PROGRESSIVELY SEVERE ARTERIOSCLEROSIS IN FEMALE BREEDER RATS

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In previous reports we described the development of naturally occurring arteriosclerosis in both male and female rats after repeated breeding[1–4]. Microscopic arterial lesions begin to appear in male and female breeders as early as the first or second breeding. Such lesions appear first in the abdominal aorta and consist of focal sub-intimal accumulations of acid mucopolysaccharides, endothelial hyperplasia and fibrosis. With advancing arterial disease the media show pooling of acid mucopolysaccharides about individual elastic fibres, elastosis, ground substance degeneration and calcification. As the enzyme, β-glucuronidase, may be involved in mucopolysaccharide metabolism[5–7], we undertook to determine whether it might be involved in the pathogenesis of the ground substance degeneration and arteriosclerosis in breeder rats. Accordingly, this enzyme was assayed in serum, adrenal glands and aortas of virgin animals, which were free of arterial disease, and of breeder rats having

varying degrees of microscopic and grossly visible arteriosclerosis. In addition, combined histochemical and histological techniques were used in an attempt to determine whether β-glucuronidase activity could be demonstrated in areas of mucopolysaccharide accumulation in and about arterial lesions.

The animals used in this investigation were Sprague–Dawley rats obtained from the Sprague–Dawley Farms, Madison, Wisconsin. The animals were housed in air-conditioned quarters, were given tap water to drink and were allowed free access to food at all times. The diet consisted of a pelleted commercial rat diet (Texlad, Inc., Monmouth, Illinois) which has a relatively low fat content (4 per cent).

Male and female virgin rats served as controls and were comparable in age to the breeder rats. The degree of development of arteriosclerosis of the aorta was determined by gross examination at the time of autopsy. Since male breeder rats develop only microscopic lesions in the aorta, the arterial samples removed from these animals were considered as a separate class. Female breeders, however, develop varying degrees of grossly visible arteriosclerosis which are readily detected at autopsy. The arteries and tissues from these animals were separated into four classes: clear, minimal, moderate and severe, in keeping with the severity of gross arteriosclerosis found in the aorta[1]. To avoid the stress of anaesthesia at the time of autopsy, all animals were decapitated instantly by means of a guillotine. Arteriovenous blood was collected from the large severed vessels in the neck; samples of serum were prepared by prompt centrifugation. The adrenal glands were quickly removed, trimmed free of fat and extraneous tissue, homogenized in ice-cold water and filtered. The entire aorta extending from the heart down to the bifurcation into the iliac arteries was removed, weighed, stripped of extraneous tissue, cut up with fine scissors, homogenized in ice-cold water and filtered. In some investigations, specific segments of the aorta, for example, arch, thoracic and abdominal, were removed and treated in the same manner. The arch of the aorta consisted of the ascending and descending limbs of the aorta free of the innominate and carotid artery branches. The thoracic aorta consisted of that portion of the aorta between the point at which the superior vena azygos crosses the aorta down to the folia of the diaphragm. The abdominal aorta extended from the diaphragm, free of side branches, down to the bifurcation into the iliac arteries.

The method of Fishman *et al.*[8] was used with only minor modifications to determine the β-glucuronidase activity in serum or in the supernatants from homogenates of adrenal or aortic tissue. Buffered serum or an aliquot of tissue homogenate was incubated with phenolphthalein glucuronic acid as described in the original method[8] and the amount of phenolphthalein released was determined. The activity was then calculated as the amount (μg) of phenolphthalein liberated per hour—1 μg/h equalling one unit. Serum samples were incubated for 20 h, adrenal homogenates, 2·5 per cent (w/v), for 2 h, and aortic homogenates, 5–10 per cent (w/v), for 20 h.

In order to demonstrate histochemically the presence of β-glucuronidase, tissue was fixed overnight in cold chloral hydrateformalin as prescribed by Fishman and Baker[9]. Cryostat sections were cut at 8μ and stained according to the method described by Seligman *et al.*[10].

Segments of the aorta and adrenal glands were all fixed in 10 per cent buffered neutral formalin (Lillie) embedded in paraffin and sectioned at 3μ. Frozen sections were cut at 5–10μ. Haematoxylin and eosin were used for routine examination; Gomori's aldehyde fuchsin stain for elastic tissue; alcian blue and toluidine blue for demonstration of metachromasia and acid mucopolysaccharides as well as the Hale stain; the von Kossa method for calcium; and Sudans II and III, oil red O and Sudan black *B* for the demonstration of lipids in the frozen sections.

No significant differences were found in the serum levels of β-glucuronidase between virgin rats without arterial disease and breeder rats with either microscopic or gross arteriosclerosis (Table 1). Although the adrenal glands contained a considerable amount of β-glucuronidase activity, no significant differences could be found between animals with and without arterial disease (Table 1). Furthermore, no difference could be demonstrated between the β-glucuronidase activity of adrenal glands from male or female rats (Table 1).

Table 1. β-Glucuronidase Activity in the Serum and Adrenal Glands of Virgin and Breeder Rats

Sample	Virgins, No arterial disease, Males*	Virgins, No arterial disease, Females*	Breeders, Microscopic arteriosclerosis, Males*	Breeders, No grossly visible arteriosclerosis, Females*	Breeders, Gross arteriosclerosis, Females*
Serum† (units/0·1 ml. ± *S.D.*)	1·13 ± 0·17	1·35 ± 0·26	1·12 ± 0·14	1·21 ± 0·16	1·26 ± 0·23
Adrenals† (units/mg ± *S.D.*)	3·05 ± 0·46	3·23 ± 0·84	2·33 ± 0·78	3·54 ± 0·50	3·42 ± 0·72

* Number of samples for each determination was 5.
† One unit of activity equals 1 μg of phenolphthalein released per h.

Significant increases, however, were found in the β-glucuronidase activity of aortas taken from female breeders with advanced gross arteriosclerosis (Fig. 1). These increases paralleled the severity of arteriosclerosis; that is, aortas from female breeders with moderate or severe gross arteriosclerosis had the greatest enzyme activity (Fig. 1). There was no significant difference in enzyme activity of the aorta between male virgin controls with no arterial disease and the male breeders with microscopic arterial lesions (Fig. 1).

Separation of the aorta of female breeder rats with varying degrees of gross arteriosclerosis into three anatomical segments, that is, arch, thoracic and abdominal, revealed striking differences in enzyme activity in these segments (Fig. 2), which coincided with: (*a*) the anatomical progression of arteriosclerosis as it develops and spreads in these animals; (*b*) the increasing severity of the disease.

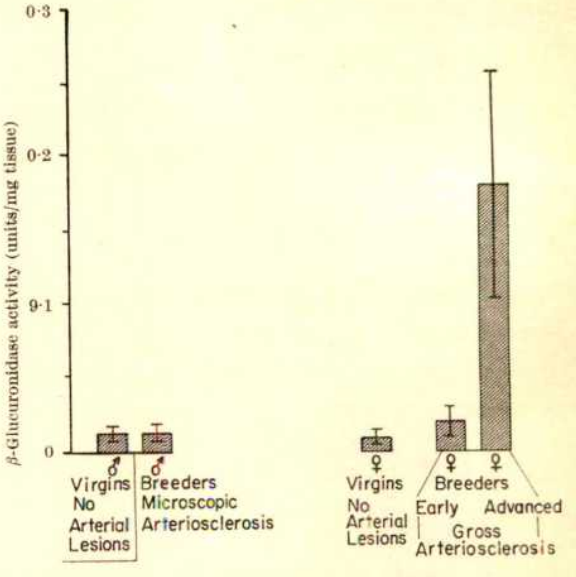


Fig. 1. Average β-glucuronidase activity of whole aortas (± *S.D.*) removed from male and female virgin rats having no arteriosclerosis (controls) compared with aortas removed from male breeders having microscopic arterial lesions only and with female breeders having either early or advanced gross arteriosclerosis. One unit of β-glucuronidase activity equals 1 μg of phenolphthalein released per h. Statistical comparison (using the '*t*-test') shows that there is no significant difference ($P > 0.05$) between the aortas of virgin rats and aortas of breeder rats having only early gross arteriosclerosis. However, highly significant differences were found among the aortas of virgin females with no arteriosclerosis and female breeders with advanced gross arteriosclerosis, $P = \ll 0.005$. (Five samples of aorta were used in each of the classes depicted)

In the female breeder rats the aortas of which showed no grossly visible lesions, that is, 'clear' (these grossly clear aortas, however, contain microscopic arterial lesions), no real differences could be determined between the three aortic segments. However, in female breeders with minimal gross arteriosclerosis, where the gross arterial plaques can be seen in the abdominal aorta only, β-glucuronidase activity was significantly elevated in only the abdominal aorta (Fig. 2).

In female breeders with moderate arteriosclerosis, where grossly visible plaques could be detected first in the abdominal and secondly in the arch segments of the aorta, the β-glucuronidase activity was significantly elevated in these two segments, specifically (Fig. 2). The thoracic aorta, which displays no grossly visible plaques in female breeders with moderate arteriosclerosis, showed no significant increase in β-glucuronidase activity (Fig. 2).

Female breeders with severe gross arteriosclerosis showed a significant increase in β-glucuronidase activity in all segments of the aorta (Fig. 2) in keeping with the presence of advanced arteriosclerosis throughout the aorta of such animals.

In general, a clear, step-wise trend toward increased aortic β-glucuronidase activity was noted within each of the aortic segments concomitant with increasing severity of arterial disease, that is, clear, minimal, moderate and severe.

Chemical analyses of aortic β-glucuronidase activity agreed well with the histochemical demonstrations of the enzyme. The enzyme was demonstrable histochemically

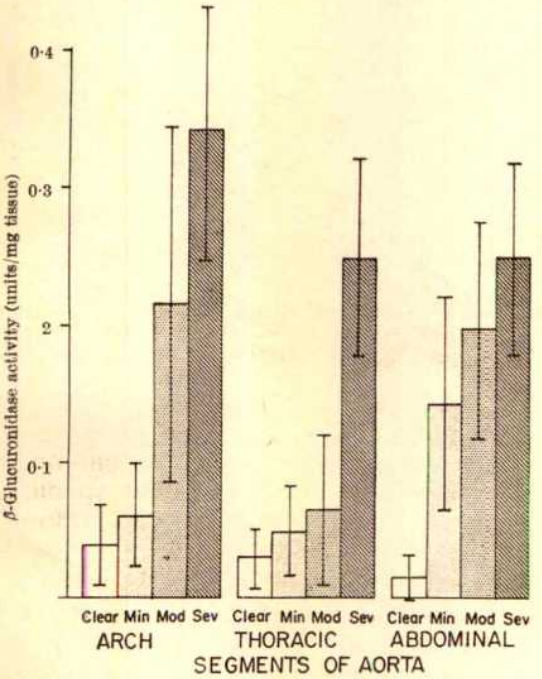


Fig. 2. Average β-glucuronidase activity of the arch, thoracic, and abdominal aortic segments (± *S.D.*) of aortas removed from female breeder rats having varying degrees of gross arteriosclerosis ranging from clear to minimal to moderate to severe. One unit of β-glucuronidase activity equals 1 μg of phenolphthalein released per h. Statistical treatment of the data by the method of analysis of variance showed that there was a highly significant difference in aortic β-glucuronidase activity ($P = < 0{\cdot}01$) among the various classes of arterial pathology in all aortic segments. Grouping of the arterial pathology classes within each aortic segment into those manifesting early versus advanced arteriosclerosis, for example, within the abdominal aorta, clear versus minimal + moderate + severe; within the aortic arch, clear + minimal versus moderate + severe; within the thoracic aorta, clear versus minimal + moderate + severe, statistical application of the '*t*-test' showed that, in all cases, there was a highly significant increase in aortic β-glucuronidase activity in the arteriosclerotic aortas commensurate with advancing severity of the arteriosclerosis ($P = < 0{\cdot}001$). (Seven separate samples were used throughout for each of the varieties of aortic samples depicted, except in the case of those with 'severe' arteriosclerosis where nine samples were used for each case, that is, a total of ninety individual aortic samples were investigated)

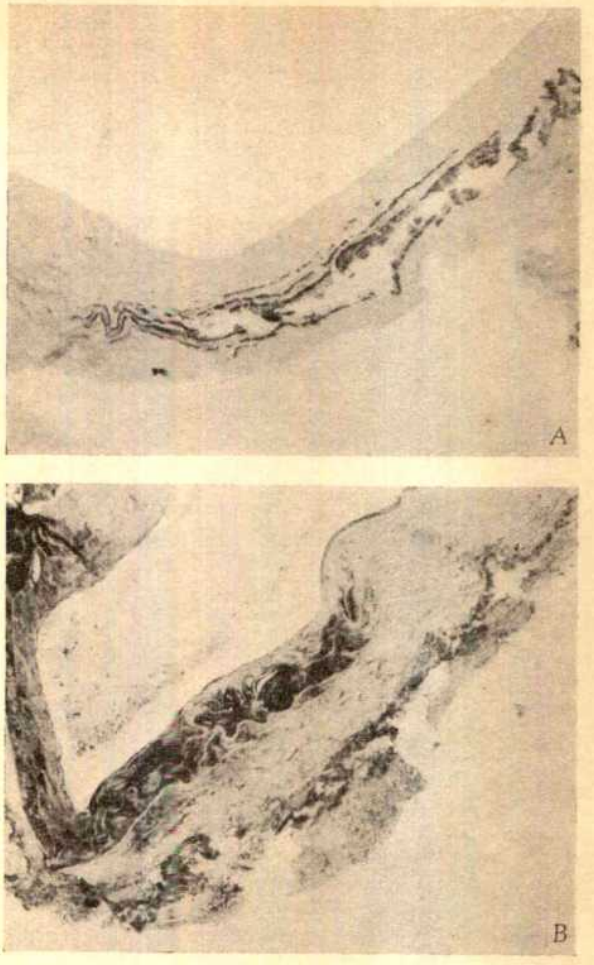


Fig. 3. *A*, Cryostat section of an aorta taken from a female breeder rat which had moderate gross arteriosclerosis. Positive β-glucuronidase activity (deep black material in figure) is found concentrated about individual elastic fibres. These same sites also contain much acid mucopolysaccharide (compare 3*B*) and calcium (× 37·5). *B*, Paraffin section stained for acid mucopolysaccharides (Hale stain), pooling of mucopolysaccharide (deep black material in figure) about degenerating elastic fibres which appear as white wavy lines (× 75)

in those arterial segments with the more advanced and complicated arteriosclerotic lesions. The aortas of virgin rats free of arteriosclerosis, and of breeder rats with microscopic arterial lesions only, showed no trace of β-glucuronidase activity.

The most intense positive reaction for β-glucuronidase was concentrated in the media, particularly in areas of degenerating ground substance and around elastic fibres which were undergoing dissolution (Fig. 3). Medial sites of dystrophic calcification also showed substantial enzymatic activity. In foci of cartilaginous metaplasia, the individual cartilaginous cells displayed a halo which reacted positively for β-glucuronidase.

In some aortas, particularly those with advanced arteriosclerosis, a diffuse positive reaction for the enzyme was observed in the media often accompanied by a very strong positive reaction in the adventitia. Those medial foci which were intensely basophilic (with haematoxylin), strongly metachromatic, and which reacted positively with alcian blue and to the Hale stain showed the greatest enzyme activity. Since the spleen has a very high level of β-glucuronidase activity, sections of spleen were tested simultaneously as control tissues together with the aortic tissue. The sections of spleen were intensely positive for the enzyme.

Unlike aortic tissue, the adrenal glands showed a much more positive reaction for β-glucuronidase. The zona (z.) glomerulosa showed only slight positive enzyme activity

in virgin rats and no activity in breeder rats. In the virgin rats, male and female, the outer portion of the z. fasciculata was intensely positive for the enzyme, but the z. reticularis and inner portions of the z. fasciculata were completely free of enzyme activity.

In breeder rats, both the z. fasciculata and the z. reticularis displayed enzyme activity but much less intense than that observed in the outer z. fasciculata of virgin rats.

The details of the histological changes involved in the pathogenesis of arteriosclerosis in breeder rats have been reported[1-4]. The earliest changes seen in the aortas of breeder rats consisted of a focal aggregation of basophilic cell elements both in the intima and media. These cells are surrounded by a metachromatic substance, probably acid mucopolysaccharide, and are either mesenchymal cells or smooth muscle cells. Large quantities of acid mucopolysaccharides appear in the intimal space and become transformed into fibrotic plaques.

With advancing arteriosclerosis the media show generalized accumulation of acid mucopolysaccharides between medial muscle cells and concentrated pooling about individual elastic fibres. In scattered foci the elastic fibres and ground substance undergo degeneration followed by haemorrhage and necrosis. The accumulation of acid mucopolysaccharide is intense in those loci which become filled-in or repaired either by cartilaginous metaplasia or dystrophic calcification.

The finding of significant increases in aortic β-glucuronidase activity with increasing severity of arteriosclerosis in breeder rats is interesting because changes in β-glucuronidase activity may be associated with: (*a*) mucopolysaccharide metabolism[6,7]; (*b*) active cell proliferation, for example, arteriosclerosis[6,7] or cancer[5,11,12]; (*c*) cyclic changes within the female sex organs accompanied by a rise and fall in enzyme activity during menstruation and pregnancy[5,9,13]; (*d*) increased enzyme activity in various endocrine glands and in serum in response to adrenal steroids[14-16], stress and Cushing's disease[17]. The arteriosclerosis which develops in breeder rats is similarly also associated with: (*a*) marked alterations in the mucopolysaccharides of aortic ground substance; (*b*) a remarkable sex difference in the nature of the development of the arteriosclerosis and the mucopolysaccharide changes; (*c*) marked activity of the reproductive system, particularly apparent in the female; (*d*) equally marked changes in adrenal steroid function[18] and histology[19], accompanied by degenerative changes associated with hyperadrenocorticism.

The increase in arterial enzyme activity was not reflected through any apparent change in serum levels. However, these breeder rats were examined several weeks *post partum* when serum enzyme activity would be at its lowest level[11,13,20-22].

The results of the chemical analyses of adrenal β-glucuronidase activity provided no clue as to how this enzyme in the adrenal cortex might participate either in changes in cortical function *per se* or in the enzyme changes observed within the arterial wall.

However, the histochemical testing of adrenal gland slices revealed specific zonal differences in the concentration and distribution of β-glucuronidase, that is, absence of β-glucuronidase in the z. reticularis of adrenals of virgin rats which have no arterial lesions compared with its presence in this zone in arteriosclerotic breeder rats. This condition could be a reflexion of altered 17-ketosteroid production in breeders.

The significant increase in aortic content of β-glucuronidase in female breeders with increasing severity of gross arteriosclerosis is particularly meaningful and provocative. Kirk[6] reported that although the activity of this enzyme was relatively low in normal human aortas, it did become elevated in arteriosclerotic aortas. Kayahan[23] claims that β-glucuronidase activity is low in the serum of animals prone to develop atherosclerosis and high in those resistant to atherosclerosis. In our work the enzyme activity consistently coincided with the anatomical progression of the disease in breeder rats. This is especially well illustrated in the case of female breeders with moderate arteriosclerosis (see Fig. 2). Although high enzyme activity was demonstrated in both the plaque-ridden arch and abdominal aortic segments, the thoracic segment, free of any gross plaques and serving as an internal control, was devoid of any significant enzyme activity.

The close association of enzyme activity with ground substance degeneration and acid mucopolysaccharide accumulation is in accord with the findings of Zemplényi *et al.*[7]. These investigators also found, just as we did, increased enzyme activity in medial smooth muscle cells and endothelium. Furthermore, they found increased β-glucuronidase activity in cholesterol-fed rabbits preceding the development of any recognizable arterial lesions[7].

The appearance of arterial lesions in first the abdominal and later the arch and thoracic segments of the aorta of female breeders may be due to physico-chemical differences between the arterial segments conditioned by female sex hormones. In this connexion, Malinow *et al.*[24] and Christie and Dahl[25] have shown that there are cyclic changes in aortic oxygen consumption associated with sexual activity in female rats and that the aerobic and anaerobic metabolism of the rat aorta is under the influence of gonadal hormones. Similarly, Bertelsen[26] has shown that mucopolysaccharides, the sulphated mucopolysaccharides in particular, tend to accumulate in tissues having a low oxygen tension. We have also observed considerable differences in calcium, hydroxyproline (as an index of collagen content) and mucopolysaccharide between aortas of male and female breeder rats, between aortas with early and advanced arteriosclerosis and between the various segments of the aorta[27].

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

High-resolution Spectra of Decametric Radio Bursts from Jupiter

A PREVIOUS experiment relating to the fine structure in the dynamic spectra of decametric radiation from Jupiter[1,2] was repeated during the 1964 apparition of the planet, but using different techniques of observation.

The main instrument was a high-resolution multi-channel spectrograph. The centre channel was tuned to 19·0 Mc/s and the others at 50-kc/s intervals on both sides of it—their 3 and 40 dB band-widths being 5 and 50 kc/s, respectively. The number of channels varied from 10 to 14. The dynamic spectra were recorded photographically from intensity-modulated indicators, the time constant of integration being 30 msec. The aerial was a two-element collinear array with driven reflectors.

Two phase-switching interferometers were used to identify the Jovian radio bursts. One was of a panoramic type sweeping, repeatedly every 6 sec, the range 20–15 Mc/s. The dynamic spectra with phase were recorded on colour film from an intensity-modulated white-phosphor cathode-ray tube display, in front of which a colour disk with red and green segments was synchronized to rotate with the broad-band phase-switch. The other interferometer was of a single-frequency type (working at 19·3 Mc/s) driving a pen recorder. The aerials consisted respectively of two horizontal biconical dipoles with reflecting screens, and two three-element Yagi aerials, the baselines extending a few wave-lengths in the east–west direction.

The multi-channel spectrograph was operated for 4–5 h each night from September 1 to December 15, 1964, and the other receivers continuously over the same period. Almost no local interference was experienced at either of the two isolated sites of observation and the low night-time values of the ionospheric critical penetration frequency resulted in the absence of long-range interference during the Jovian transits.

Only the strongest bursts were recorded with the high-resolution equipment, the total number of nights with activity being 33. Most of the recordings showed roughly similar variations of intensity with time from channel to channel, indicating radiation band-widths of the order of 500 kc/s or more, the durations ranging from a few seconds to a few tens of seconds. On 14 nights recordings were obtained in which the variations were not similar at all, indicating fine structure composed of pulses with band-widths of the order of 50 kc/s or less. The duration of pulses varied from the time resolution of the instrument to a few seconds. A typical spectrum is reproduced in Fig. 1.

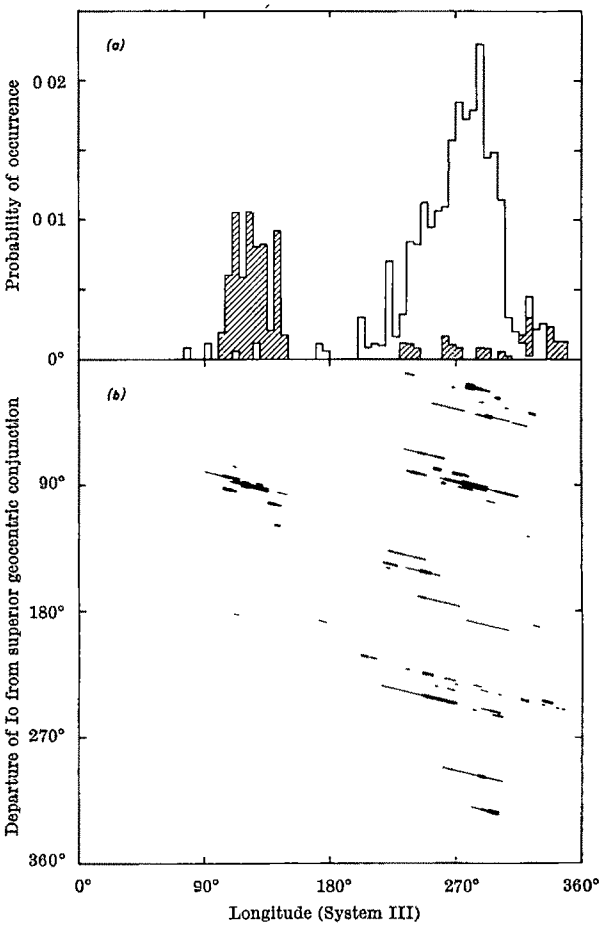


Fig. 2. (*a*) Probability of occurrence plotted against System III longitude. Recordings that are dissimilar from channel to channel are denoted by shaded areas and those that are on average similar by unshaded areas. (*b*) The radio storms observed in high resolution plotted on System III longitude and the position of the satellite Io

The probability of occurrence as a function of System III central meridian longitude[3] is shown in Fig. 2*a*, the shaded profile denoting narrow-band structure. The relatively low over-all gain of the multi-channel installation results in low values of probabilities. Following the precedence of Bigg[4] the storms are plotted in Fig. 2*b* on the co-ordinate system of the longitude of the Jovian satellite Io in its orbit, measured from superior geocentric conjunction[5], and the radio longitude. Here the width of the lines is closely related to the total duration of the bursts in equal intervals of observing time.

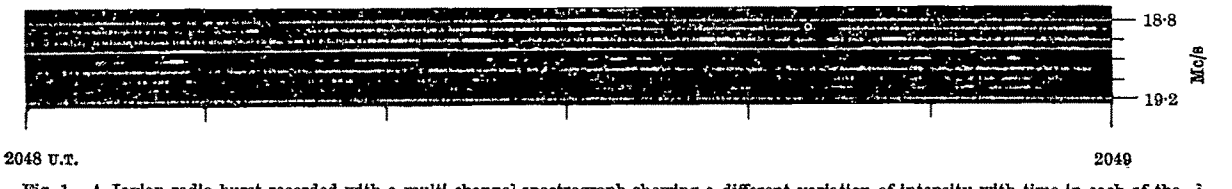


Fig. 1. A Jovian radio burst recorded with a multi-channel spectrograph showing a different variation of intensity with time in each of the channels, tuned at 50-kc/s intervals. System III longitude is 122°

It is worth noting that the trains of short-duration pulses observed in 1963 (refs. 1 and 2), which occurred at radio longitudes of the central meridian of 140°–190° and Io's position of 55°–75°, seem to be a different spectral phenomenon, although they were not recorded with certainty in 1964.

It is possible that these observations indicate some variations in the spectral fine structure depending on the positions of the radio system and the satellite Io. Various authors[6,7] have discussed the Jovian decametric emissions presuming some kind of exospheric disturbances; the extension of the present experiment to weaker radio storms would probably have some interest in studying the nature of the disturbances produced by the satellite.

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ASTROPHYSICS

Interstellar Grains and the Diffuse Galactic Light

Present knowledge of the physical nature of the interstellar grains is based principally on interpretations of the observed extinction and polarization of starlight. Some scattering of the galactic radiation field by the interstellar grains must occur and it is recognized that a precise determination of the brightness of such a diffuse galactic light can also contribute to our knowledge of the grains. Since the first investigations of the diffuse light by Elvey and Roach[1] in 1937 there have been further studies by Henyey and Greenstein[2], van Houten[3] (using the measurements of the brightness distribution in the Milky Way by Elsässer and Haug[4]) and Roach and Smith[5]. Unfortunately the results of these four investigations are in considerable disagreement. Two important sources of this disagreement arise from uncertainties in assessing (1) the contributions to the observed brightness of the airglow, zodiacal light and light scattered by the Earth's atmosphere; (2) the contributions from faint stars, the integrated starlight.

This communication reports a new determination of the brightness, D, of the diffuse galactic light based on measurements secured from a sounding rocket. These measurements were obtained from altitudes above 180 km and consequently corrections for scattering and extinction in the atmosphere and for contamination by airglow (see Packer[6]) are not required. The night-sky photometer, containing a standard B filter and with a field of view of 2·7 square degrees, was flown aboard an *Aerobee* 150*A* rocket launched from the White Sands Missile Range on September 15, 1964, at 0235 h M.S.T. Observations were made along three great circle scans crossing the galactic plane at longitudes l^{II} = 53°, 66° and 164°. To derive D, it was necessary to subtract the zodiacal light and integrated starlight contributions from the measured brightness. The zodiacal light at low galactic latitudes was derived as follows: the zodiacal light away from the galactic plane was determined before and after the galactic crossing by subtracting the integrated starlight (well determined for $b^{II} > 20°$) from the total brightness; the zodiacal light at low galactic latitudes was then obtained from the foregoing by interpolation. The zodiacal light changed only slowly along the three scans so that this procedure introduced a small error in D of less than 10 (expressed in units of $n(B)$ = the equivalent number of stars of magnitude B = 10·0 per square degree).

It has already been noted that there is an uncertainty in the integrated starlight contribution. Two independent determinations by Bottlinger[7] and by Roach and Megill[8], based on different star counts, show good agreement only at latitudes $b^{II} > 20°$. The sources of this disagreement have been discussed by Henyey and Greenstein[2], van Houten[3] and Roach and Smith[5], but no definite conclusions have been drawn. In interpreting the present measures we shall assume that the integrated starlight contribution lies in the range of values derived by Bottlinger[7] and Roach and Megill[8]. The resulting values of D are given in Table 1. The numbers in columns 4 and 5 with and without parentheses correspond to values based on the starlight correction from Bottlinger[7] and Roach and Megill[8] respectively. The maximum values of D for the three galactic crossings are:

l^{II}	b^{II}	D (units of $n(B)$)
53°	−8°	76 ± 20
66°	−4°	159 ± 29
164°	−6°	61 ± 14

We remark that the diffuse light is centred several degrees south of the galactic plane, as found earlier by Henyey and Greenstein[2].

Before comparing these values with the results of earlier studies we draw attention to the conclusion of Roach and Smith[5] that the calibrations used by Elvey and Roach[1] and by Elsässer and Haug[4] most probably give brightnesses too high and too low respectively (for both the zodiacal light and the integrated galactic light). The present investigation supports this conclusion. In Table 2 we have listed the results of three determinations of the diffuse light, D, omitting the results of Elvey and Roach[1] and Elsässer and Haug[4] because of the uncertainty in calibration. The mean value of all determinations in Table 2 is $\bar{D} = 81 \pm 11$.

In concluding this communication we use the values of D in Table 2 to place a lower limit on the mean albedo

Table 1. Surface Brightness of the Diffuse Galactic Light at Three Galactic Longitudes

l^{II}°	b^{II}°	Observed integrated galactic light	Integrated starlight		Diffuse galactic light, D	
46	−12·3	118	80	(73)	38	(45)
50	−9·9	160	92	(83)	68	(77)
53	−7·7	175	106	(91)	69	(84)
57	−5·5	177	120	(98)	57	(79)
61	−2·8	164	147	(107)	17	(57)
65	−12·3	111	82	(75)	29	(36)
66	−7·0	192	99	(84)	93	(108)
66	−3·6	269	125	(95)	144	(174)
67	−1·7	230	141	(97)	89	(133)
67	4·0	134	95	(74)	39	(60)
67	9·3	83	60	(53)	23	(30)
165	−1·2	138	120	(80)	18	(58)
164	−5·9	119	63	(52)	56	(67)
163	−10 4	91	44	(40)	47	(51)
163	−15·0	59	39	(36)	20	(23)

The brightness unit is the equivalent number of stars of magnitude B = 10·0 per square degree = $n(B)$.

Table 2. Brightness of the Diffuse Galactic Light found by Different Authors (in Units of $n(B)$) and the Corresponding Minimum Albedo γ_{min} at $\lambda \simeq 0{\cdot}45\mu$

Authority	l^{II}° →	40	53	66	140	164	Average
Henyey and Greenstein[2]		106 ± 34			52 ± 20		
Roach and Smith[5]							32 ± 10
Wolstencroft and Rose			76 ± 20	159 ± 29		61 ± 14	
	γ_{min} →	0 55 ± 0·30	0·45 ± 0 16	0 65 ± 0·18	0 51 ± 0 32	0·53 ± 0·17	0·24 ± 0 13

of the interstellar grains. If the grains are perfect forward scatterers they will produce the maximum diffuse light for a given albedo γ. This fact can be used to place a lower limit on γ. In this case the relation between γ and the brightness of the diffuse light D and integrated starlight I is:

$$\gamma = \frac{D}{D+I} \cdot \frac{1}{1-e^{-\tau_0}} = \gamma_{min}$$

(see Henyey and Greenstein[2]), where τ_0 is the optical depth in the direction of observation. We do not attempt to place an upper limit on γ since its value is critically dependent on two unknowns—the asymmetry of the phase function and the value of τ_0. The values of γ_{min} (at $\lambda \simeq 0{\cdot}45\mu$) corresponding to $\tau_0 = \infty$ are given in Table 2. The mean value of these determinations is $\bar{\gamma}_{min} = 0{\cdot}49 \pm 0{\cdot}11$, which is close to the value of $\bar{\gamma}_{min}$ based on the present measurements alone. We conclude that there is an even chance that $\gamma > 0{\cdot}49$; it is practically certain that $\gamma > 0{\cdot}38$. A more detailed description and discussion of this work will be given in a forthcoming publication.

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High-energy Astrophysics and a Possible Sub-nuclear Energy Source

Energy is always released when, due to the natural interactions, more bound states are formed from less bound states. This happens, for example, during the gravitational contraction of a given mass, the energy release being related to the long-range gravitational attraction and to the macroscopic properties of the matter. This is also true of nuclear reactions, when the process is connected with the nuclear structure of the matter and the short-range nuclear interactions.

As is well known, these two processes seem to be sufficient to explain the stellar energy sources. However, the enormous energy release in some extragalactic (and probably cosmological) phenomena, such as the radiogalaxies and the quasi-stellar sources, poses once again the problem of energy sources in astrophysics.

The energy required in these violent events can reach about 10^{60}–10^{62} ergs and perhaps even more, corresponding to about 10^6–10^8 $M_\odot c^2$. The associated time-scale is probably not larger than 10^5–10^6 years. Since the efficiency of the nuclear reactions is rather low (less than about 1 per cent of the mass can be converted to energy), the release of 10^{60}–10^{62} ergs would require the nuclear transformation of more than 10^8–10^{10} $M_\odot$. This seems rather unlikely on such a short time-scale.

The other possibility, that is, the release of gravitational energy in the contraction of large masses, has been investigated by many research workers (see, for example, refs. 1–3). The problem is of interest *per se* and poses some fundamental questions in physics[4]. However, the binding energy of a configuration in general relativity is different from that derived by the use of Newtonian theory, and the maximum amount of energy available from a contracting mass M is not given by $G\frac{M^2}{R_s} = \frac{1}{2}Mc^2$ (R_s is the Schwarzschild radius $\frac{2GM}{c^2}$). The energy released due to the contraction from infinity to the radius, R, is given by the binding energy: $B.E. = (M_0 - M)c^2$, where M is the observable mass of the configuration with radius R, and M_0 is the original mass when the particles are dispersed to infinity. Many authors have shown that for some configurations we have $M > M_0$ (refs. 5–7 *et al.*). It is clear that these configurations cannot be obtained by spontaneous contraction.

More clearly, Fowler[5] showed that the binding energy of a contracting sphere in a post-Newtonian approximation contains a negative term and that, because of the presence of this term, the binding energy does not monotonously increase when the radius of the configuration decreases. The binding energy increases up to a certain radius, but further contraction gives rise to less bound states. Thus the energy available through the collapse is much less than $\frac{1}{2}Mc^2$ and its exact value depends on a detailed investigation of the properties of contracting relativistic configurations.

From another point of view, no direct evidence exists for the occurrence of the gravitational collapse, since the evidence is only for explosive phenomena.

It is difficult to understand why the contraction of a given system with a total mechanical negative energy would give rise to a subsequent expansion of the same system, since the expansion demands that the energy be positive. It is necessary to assume that during the collapse of a central unobserved configuration the energy is transferred to the outer region. The sum of the energies in the internal and external regions is always negative if no other energy source is available. For these reasons, it is interesting to consider other possible energy sources.

A first proposal, initially suggested by Burbidge and Hoyle[8] and extensively investigated by Alfvén in a recent paper[9] concerns the possibility of matter–antimatter annihilation. There is another possibility for energy release in a sub-nuclear process which is based on the idea of a compound structure of the elementary particles.

We shall assume the quark's model of hadrons in the $SU(3)$ and $SU(6)$ theory of strong interactions. However, these considerations could be made in the framework of any theory based on the idea that the elementary particles are bound states of more fundamental unities.

In the quark's model the mesons are composed of a quark and an anti-quark and the baryons are the bound state of three quarks (for further details see, for example, ref. 10).

Since the experimental search for quarks gives a lower limit for their mass of about 5 GeV, the formation of the normal matter starting from the quarks would be extremely exoenergetic. However, this process is possible only in a situation of very high density, during which the distance between the quarks is of the order of, or less than, the range of the forces which cause the superbinding.

The order of magnitude of this distance is given by the Compton wave-length of the bound state. For the proton the Compton wave-length is:

$$\lambda_c = \frac{\hbar}{pc} \sim 1{\cdot}3 \times 10^{-13} \text{ cm}$$

which corresponds to a minimum density for the quark's matter of about 2×10^{14} g cm^{-3}.

A knowledge of the statistical properties of the quarks would make it possible to compute the relative concentration of quarks and hadrons under given physical conditions. This can be done in a way similar to that used by Ambartsumian and Saakian[11] to analyse the composition of a superdense gas of elementary particles.

Since this primordial matter would have a density much greater than that corresponding to the gravitational radius of large masses, the cosmic explosions could represent the delayed Friedman expansion of some regions in the primeval universe as suggested by Novikov[12].

It is, however, difficult to suppose that this process is the general cosmogonical process. If all matter were created in this way, the universe would be extremely hot and there would be a large diffuse flux of X-rays. I hope to be able to give more details on this point in the near future.

Finally, I would like to point out that if the quarks exist at low concentration in the stars, their mutual binding is rather unlikely under normal stellar conditions. However, at the end of stellar evolution very high densities and temperatures can probably be reached and the quarks can 'burn'. The related emission of high-energy particles from the super-dense star could produce phenomena similar to those observed in cosmic ray physics and in some celestial regions (X-rays, optical- and radio-emission).

I was the recipient of a European Space Research Organization fellowship during the course of this work. I thank Prof. E. Schatzman for stimulating discussions and hospitality at the Institut d'Astrophysique, Paris. I also thank Prof. L. Gratton and the members of the Astrophysical Laboratory, Frascati, for discussions on high-energy astrophysics, and Dr. F. Strocchi for advice.

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PHYSICS

Auroral Effects and Excess Energy Electrons

Auroral displays are usually attributed to increased leakage of electrons from the outer radiation belt. The mechanism responsible for this increased leakage is not yet understood, but the leakage is known to occur following the arrival of corpuscular radiation from the Sun. The time delay between solar eruptions and the onset of auroral phenomena shows that any solar electrons involved must have energies below 100 eV. Because, on the other hand, auroral electrons typically have energies in the range of several tens of keV, an electron acceleration mechanism must exist, presumably within the radiation belt. The purpose of this communication is to point out similarities between auroral effects and laboratory observations of electron leakage from a magnetic mirror, accompanied by electron acceleration, and to suggest that the same basic mechanism may play a part in both cases.

Previously it was shown[1] that through space-charge oscillations electrons confined in a Penning type structure can acquire abnormally high energy, and that such excess-energy electrons readily diffuse across magnetic lines of force[2]. These effects appear if the electron current injected into the system exceeds a critical value. The present experiments extend these observations to electron confinement in a magnetic mirror.

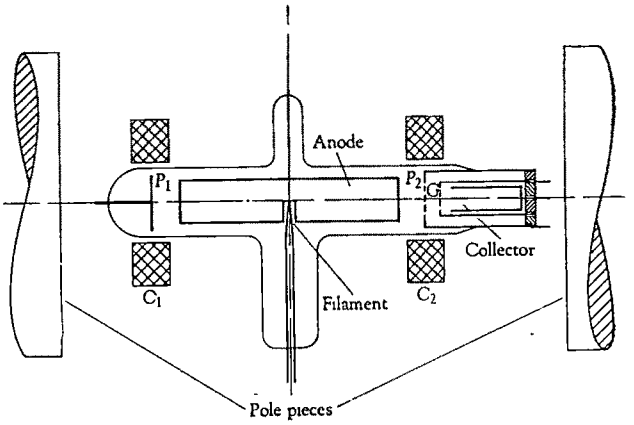


Fig. 1. Experimenta larrangement

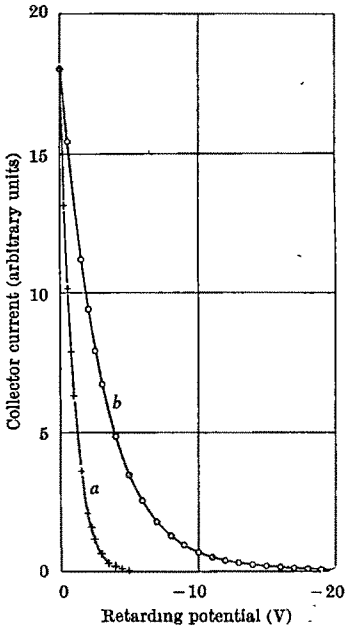


Fig. 2. Retarding potential curves. *a*, Without mirror field; *b*, mirror coils energized

The experimental arrangement is shown in Fig. 1. Thermionic electrons from a filament are injected into an open anode cylinder, which they are prevented from reaching by applying a sufficiently strong uniform axial magnetic field. The electrons therefore move along the field lines to the end plates P_1 and P_2, which are positive with respect to the filament. A sample of these electrons passes through small holes in P_2 and is subjected to a retarding potential analysis by means of the grid G, which is placed at a variable potential V_r, negative with respect to the filament. Fig. 2*a* shows a typical retarding potential curve obtained in this way. The collector current becomes zero at $V_r \simeq -5$ V, a value which can be accounted for by the heater voltage (about 4 V) and the initial velocities of thermionic emission. By energizing the two coils C_1 and C_2 the magnetic field can be made to assume mirror geometry, and the electrons then tend to be confined within the anode cylinder. When a certain degree of confinement has been achieved, space-charge oscillations suddenly set in and impart excess energy to the electrons. A typical retarding potential curve under these conditions is shown as Fig. 2*b*, where it is seen that the average energy of the electrons passing through the mirror field has been considerably increased, and that excess energies up to at least 20 eV are measurable. The magnitude of the excess energy depends on the injected electron current, the

magnetic field configuration and presumably also the geometry of the system.

The effects described resemble those encountered in auroral phenomena. It seems likely that the arrival of comparatively slow electrons from a solar flare in the radiation belt raises the charge density within the belt beyond the threshold-level required for the appearance of excess-energy electrons, and these high-energy electrons leak out into the auroral zone.

I thank Dr. W. Schwietzke for his interest in the work, and Messrs. L. Bessant and I. Varga for assistance in the construction and operation of the apparatus.

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Quasars, Anti-matter and Optical Asymmetry

With reference to recent letters on quasars, anti-matter and optical asymmetry[1,2], it should be noted that Vester directed attention to the parallel between the asymmetry of matter and the optical asymmetry of metabolites, a few weeks after the discovery of parity non-conservation in weak interactions[3]. The relationship between asymmetry at the level of elementary particles and at the molecular level has been discussed in a number of publications[4-8]. In particular, the problem of the origin of optical activity in connexion with the origin of life has been considered, and it has been suggested that the optical asymmetry found on Earth is related to the structure of matter itself, that is, it is a reflexion of the asymmetry of our part of the Universe.

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Rheological Behaviour of Granular Material

Instruments normally used for measuring viscosity are used from time to time for studying the flow properties of granular materials, especially rotational viscometers. Wieghardt[1] used, for sand, a large rotating cylindrical vessel, and measured the torque on a partly immersed long, smooth metal cylinder of small diameter. Benarie[2], working with powders, made use of an ordinary viscometer with a rotating bob, which had radially arranged baffles to avoid slippage. Kuno and Kurihara[3] in similar work used a MacMichael viscometer, rotating the outer cylinder, which also had baffles.

The aim of such investigations was to measure or compare the internal friction of granular materials, to eliminate wall-friction or slippage and to ensure a regular and well-controlled type of flow.

We carried out some experiments with a Rotavisko meter, using a special rotating body—a continuous helical screw—which conveyed the material upwards (Fig. 1). The screw was short-pitched, and had four pitches on a length of 6·2 cm. The speed of rotation could be varied over a very broad range—from 0·06 to 486 r.p.m. Readings of the torque were referred to in arbitrary units (one unit being 4·42 g cm). Smooth glass beakers of varying diameters were used as the containers, allowing an annular width of between 0·9 and 3·7 cm.

Our experiments were carried out on dry pearl barley—a roundish grain with dimensions of 0·16 × 0·25 × 0·27 cm and a kernel-weight of 6·7 mg. Some experiments were undertaken with a barley product in platelet shape (0·12 × 0·50 × 0·77 cm and a kernel-weight of 30·5 mg). Both materials could, for our purposes, be considered as free flowing and cohesionless. Due to the large size of the particles there was none of the complications associated with the formation of agglomerates, or with irregular and varying slippage planes in the mass of material, such as frequently occur in powders.

On rotating the screw in the granular mass, the particles could be seen to be taken up, to descend at the outer parts of the cylinder, to move inwards and to be picked up by the screw again, over the whole length—but for the most part in the upper half of the screw. The screw was filled largely, but not completely, with particles. The torque exercised on the rotating body would seem to be brought about largely by the friction between the ascending and the descending grains. At the centre, around the axis, a small bulge was observed. Compared with the static position, the surface rose 0·5–1·5 cm, depending on the speed of rotation. This rising indicates the dilatation which is necessary for the flow of a granular material. The movement of the grains was rather regular. With smaller annuli, or very high speed of rotation, turbulent eddies and slippage along the outer wall of the vessel were observed.

Readings were jerky, but this is not surprising for such large particles. The spread from moment to moment was larger with a small annulus, lower speeds and larger particle size. On the other hand, it could be reduced to ± 5–10 per cent when the annulus was large, the speeds high and the particles smaller. Ten or twenty readings were taken over a 5-min period, and the mean value calculated. Only a few minutes were required to reach the steady state. When rotation was interrupted and then resumed after some time, the same mean reading was obtained.

Apart from measurements taken when the screw was rotating, readings were taken after stopping it. Within a minute or so a constant value was found. This reading

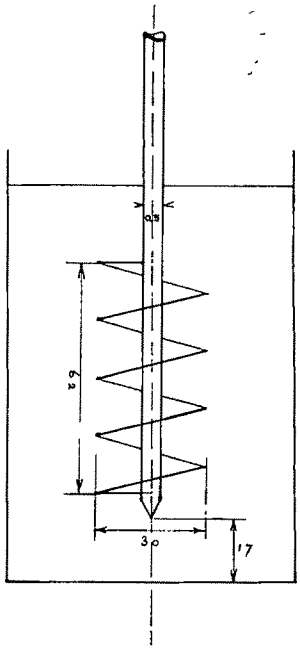


Fig. 1. Rotating screw

could be zero at very high speeds, 55–70 per cent of the rotating reading at average speeds, and frequently rose to 95 per cent for the lowest speeds of rotation. It could not, however, be taken as the true yield value of the granular material. On rotating, the mass was dilated and the packing density became lower than the static value. It took some time to settle and in this time the reading of the torque decreased to some extent.

The reading of the friction value was found to be independent of the speed of rotation. At 0·06 r.p.m. some irregularities were found; from 0·18 to 18 r.p.m. the reading barely increased (for larger annuli); but from 50 r.p.m. onwards larger values were found. The increase from 0·18 to 486 r.p.m., however, was not more than 1·5 fold. This can be explained by turbulent flow and eddying. It would seem that the internal friction, using the method described here, was largely independent of the velocity gradient—as would probably be so for any free-flowing granular material.

The influence of the width of the annulus was surprisingly small. With the round barley, annuli of 0·9–2·4–2·9–3·7 cm gave readings, at 6 r.p.m., of 9·5–7·8–8·3–8·6. At lower speed the difference was still smaller and at high speed it was somewhat larger. Even with the larger barley platelets the annuli of 2·4–2·9–3·7 cm did not have any great effect. The apparatus could thus also be kept within modest dimensions for larger particles.

The effect of the height to which the vessel is filled is given, for round barley, in Fig. 2. The reading of the torque has been plotted against $(\frac{1}{2}L + h)$, where L is the length of the screw (6·2 cm) and h the head above the screw. The friction was found, for the main, to be directly proportional to the pressure.

Comparing the two different types of barley, the smaller roundish and the larger platelet-shaped particles, we found torque (friction) readings of 7·9 and 8·7 respectively. Dividing by the loose bulk densities of 0·84 and 0·57 g/ml., we have calculated 'corrected' readings of 9·4 and 15·3. The platelets had a considerably higher internal friction coefficient. In these tests an annulus of 2·9 cm was used, and a head, $h = 0$; the mean readings at six speeds between 0·18 and 18 r.p.m. were calculated.

The addition of 3·0 and 5·7 per cent arachide oil to the round barley grains gave some cohesion to the particles. Under the same test conditions the friction readings dropped to 78 and 69 per cent respectively.

With the present instrument, it would be very difficult to calculate the friction coefficients in absolute units. It would be possible, however, to calibrate the apparatus by means of particles such as uniform glass spheres.

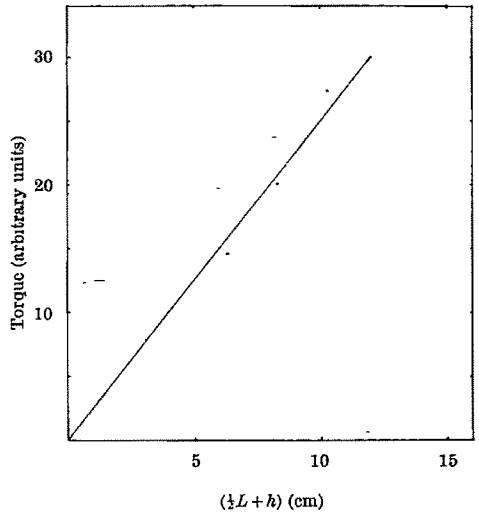


Fig. 2. Relation between head and friction

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METALLURGY

Instrumented Impact Testing of Zircaloy

Coleman and Hardie's recent letter[1] on slow bend testing of hydrided zirconium poses some interesting questions with regard to the interpretation of the transition behaviour of zircaloy-2 (zirconium + 1·5 per cent tin) in impact tests. By instrumenting the Charpy machine to obtain the load/time relationship during fracture[2], the modes of brittle and ductile behaviour can be identified in a similar manner to slow bend testing. Using this technique, load/time curves have been obtained on hydrogen-free and hydrided (180 p.p.m. and 700 p.p.m.) zircaloy Charpy V specimens throughout the transition range.

Tracings of the load–time curves are shown in Fig. 1 together with the energy absorbed during the test. The energy transition of hydrogen-free zircaloy can be attributed to the rate of fall in load (corresponding to the advance of a crack through the specimen) which increases as the test temperature is lowered. This implies a change in ductility of the material at the root of the crack. At −196° C the load drop is extremely rapid, the crack presumably becoming self-propagating, as in Coleman and Hardie's work, due to the stored elastic energy in the specimen. At higher temperatures, continuous application of load is necessary to drive the crack through the specimen. The 'crack speeds' were calculated from the time interval between the point of maximum load and zero load, taking the maximum slope of the falling load–time trace, and are shown in Fig. 2. The 'crack speeds' for the self-propagating cracks possibly represent minimum values due to the limitation of the recording equipment.

The transition from slow crack growth to self-propagation occurs at much higher temperatures with the hydrided material. Within the transition range, curves similar to Coleman and Hardie's type *B* were obtained. It is considered that the load drop does not represent a general yield behaviour, but rather, at these large bend angles, the occurrence of a self-propagating crack down the centre of the specimen. The tail after the rapid load drop (250° C, 180 p.p.m.; 350° C, 700 p.p.m.) possibly repre-

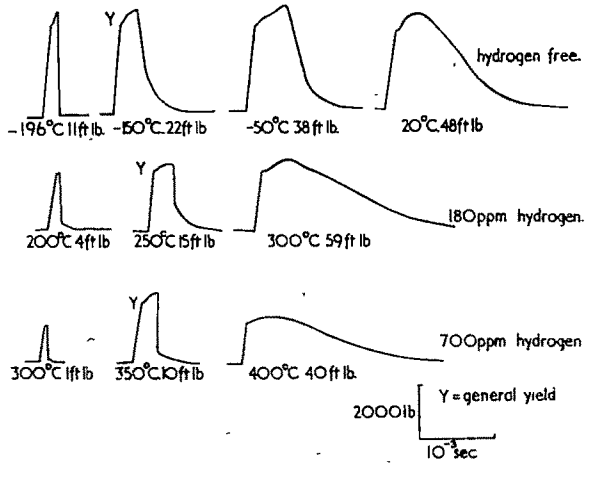


Fig. 1

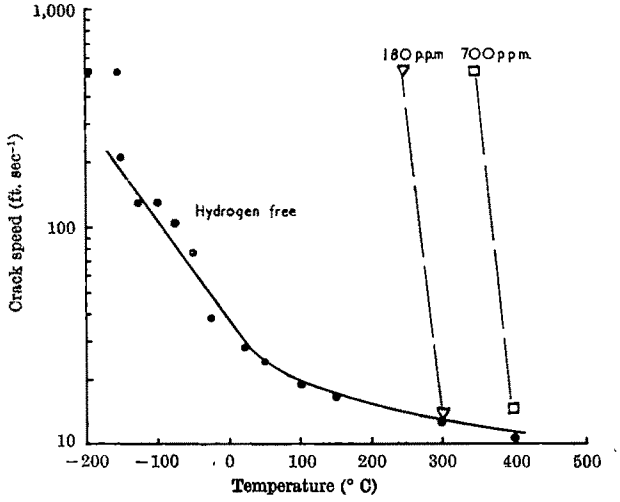


Fig. 2

sents the tearing of surface shear lips after the arrest of the rapid initial crack. The observation of this surface behaviour would possibly account for Coleman and Hardie's low value of the crack speed.

It is concluded that the wide transition in unhydrided zircaloy is due to a gradual decrease in ductility which facilitates easier ductile crack propagation. The intervention of rapid, self-propagating fractures in the hydrided material results in a much narrower transition range.

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CHEMISTRY

Diffusion of Carbon Dioxide through Thin Layers of Solution

WE have adapted the Millipore filter principle of Scholander and Hemmingsen[1,2] to an extremely simple apparatus, and have used this to investigate the diffusion of carbon dioxide through buffer solutions with and without carbonic anhydrase. While the accuracy of absolute determinations of diffusion coefficient using this method is limited by uncertainty as to the thickness of the layer of fluid, the porosity of the Millipore filter, and the precise $[CO_2]$ gradient, it is clearly an adequate method for demonstrating large ratios of fluxes.

In order to measure the diffusion coefficient of carbon dioxide in aqueous solution it is customary to work at low *p*Hs to reduce the concentration of bicarbonate to a negligible amount[3]. At *p*H 7·4 the concentration of bicarbonate in equilibrium with dissolved carbon dioxide is twenty times the concentration of the latter and, as a consequence, in such a system the apparent diffusion coefficient of carbon dioxide should be increased by the concomitant diffusion of bicarbonate ion, which is reported to be as rapid as that of carbon dioxide[4]. It seemed possible that, during the rapid transit of carbon dioxide through the thin (0·015 cm) layer of solution in the Millipore filter, chemical equilibrium between dissolved carbon dioxide and HCO_3^- might not be established and the theoretical facilitation would not be realized. In the presence of excess carbonic anhydrase, however, chemical equilibrium should be closely approached and the apparent diffusion coefficient of carbon dioxide increased.

The diffusion cell of Mochizuki and Forster was used[5] (Fig. 1). Since carbon dioxide diffuses much faster than carbon monoxide, nitrogen or oxygen for the same partial pressure gradient the gas sampling technique previously used for measuring the flux of carbon monoxide could not be used. Instead a 0·1-ml. pipette containing a drop of paraffin oil was connected between the two chambers so that the movement of gas from one to the other with time could be followed. One chamber was filled with air, the other with expired gas (I. L.), which simplified the procedure and which varied less than several tenths of 1 per cent from the average value of 36 mm mercury when analysed on numerous occasions.

In this system the net flow of gas across the film is measured. However, the permeability of the film for carbon dioxide is about twenty times that for the other gases present, namely oxygen and nitrogen, owing to its greater solubility. The rate of exchange of these two gases will be only 4 per cent of the rate for carbon dioxide under the conditions in these experiments, and therefore we have neglected it.

In performing an experiment, the two chambers were filled with air and 5 per cent carbon dioxide mixture respectively with the stopcocks open to permit the pressures to equilibrate with atmospheric, the stopcocks then closed, and the time taken for the oil droplet to pass about eight successive 0·01 ml. divisions recorded. Since the total change in volume was 0·2 ml., this represents about 40 per cent of the process, and the back pressure cannot be ignored. It can be shown that the net change in volume (v) should be described by the following approximate equation:

$$v = 713 \frac{P_{A,0}}{\left[\frac{1}{V_A} + \frac{1}{V_B}\right]} \left(1 - \exp\left(-D\,713\left[\frac{1}{V_A} + \frac{1}{V_B}\right]t\right)\right) \quad (1)$$

$P_{A,0}$ is the partial pressure of carbon dioxide initially introduced into chamber A in mm mercury. 713 is the barometric pressure less the vapour pressure of water at 37° C, and considered constant. V_A and V_B are the volumes of the two chambers, which were 11·5 and 6 ml. respectively. The carbon dioxide was usually placed in the 11·5 ml. chamber. D is the transfer coefficient for carbon dioxide in the film in ml. (standard temperature pressure dry)/(min × mm mercury); t is the time in sec. The experimental results fitted this equation satisfactorily, so it was used to calculate D in conjunction with a semi-log plot. The equation is based on the simplifying assumption that the total gas volume of the chambers does not alter; however, this introduces an error of less than 3 per cent in the results. All experiments were performed at 37° C.

Pertinent results are summarized in Table 1. Although the absolute value of the apparent D is unreliable for the reasons already mentioned, its value in water is close to that extrapolated to 37° C from values reported in the literature[3] at 20° C, namely $2{\cdot}4 \times 10^{-5}$ cm²/sec, as is the value in N/100 hydrochloric acid. Attempts to take measurements at lower *p*H resulted in changes in the physical properties of the Millipore filter. There was no significant facilitation of carbon dioxide transfer in buffer at *p*H 7·9. However, on the addition of a fraction of a

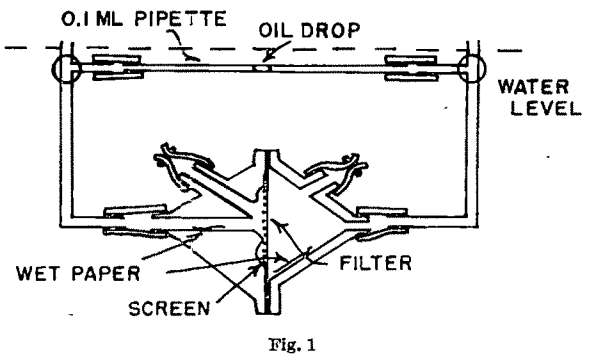


Fig. 1

Table 1. Apparent Diffusion Coefficient for Carbon Dioxide in Various Fluids

Fluid	No. of measurements	Apparent D (cm^2/sec)	$\pm S.E.$ mean (cm^2/sec)
Water	4	2.8×10^{-5}	$+0.16 \times 10^{-5}$
Water + $\frac{N}{100}$ HCl	3	3.1×10^{-5}	$\pm 0.47 \times 10^{-5}$
0·4 M Barbiturate buffer pH 7·9	9	3.2×10^{-5}	$\pm 0.15 \times 10^{-5}$
0·4 M Barbiturate buffer pH 7·9 + carbonic anhydrase	9	$*4.5 \times 10^{-5}$	$\pm 0.30 \times 10^{-5}$
0·4 M Barbiturate buffer + human blood	4	$*3.8 \times 10^{-5}$	$\pm 0.30 \times 10^{-5}$
Whole human blood: lysed	2	$*6.1 \times 10^{-5}$	—

* Probability that the value is greater than that for water is more than 95 per cent.

milligram of commercial carbonic anhydrase per ml., there was a 40 per cent increase in the apparent D (P less than 0·05). Addition of human blood, with its known carbonic anhydrase content, to the barbiturate buffer increased D, but not significantly. Facilitation was most marked in freeze-thawed whole human blood.

These observations are in line with A. V. Hill's comment on Krogh's estimations of the D_{CO_2} in tissue[6], which showed it was increased only 1·5 times over that in water. He suggested that the failure to find the twentyfold increase that would be expected on the basis of the $[HCO_3^-]$ gradient was due to the impermeability of cell membranes to the ion. However, ions cannot move in solution without the simultaneous movement of those of opposite charge in the same direction, or those of similar charge in the opposite direction. Thus in our system the bicarbonate ion could move across the membrane carrying with it the buffer cation, the $[H^+]$ being extremely small, which would set up a back gradient of buffer cation. Regardless of the identity of the ions that flow, the net effect would be to slow the overall process, which may be the explanation for the relatively small amount of facilitation found, about one-twentieth that expected from the experimental gradients of $[CO_2]$ and $[HCO_3^-]$.

The increase in apparent D_{CO_2} resulting from the addition of a miniscule amount of carbonic anhydrase to the buffer indicates that the hydration or hydrolysis of carbon dioxide is rate limiting in the absence of a catalyst. It is also pertinent that the diffusion of one weak electrolyte is decreased in the presence of others[7].

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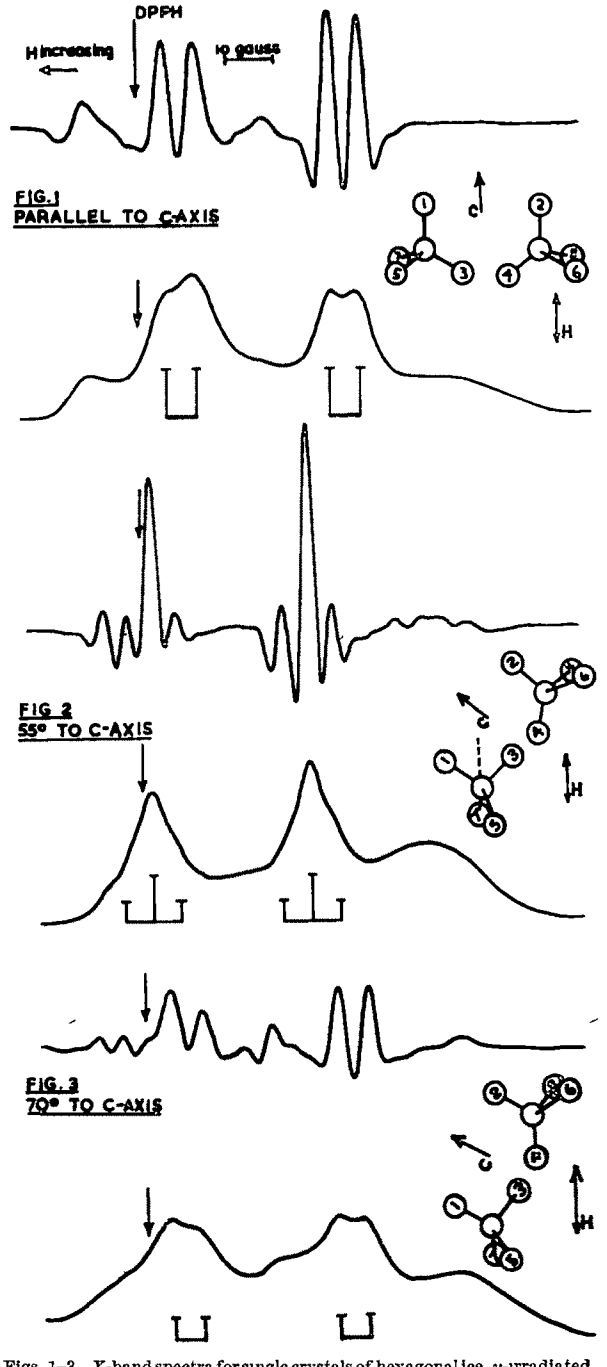
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New Structure in the Electron Spin Resonance Spectrum of γ-irradiated Single Crystals of Ice

Electron spin resonance spectra obtained here for single crystals of ice, γ-irradiated and measured at 77° K, show structure not previously reported for the resonance near $g = 2$, which is generally assumed to be due to OH radicals. Earlier work by other authors has been directed mainly to the investigation of polycrystalline ice[1], except for a report[2] giving results on single crystals rather different from those reported here, and a recent communication[3] published near the end of these preliminary experiments.

The spectra obtained in the present work, which was at X-band, are in general complex and diffuse. However, the occurrence of quite sharp peaks for certain orientations of the crystalline axes with respect to the magnetic field, and also of a small, partly resolved splitting superimposed on the main OH doublet, suggests that the OH radical is at a normal oxygen site in the crystal lattice.

Single crystals from both ordinary water and heavy water (D_2O) were grown by the method of Adams and Lewis[4], and cylindrical samples were cut with their axes along the three symmetry axes of the crystal; these were then irradiated at 77° K with doses varying from one to ten Megarads in a cobalt-60 γ-source. Spectra were taken at 5° intervals for rotation about each of these axes, when arranged perpendicular to the applied field. Rotation about the c (unique) axis exhibited the expected six-fold symmetry with alternate mirror planes every 30°, while



Figs. 1–3. X-band spectra for single crystals of hexagonal ice, γ-irradiated and measured at 77° K. In each case the second derivative spectrum is shown with the absorption spectrum immediately below it. The calculated neighbour proton splitting is indicated under each absorption curve. The orientation of the magnetic field H with respect to the crystal c-axis and bond directions is also shown. For the doublet in Figs. 1 and 3, $g = (2.0207 \pm 0.0005)$, $A = (36.8 \pm 0.3)$ gauss. For the doublet in Fig. 2, $g = (2.0151 \pm 0.0005)$. $A = (34.3 \pm 0.3)$ gauss

on rotation about the other two axes each showed two mirror planes at right angles to one another[2].

Hexagonal ice has its oxygen atoms on a wurzite-type lattice, with one hydrogen atom to every bond. Each oxygen is surrounded tetrahedrally by four hydrogen bonds, with two of the hydrogen atoms bound covalently to it at a distance of 1 Å, and the other two loosely bound at a distance of 1·75 Å (ref. 5).

Three examples of second derivative spectra obtained are shown in Figs. 1–3, with the electronically integrated spectra below them. The bond symmetry for each orientation is shown to the right. Fig. 1 shows the spectrum obtained with the crystal *c*-axis parallel to the applied field, H. In Fig. 3 (*c*-axis at 70° to H). the bond co-ordination of Fig. 1 is repeated around half the oxygen sites, and part of the spectrum in Fig. 1 is seen to recur at half intensity. In Fig. 2 (*c*-axis at 55° to H), the tetragonal axis of the bonds for half the oxygen sites coincides with H, so that there are four bonds making equal angles of 55° with the field, and at this angle a sharp resonance is again observed. These results suggest that the various orientations have a symmetry very similar to that of the hydrogen bonding of the lattice—in contradiction to the model proposed by McMillan *et al.*[2]. It is thought possible that the radiation simply removes one hydrogen atom from the lattice. This would leave a hydroxyl radical either along one of the bond directions or, more probably, deflected from this direction towards the plane containing the three oxygen atoms to which the radical is still hydrogen bonded. Present analysis favours the latter model.

The small additional splittings observed are accounted for by a dipole–dipole interaction between the spin-electron and neighbouring protons. The result of a simple calculation in which it is assumed that the spin-electron is a point dipole at a normal oxygen site, with the two normal hydrogen-bonded protons at 1·75 Å distance, is shown under each spectrum. Transitions[6] of the form $\Delta m_I = 1$ were included in the calculation.

In the recent communication[3] reporting work on single crystals and powders of ice, it has been proposed that the low-field broad resonance (which clearly shows in the integrated spectrum of Fig. 2) is also due to the OH radical. Both annealing experiments and the angular variation of the D_2O spectrum observed in this laboratory tend to confirm this.

However, there is a small residual broad spectrum which remains when the OH resonance has been annealed, and this is also being investigated.

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Oxidation of *Cyclo*hexane by Lead Tetra-acetate

Possibly because of displacement by apparently more attractive substrates, the reactions of lead tetra-acetate with simple paraffins has not received much attention. Earlier discussions[1,2] suggested that reaction in warm solvent was not likely although the promoting effect of paraffins on the thermal decomposition of the reagent in hot acetic acid had been noted[3]. Recently slow reaction with alkyl and aryl*cyclo*propanes has been reported[4].

*Cyclo*hexane has been used in photoreactions, for example, with nitrosyl chloride[5], ultimately resulting in large yields of *cyclo*hexanoneoxime as a result of the rearrangement of the initially formed nitroso compound indicative predominantly of mono-substitution, and with halogens[6], characterized by the appearance of poly-substituted products.

Contrary to the earlier suggestions, it has now been found that *cyclo*hexane reacts at a reasonable rate with lead tetra-acetate, giving good yields of a mixture of acetoxy derivatives. From the reaction of the paraffin (42 g; 0·5 M) with the oxidant (222 g; 0·5 M) in acetic acid (400 ml.) only 50 per cent of unchanged material was recovered from the thermal reaction at 80° (in the dark) and less than 40 per cent from the photoreaction (sunlight) at 20°–25° C. However, as shown in Table 1, there was a considerable difference in the distribution of products of the two reactions.

In the thermal reaction, the main bulk of products is divided between *cyclo*hexyl acetate and higher boiling material with comparatively little intermediate diacetates. This result suggests that initially formed monoacetate competes with unchanged hydrocarbon as a substrate for further oxidation.

By contrast, *cyclo*hexyl acetate appears as the main product of the photoreaction with markedly less higher boiling products, suggesting in turn that the hydrocarbon itself is the preferred substrate, that is, the tendency is to react preferentially with material of least degree of acetoxylation. This point was examined more directly by using *cyclo*hexyl acetate as initial substrate.

The acetate (71·2 g; 0·5 M) with lead tetra-acetate (222 g; 0·5 M) in acetic acid (400 ml.) was oxidized in a photoreaction. Products of the reaction, analysed as before, are indicated in Table 2.

The contrast with the thermal reaction in distribution of products is maintained. The total yield of diacetates is now far greater than that of higher boiling materials, which is again indicative of preferential attack on lower acetoxylated material—in this case the *cyclo*hexyl acetate. It seems likely that the build-up of higher acetates occurs mainly after the yield of diacetates has assumed competitive proportions.

A further point of specificity appears within the diacetate products. It is clear that much less attack occurs at the 2-position—the combined yields from the 3- and 4-positions giving a ratio of 13 : 1 with respect to distribution of products. This result can most probably be attributed to steric hindrance to attack at the 2-position.

In essence these results suggest that the thermal and photo-oxidations proceed by different mechanisms. The thermal reaction is subject to substituent influence, so that as acetoxylation proceeds the products tend to become the preferred substrates, thus yielding greater amounts of higher boiling materials without build-up of intermediate fractions to any great extent. It is very important, however, to note that this course of reaction only applies so long as unchanged *cyclo*hexane is present. An attempted thermal oxidation (80° C) of *cyclo*hexyl acetate in the dark showed no evidence of reaction even after a heating period

Table 1. Oxidation of *Cyclo*hexane (0 5 M) by Lead Tetra-acetate (0 5 M) in Acetic Acid

Oxidation product*	Thermal oxidation at 80° C (g)	Photo-oxidation at 20°–25° C (g)
*Cyclo*hexane not converted and methyl *cyclo*hexane formed	21 1	15·6
*Cyclo*hexyl acetate	8 5	16·95
Cis and *trans* 1,2-diacetates	1 9	1·5
1,3-diacetates	0·16	0 33
1,4-diacetates	0 28	0 37
Higher boiling acetates	10 0	3 9

* Analysis of neutral esters by gas chromatography using di-isodecyl-phthalate (20 per cent w/w) on 'Celite' as stationary phase, and helium (50 ml./min) as carrier gas. The column temperature was 170° C

Table 2. Photo-oxidation of *Cyclo*hexyl Acetate with Lead Tetra-acetate at 20°–25° C

Product	Yield (g)
Unconverted *cyclo*hexylacetate	44 7
Cis and *trans* 1,2-diacetates	1 3
1,3-diacetates	6·7
1,4-diacetates	9·6
Higher boiling acetates	8·3

of 170 h. Reaction was promoted by the addition of *cyclo*hexane.

The products of the photoreactions possibly result from an initial attack on the substrate by a methyl radical arising from the direct decomposition of the tetra-acetate[7]. Analysis of gas evolved during the photo-oxidation of *cyclo*hexyl acetate showed a methane/carbon dioxide ratio close to 1. The recovered hydrocarbon from the photoreaction of *cyclo*hexane included a small proportion of methyl *cyclo*hexane.

On the other hand, the mechanism of the thermal reaction is less obvious but must involve an association of paraffin, oxidant and ester. Work on this aspect is continuing.

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Isolation of Ammonium Thiophosphite by means of Preparative Ion-exchange Chromatography

Although the existence of thio-derivatives of lower phosphorus anions has been accepted for some time, only one claim has been made of their isolation—by Lemoine[1,2] in 1881 and 1884. It now seems unlikely that his method of slow crystallization from a phosphorus sulphide hydrolysis mixture yielded single products, especially since he had no means of checking the purity.

A preliminary investigation showed that all of P_4S_3, P_4S_5, P_4S_7, and P_4S_{10} gave thiophosphorus species, in addition to simple phosphorus oxyanions when submitted to hydrolysis by solutions of sodium hydroxide or sulphide. Further investigation with anion-exchange columns coupled to a Technicon autoanalyser[3] established that one unidentified species was eluted just before monothiophosphate (PO_3S^{3-}) at *p*H 11·4 (see Fig. 1).

A linear gradient of increasing chloride concentration was used and comparison of the retention times of phosphite (HPO_3^{2-}) and phosphate (PO_4^{3-}) led to the prediction that this unknown anion was monothiophosphite (HPO_2S^{2-}).

Samples of this species were separated from the other phosphorus compounds by heavily overloading a large column (50 cm long, 3 cm diameter) of anion-exchange resin 'De-Acidite *FF*' and eluting with a constant strength of ammonium chloride buffered to *p*H 10 with ammonia. The resultant solution was evaporated down to a few millilitres at room temperature (20° C) under low vacuum and then adsorbed on to cellulose. Washing with a mixed solvent (42·5 isopropanol : 42·5 2-butanone : 15 conc. ammonia) removed the chloride, and the pure phosphorus salt was stripped off with ammoniated water. A white microcrystalline salt was precipitated from this solution with excess alcohol and the purity of the precipitated salt was checked using both thin-layer and anion-exchange chromatography.

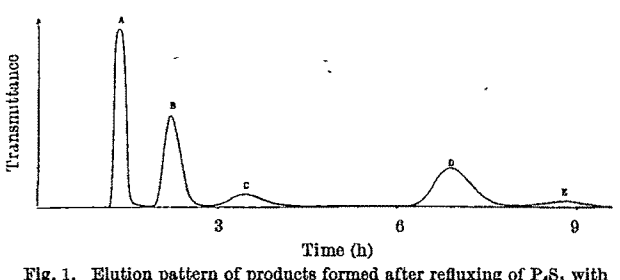


Fig. 1. Elution pattern of products formed after refluxing of P_4S_3 with excess 1 N sodium hydroxide for several minutes. *A*, Hypophosphite, *B*, phosphite; *C*, phosphate; *D*, unknown (monothiophosphite); *E*, monothiophosphate

The salt proved to be unstable towards water vapour, losing hydrogen sulphide in acid solution with the consequent formation of ammonium phosphite. Concentrated nitric acid and bromine water both react powerfully with the salt; a yellow solid is produced—presumably elemental sulphur. Its strong reducing properties are illustrated when silver nitrate is changed into the metal and a nickel spatula into black nickel sulphide.

A precursory examination of the infra-red spectrum reveals a sharp peak at 2,350 cm^{-1} which corresponds to the characteristic P—H stretching frequency. Weaker absorptions at 1,005 cm^{-1} and 415 cm^{-1} may be tentatively assigned to P—H deformation and P—S stretching vibrations respectively. A strong absorption due to the ammonium ion was seen at 1,415 cm^{-1}.

Analysis of the compound showed the phosphorus : sulphur : nitrogen ratio to be 1·00 : 1·01 : 2·03. For the empirical formula $(NH_4)_2HPO_2S$:

	P	S	N	H
Calculated	23·4	24·3	21·2	6·87
Found	22·7	23·8	20·8	7·01

The elution position under anion-exchange was sufficient evidence that there was one phosphorus atom per molecule. Moreover, the anhydrous nature was indicated when the salt decomposed on heating without melting, and no water was detected in the decomposition products

From the foregoing evidence the salt appears to be anhydrous diammonium monothiophosphite and its structure probably resembles that of trisodium monothiophosphate[4], that is:

$$[NH_4^+]_2 \quad \left[\begin{array}{c} H \\ | \\ S—P—O \\ \| \\ O \end{array} \right]^{2-}$$

Further investigations are in progress.

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Synthesis of Alkyl-β-D-xylopyranosides in the Presence of Mercuric Salts

Synthesis of alkyl-β-D-glycosides has been achieved mainly through the reaction of poly-*O*-acetylglycosyl-halides with alcohols in the presence of a heavy metal (Koenigs-Knorr).

Except with simple alcohols, the most common procedure has been to dissolve the *O*-acetylglycosylbromide in an inert dry solvent, after adding a drying agent and silver oxide (dry and freshly prepared) and then adding an excess of the appropriate alcohol.

In recent years, mercuric salts have frequently been used. While there seems to be a general tendency for the reaction to proceed with Walden inversion, either anomer may be produced, depending largely on the aglycon concentration. Although the time of reaction is much shorter and a commercially available catalyst is used, mercuric

Table 1. ACETYLATED β-D-XYLOPYRANOSIDES

	M.p. (° C) cryst. solvent	$(\alpha)_D^{25}$	Yield	Analytical value C	H	CH_3CO
Ethyl	106–108	−61° 5	48	51 4	6·6	42·5*
$C_{13}H_{20}O_8$	Ethanol	*c*, 4		51·3	6·7	42·3†
Propyl	108–109	−60° 6	40	52·8	6·9	40·5
$C_{14}H_{22}O_8$	Ethanol–water	*c*, 4		52 7	6·9	40·0
Isopropyl	122–124	−62° 5	35	52·8	6 9	40·5
$C_{14}H_{22}O_8$	Ethanol–water	*c*, 4		52 8	6·8	40·2
n-Butyl	100–101	−58° 0	41	54·2	7 2	38·5
$C_{15}H_{24}O_8$	Ethanol–water	*c*, 4		54·1	7·0	38·5
tert-Butyl	130–132	−44° 8	60	54·2	7 2	38·5
	Ethanol–water	*c*, 2		54·1	7·1	38 0
n-Amyl	54–55	−58° 7	60	55·5	7 5	37·0
$C_{16}H_{26}O_8$	Ethanol–water	*c*, 2		55 2	7·6	36·8
n-Hexyl	59–60	−56° 8	40	56·6	7 8	35 5
$C_{17}H_{28}O_8$	Methanol–water	*c*, 2		56·4	7 9	35 6
n-Heptyl	55–56	−55° 0	30	57 8	8 0	34 2
$C_{18}H_{30}O_8$	Methanol–water	*c*, 2		57·6	8 1	34 3
n-Octyl	52–53	−53° 4	30	58 7	8·3	33·0
$C_{19}H_{32}O_8$	Ethanol–water	*C*, 2		58·4	8 3	33 1

* Calculated.
† Found.

Table 2. β-D-XYLOPYRANOSIDES

	M.p. (° C) cryst. solvent	$(\alpha)_D^{25}$	Yield	Analytical value C	H
Ethyl	95–96	−37° 0	75	47 1	7·9*
$C_7H_{14}O_5$	Acetone	Water, *c*, 2		47·2	7·9†
Propyl	92–93	−58° 6	80	50·0	8·3
$C_8H_{16}O_5$	Acetone	Methanol; *c*, 2		50 1	8·4
Isopropyl	115–116	−66° 0	80	50 0	8 3
	Methylethylketone	Methanol, *c*, 4		49 9	8·4
n-Butyl	89–90	−54° 0	87	52 5	8 7
$C_9H_{18}O_5$	Acetone	Methanol; *c*, 4		52·5	8·7
tert-Butyl	122–123	−28° 5	80	52·5	8 7
	Acetone	Methanol; *c*, 2		52 3	8 8
n-Amyl	88–89	−54° 5	81	54 5	9·1
$C_{10}H_{20}O_5$	Acetone	Methanol, *c*, 2		54·5	9 1
n-Hexyl	88–89	−50° 9	78	56 4	9 4
$C_{11}H_{22}O_5$	Acetone	Methanol; *c*, 2		56·4	9·3
n-Heptyl	84–85	−48° 4	82	58·1	9·7
$C_{12}H_{24}O_5$	Acetone	Methanol; *c*, 2		57·9	9 6
n-Octyl	91–92	−45° 1	80	59 5	9·9
$C_{13}H_{26}O_5$	Acetone	Methanol; *c*, 2		59 6	9 9

* Calculated.
† Found.

salts apparently are not widely used, and this is especially so for higher alcohols.

In the present work we prepared the tri-*O*-acetyl-alkyl-β-D-xylosides by condensation of tri-*O*-acetyl-α-D-xylopyranosylbromide with the appropriate alcohol (up to *n*-octanol) and used mercuric acetate as a catalyst. In each case the β-anomer was formed in good yield. Thus, even with higher alcohols, the more simple reaction with mercuric salts can be used.

The alkyl-β-D-xylosides were prepared by catalytic deacetylation of the corresponding acetates.

Preparation of the alkyl-tri-O-acetyl-β-D-xylosides. The tri-*O*-acetyl-α-D-xylopyranosylbromide was prepared by the method of Barczai-Martos and Körosy[1]. In the condensation reaction all the apparatus and reagents had to be thoroughly dry. The presence of water would have resulted in the production of a considerable amount of by-products, chiefly 2,3,4-tri-*O*-acetyl-D-xylose and a xylobiosacetate, tentatively identified as β-D-xylopyranosyl-β-D-xylopyranoside-hexa-acetate.

Acetyl groups were determined by the method of Kunz[2]. Mercuric acetate (16 g, 0·05 mol.), absolute chloroform (200 ml.), 'Sikkon' (18 g) and the appropriate alcohol (1–1·5 mol., except for heptanol and octanol, where 0·2 mol. was used) were stirred for 15 min at room temperature. During stirring, acetobromoxylose (33·9 g; 0·1 mol.) was added and the mixture refluxed on a water bath for 15 min. After cooling, the solution was filtered, thoroughly washed with water, dried, using sodium sulphate, and evaporated *in vacuo*. The resulting syrups were crystallized from the appropriate solvent.

The amyl-, hexyl-, heptyl- and octyl-xylosideacetates were purified by dissolving the final syrups in petroleum ether (b.p. 60°–80°), filtering of an insoluble residue (tri-acetylxylose and xylobioseacetate) and evaporating the filtrate *in vacuo*. The residue was dissolved in 95 per cent ethanol and kept at 4°. The products crystallized very slowly when water was added drop by drop over a period of several days.

Table 1 shows the alkyl-tri-*O*-acetyl-β-D-xylosides which were prepared in this way (all rotations were determined for chloroform solutions).

Preparation of alkyl-β-D-xylopyranosides. The xylosides were prepared by catalytic deacetylation with sodium-methoxide as described by Thompson and Wolfrom[3]. Table 2 shows the β-D-xylopyranosides which were prepared.

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Partial Specific Volumes of Solubilized Collagens and their Derived Gelatins

DETERMINATION of molecular weight by the method of sedimentation diffusion[1] necessarily involves the measurement of solute partial specific volume, $\bar{v}$. Owing to the highly viscous nature of solutions of soluble collagen, the determination of $\bar{v}$ for such materials is not easy. Previous investigators[2,3] have adopted a value of 0·70 ml/g for $\bar{v}$ of collagen; this is the value obtained for the much less viscous parent gelatin that is formed when collagen is denatured. It is thus implicitly assumed that there is no change in $\bar{v}$ on denaturation and we considered that this matter required experimental investigation. Accordingly, the normal pycnometric procedure for obtaining $\bar{v}$ has been utilized and found satisfactory for very dilute collagen solutions, despite the high viscosity. In 0·15 M citrate buffer, *p*H 3·67, at 20° C, typical values are:

calfskin acid soluble collagen, $\bar{v} = 0{\cdot}645 \pm 0{\cdot}010$ ml./g
calfskin soluble encollagen, $\bar{v} = 0{\cdot}625 \pm 0{\cdot}010$ ml./g
ossein soluble encollagen, $\bar{v} = 0{\cdot}650 \pm 0{\cdot}010$ ml./g

After thermal denaturation, the parent gelatin of acid soluble calfskin collagen gave $\bar{v} = 0{\cdot}695 \pm 0{\cdot}005$ ml./g at 40° C. This is in good agreement with the values previously obtained[2,3] for denatured ichthyocol and with the estimates computed from the amino-acid composition of gelatin[4,5] and the specific volumes of the component amino-acid residues[6].

The present results clearly demonstrate a significant change in $\bar{v}$ on denaturation of collagen, and the lower value in the undenatured state indicates not only a compact structure but also that strong intramolecular compressions must exist. At least a partial release of such compressions accompanies denaturation.

In the presence of 1 M sodium salicylate, a chemical denaturant, $\bar{v}$ for the calfskin parent gelatin is $0{\cdot}755 \pm 0{\cdot}010$ ml./g, a value much higher than that obtained by thermal denaturation. It is suggested that this result may reflect the release of highly compressed water from the protein by the binding of salicylate ions, though other mechanisms are not excluded. Rice *et al.*[7] have recently reported a small difference in $\bar{v}$ for calfskin collagen, according to whether the solvent is citrate or phosphate buffer. It would seem, therefore, that additional careful investigations of the variation of $\bar{v}$ with environment are required both for the highly compact collagen and for gelatin.

The introduction of the appropriate, new collagen values into the usual expression for molecular weight in terms of sedimentation and diffusion coefficients yields values some 17 per cent lower than previously obtained, in agreement with the indications of osmotic pressure work. By argu-

ments which will be presented fully elsewhere, the physicochemical investigations taken together tend to the view that a single (α −) chain of parent gelatin possesses a molecular weight of 90,000 ± 5,000.

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BIOPHYSICS

Efficiency of Ion Transport Mechanisms

It has often been assumed, as in the theory of active transport of ions, that metabolic energy is required to transfer sodium ions from a low intracellular concentration to a higher concentration in blood serum or an external fluid[1–3]. On that assumption, the efficiency of any transport mechanism would be expressed as the ratio of output of work to input of energy. Theoretical calculations have been based on the following approximation, derived from the laws of ideal solutions:

$$\text{Ideal molal work} = RT \ln \frac{c_{Na}'}{c_{Na}''} \quad (1)$$

The work per unit time divided by the rate of energy conversion yields a value for the efficiency of the assumed mechanism. If the external concentration, c_{Na}', is 140 m.equiv./l. (as in blood serum), and if c_{Na}'' is taken as 28 m.equiv./l., then:

$$\text{Ideal molal work} = 1{\cdot}0 \text{ kcal per equivalent at } 37^\circ \text{ C} \quad (2)$$

If such an assumed process were to be continuous and irreversible, it would be accompanied by a large number of irreversible side-effects which do not appear to occur in Nature. The concentration of extracellular sodium would increase as intracellular sodium is depleted. There would be a simultaneous change in the electrolyte balance, which would be compensated only by a cyclical return of sodium ions from the higher concentration c_{Na}' to the cellular concentration c_{Na}''. To maintain a steady invariant state in cytoplasm and serum, it is necessary not only that c_{Na}' and c_{Na}'' remain constant, but also that the concentrations of chloride, potassium, and the other physiological ions remain fixed. A true calculation of the efficiency of any ion transport mechanism must consider the conditions of intracellular and extracellular invariance. For sodium the free energy change, ΔG, of the reversible isothermal process is zero:

$$\Delta G_1 = RT \ln \frac{c_{Na}'}{c_{Na}''} = (\Delta\mu_{Na})_1 \quad (3)$$

$$\Delta G_2 = RT \ln \frac{c_{Na}'}{c_{Na}''} = (\Delta\mu_{Na})_2 \quad (4)$$

$$\Delta G_{total} = (\Delta\mu_{Na})_1 + (\Delta\mu_{Na})_2 = 0 \quad (5)$$

where $\Delta\mu_{Na}$ is the change of chemical potential of sodium ions.

Similar considerations apply to the other ions. Equations (1)-(5) are based on the ideal solution laws, often assumed to be approximately valid for cells and tissues. In a generalized formulation, non-ideal terms are required[4–6]. According to the theory of electrolyte solutions, the chemical potential, μ_i, of any ion is defined by the equation:

$$\mu_i = \mu_i^0 + RT \ln c_i f_i \quad (6)$$

where μ_i^0 is the standard chemical potential and f_i is the activity coefficient. By an appropriate definition of the standard state of a physiological system, the activity coefficient of any ion may be assigned the value 1·0, and the non-ideal deviations may be expressed by a change of μ_i^0 between two different phases. Thus the change of chemical potential, $\Delta\mu_{Na}$, between blood serum and cytoplasm is given by:

$$\Delta\mu_{Na} = \Delta\mu_{Na}^0 + RT \ln \frac{c_{Na}''}{c_{Na}'} \quad (7)$$

This may be expressed in the following way:

Real molal work = non-ideal molal work + ideal molal work

The magnitude of $\Delta\mu_{Na}^0$ is of the order of 2·3 kcal, or −100 mV in electrical units[5,6]. If the ideal molal work is −1·0 kcal (equation (2)), then the real value of $\Delta\mu_{Na}$ is 1·3 kcal, or −56 mV. The value is of the same order of magnitude as the ideal molal work, but of opposite sign. Therefore, when account is taken of the 'non-ideal work', there is no need of a hypothetical pump to force sodium ions up a 'concentration gradient'. Its place is taken by $\Delta\mu_{Na}^0$, the value of which depends only on non-ideal deviations from the ideal equation of state.

Over any closed cyclic process in an invariant system, $\Delta\mu_{Na}$ is zero (equation (5)). The conditions of invariance require c_{Na}' and c_{Na}'' to remain constant. The constant $\Delta\mu_{Na}^0$ depends only on the nature of the two phases, and is independent of intermediate steps or mechanisms. The condition of invariance requires that the integral of $d\mu_i$ for any ion over any pathway C depends only on the initial and final states. The value becomes zero over any path in which the initial and final states are the same. These conditions are expressed by equations (8–10).

$$\int_C d\mu_i = 0 \quad (8)$$

$$\int_C d\mu_i^0 = 0 \quad (9)$$

$$\int_C RT \, d \ln c_i = 0 \quad (10)$$

All other thermodynamic functions, such as entropy, free energy and enthalpy, have the same property: they depend only on the physicochemical state of any phase, and are independent of the paths of transport processes.

In any physicochemical system at constant temperature and pressure, the condition of invariance requires that ΔG be zero for all possible redistributions of ions. Spontaneous processes can occur only in the direction in which ΔG is negative. We have shown[4–6] that the conditions for maximal stability in a heterogeneous system are:

$$\Delta\mu_{Na} = \Delta\mu_K = \tfrac{1}{2}\Delta\mu_{Ca} = \tfrac{1}{2}\Delta\mu_{Mg} = -\Delta\mu_{Cl} \quad (11)$$

The most stable distribution is determined by the value of $\Delta\mu^0$ for each ion.

In general, the maximal work, w_{max}, is equal to the decrease of free energy. Thus:

$$\Delta G = -w_{max} \quad (12)$$

For an ideal gas:

$$\Delta G = RT \ln \frac{p_2}{p_1} \quad (13)$$

A gas pump requires a supply of energy to transport air from a low pressure, p_1, to a high pressure, p_2. However, if the air simultaneously returns to the lower pressure, ΔG is zero, and no effective work is performed. The efficiency is zero. In the atmosphere there is a pressure gradient with increasing altitude, but this cannot be converted to

useful work. The condition of maximal stability requires that ΔG be zero between any two altitudes or pressures. Air may freely circulate between any two levels with no change of free energy or performance of work.

If there is a sodium pump or other ion transport mechanism within cells, operating to perform work by increasing the free energy or chemical potential of the ions, then its maximal efficiency must be zero. The reason for this is that it operates in an invariant, isothermal, heterogeneous system. The condition for invariance requires, among other things, that an equivalent quantity of ions must immediately return to the cells. The values of ΔG and $\Delta\mu$ over any cyclical isothermal process in an invariant system may readily be shown to be zero, since they depend on only one fixed state. According to Carnot's principle, the reversible isothermal work is zero. Therefore, the efficiency of any energy-consuming mechanism operating in the reversible cycle must also be zero. Efficiency can be measured only for work-producing processes, such as muscular contraction, which involve both reversible and irreversible steps.

If there is no sodium pump, it is meaningless to calculate its efficiency.

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BIOCHEMISTRY

Structure of α-Keratin

IN a recent publication, Fraser, MacRae and Miller[1] have given a comprehensive interpretation of the X-ray diffraction photograph of α-keratin (porcupine quill tip) and, in particular, considered the quantitative aspects of the small-angle and large-angle equatorial scatter. This problem has also been discussed by Wilson[2], whose approach differs in some important respects from that of Fraser *et al.*, although based on a microfibril model of the same type. Wilson supposes that interference effects between protofibrils within a microfibril, as well as between different microfibrils, affect the X-ray intensity at small scattering angles, whereas Fraser *et al.* consider only the 'between microfibril' interference. According to them, the small-angle scatter can be ascribed solely to a difference in effective mean electron density between the microfibrils and their surrounding matrix, and for a given microfibril-matrix texture the intensity should be simply proportional to this difference. The evidence in favour of this idea is that when heavy metals, such as silver or osmium, are incorporated in the matrix material the main effect on the diffraction is to enhance the small-angle scatter, leaving the maxima of intensity at the same scattering angles as before.

On physical grounds, Wilson's theory is inadequate[3] if only because of his neglect of the side-chain scatter. Fraser *et al.* consider the side-chain scatter, but assume that it completely annuls the protofibril scatter at small angles. We believe that the truth lies somewhere between these extremes, and that the effect of the side-chains and other material between the protofibril main-chains must be to reduce, but not to eliminate, the protofibril intensity at small angles.

This belief is strengthened by our experimental results for the equatorial scatter of Lincoln wool. In a general way these are similar to those quoted by Fraser *et al.* for porcupine quill, with one important exception. This is that the relative intensities of the 45 Å and 27 Å reflexions are quite different in untreated and metal-containing wool (the intensities are roughly equal in untreated fibres, but the 45 Å reflexion is some five times stronger than the other in the silver-wool). The intensity contour, therefore, cannot depend on the relative electron densities of the matrix and microfibril in the way which Fraser *et al.* suppose; some other factor, presumably associated with the arrangement of the protofibrils in the microfibril, must modify the intensity at small angles in untreated wool.

We have examined this possibility in its quantitative implications, and have evolved a model structure which seems to have the correct scattering properties. In this, the microfibril consists of an annular array of protofibrils surrounding a core. The protofibrils have organized and less organized regions along their lengths, the former having diffraction properties like Crick triplet coiled-coils. A maximum of nine such protofibrils could be fitted into the annulus, but they need not all be organized into coiled-coils in any particular cross-section of the microfibril; we suppose, therefore, that there is an effective average number f_a (less than 9) of coiled-coils in the annulus. The microfibril core might also show coiled-coil organization and we suppose that it scatters X-rays as if it contained f_c (less than 2) coiled-coils in the cross-section. The unorganized material in the microfibril is taken to scatter like a uniform electron cloud filling the gaps between, and to some extent within, the coiled-coils. A similar cloud between the microfibrils simulates the scatter from the matrix. The small-angle scatter of such a model consists of (*a*) a part due to microfibril-matrix texture, of the type envisaged by Fraser *et al.*, and (*b*) a part due to protofibril scatter modified by interference between protofibrils in the same microfibril. Both components have maxima near 45 Å and 27 Å; in (*a*) the former is more intense, in (*b*) the latter (to an extent depending on the ratio f_c/f_a). In untreated Lincoln wool component (*a*) must be small (the mean electron densities in the matrix and microfibril being approximately equal) so that the small-angle scatter is mainly due to (*b*). In silver-stained wool only (*a*) is important (electron density of stained matrix being large). In other keratin fibres it may happen that both (*a*) and (*b*) contribute significantly, to give an intermediate type of intensity contour.

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Incorporation of Labelled Lysine into the Desmosine Cross-bridges in Elastin

THE isolation and chemical characterization of two new amino-acids, desmosine and isodesmosine, which form bridge-points between peptide chains in elastin has already been described[1–3]. The isomeric compounds are 1,3,4,5- and 1,2,3,5-tetra-substituted pyridinium salts with the empirical formula $C_{24}H_{40}N_5O_8Cl$ (as the chloride). Consideration of the structure of the two compounds and their location as cross-bridges between peptide chains led Partridge *et al.*[4] to suggest a possible biosynthetic route through ring closure on four lysine residues pre-existing in the peptide chains of a soluble pro-elastin.

A preliminary attempt to verify this suggestion experimentally has already been reported[5]. In these experiments groups of rats, 25 days old, were injected at intervals for 9 days with U-^{14}C-lysine and the animals were killed 12 h after the last injection. Lysine, desmosine and isodesmosine were isolated from purified elastin prepared from the aortae. The results showed that under the conditions of the experiments about 2 moles of lysine, instead of the expected 4 moles, were incorporated into each mole of desmosine and isodesmosine; it was remarked that this unexpectedly low result could have arisen because of a time lag between the incorporation of labelled lysine and ring-closure to form the desmosines.

About the same time a publication by Miller, Martin and Piez[6] showed clearly that the elastin from the aortae of 12-day-old chick embryos contained much more lysine and less desmosine and isodesmosine than elastin from full-grown birds. Over the age-range investigated the sum of lysine and quarter-desmosine remained constant and there was a smooth transition of the lysine/quarter-desmosine ratio with increase in age. The same authors reported that, in tissue culture experiments with chick embryo aorta, the amount of lysine incorporated into desmosine and isodesmosine increased steadily for some days after the culture had been allowed to take up a quantity of radioactive lysine from the medium. When the aortae were allowed to grow in a medium without labelled lysine for 11 days after a pulse dose of U-^{14}C-lysine, the specific activity of desmosine and isodesmosine was a little more than four times that of the lysine isolated from the same sample of elastin.

The experiments now reported confirm our tentative conclusions from the preliminary experiments[5] and show that the results of Miller *et al.*[6], obtained with avian embryo aorta in tissue culture, are valid for the growing rat.

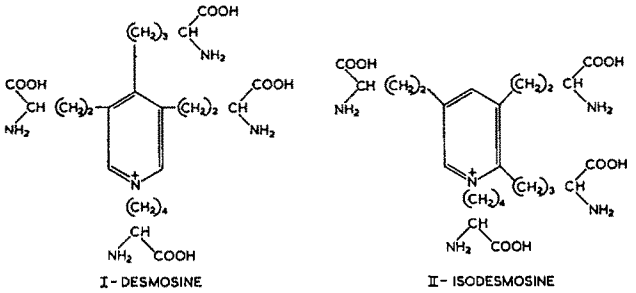


Fig. 1

The experimental procedure adopted was similar to that described earlier[5] except that after receipt of a concentrated pulse dose of U-^{14}C-lysine the groups of animals were fed a normal growth ration *ad lib.* until they were killed. Three groups of ten weanling male Sprague–Dawley rats were each kept for 8 days under the conditions of the experiment before the injections were started. Each rat received a total of approximately 0·030 mc. of U-^{14}C-lysine given in three doses per day for 10 days. The groups were killed 0·5 days, 5 days and 17 days after the last injection. The aortae were dissected from the aortic valve to the bifurcation; they were then freed from connective tissue and washed exhaustively with 0·15 M sodium chloride and dehydrated with acetone. The dried defatted aorta powder was treated with 0·1 N sodium hydroxide at 98° for 45 min[7], washed six times with hot water, and dried by treatment with ethanol and ether. The purified elastin was hydrolysed by refluxing with iron-free 6 N hydrochloric acid for 72 h in a current of nitrogen; desmosine, isodesmosine and lysine were isolated chromatographically as already described[5]. Samples (1·0 ml.) were taken from the centre fraction of each peak for scintillation counting in Bray's solution (21 ml.) containing 'Thixcin' (25 g/l.). A second sample (0·5 ml.) from the same fraction was used for the estimation of the amino-acid concentration using the Technicon 'Auto-analyser'. The results are given in Table 1.

Table 1. Incorporation of U-^{14}C-lysine into Desmosine and Isodesmosine

Sample	Days after last injection	c.p.m.	μmoles amino-acid	c.p.m./μmole	c.p.m./μmole ratio to lysine
(1)	0·5 days				
	Isodesmosine	40·8	0·062	660	1·57
	Desmosine	45·9	0·080	573	1·36
	Lysine	176·2	0·418	421	1
(3)	5 days				
	Isodesmosine	91·7	0·079	1,150	3·0
	Desmosine	83·2	0·085	977	2·6
	Lysine	199·0	0·527	377	1
(4)	17 days				
	Isodesmosine	108·5	0·113	958	4·56
	Desmosine	113·0	0·125	905	4·30
	Lysine	76·7	0·366	210	1

It will be observed that the molar specific activity of the desmosine isomers increased with time after the pulse dose of U-^{14}C-lysine and eventually reached a value of rather more than four times that of the lysine isolated from the same sample of elastin. That the figure was fractionally in excess of four was to be expected since there was considerable growth of aorta tissue during the 17 days which elapsed before the animals were killed. Since elastin from young animals contains a higher lysine/desmosine ratio[6], the activity of lysine is diluted to a greater extent relatively to the desmosine isomers. The results also show that the activity of isodesmosine was significantly higher than that of desmosine throughout the duration of the experiment. This may result from a difference in the rate of formation of the isomers and from the circumstance that mammalian elastin contains more desmosine than isodesmosine.

The incorporation of four molecules of lysine into both desmosine and isodesmosine, and the demonstration that the cyclization process is a slow one occupying up to 17 days after the uptake of the lysine, provides an adequate explanation for the observed variation in the composition and physical properties of elastin from animals at different stages of growth. However, it does not preclude the possibility that residues other than lysine may be involved in cross-bridges.

Chemical investigation of the structure of the isomeric desmosines[2,3] has resulted in the location of all the carbon atoms except for the methylene groups in the side chains on positions 3, 4, 5 in desmosine and 2, 3, 5 in isodesmosine. The demonstration of the biosynthesis of the desmosines from four lysine residues shows that the isomers arise by cyclization of straight-chain 6-carbon units and indicates (I) and (II) as the probable structures of the isomers.

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Inhibition of the Biosynthesis of the Cross-links in Elastin by a Lathyrogen

In some animal species nutritional deficiency of copper is known to result in a defect of the walls of the large arteries which leads to aortic rupture[1,2]. The defect has been attributed, at least in part, to a change in the amino-acid composition of elastin[3,4]. The lysine content of elastin isolated from copper-deficient animals is abnormally high, and there is reason to believe that this is related to a failure in the biosynthesis of linkages connecting the peptide chains.

Recently two new amino-acid isomers, desmosine and isodesmosine, have been isolated from hydrolysates of elastin and characterized as tetra-amino-tetracarboxylic acids containing quaternary pyridinium rings[5,6]. These substances appear to be in peptide linkage with at least two primary chains and thus can act as cross-links. The empirical formula of the isomeric desmosines, $C_{24}H_{39}N_5O_8$, suggested that they might arise biosynthetically by the oxidation and cyclization of four lysine side chains[7] and this was confirmed by feeding experiments with U-^{14}C-lysine[8,9]. Using chick embryo aorta in tissue culture, Miller, Martin and Piez[10] showed that added U-^{14}C-lysine is incorporated into the desmosines *in vitro* and that the molar ratio of the specific activities of the desmosines and lysine reached 4 : 1 about 11 days after the addition of a pulse dose of labelled lysine.

The symptoms of copper deficiency and lathyrism in turkey poults are highly analogous[11] and unpublished observations[12] have shown a decreased content of elastin in the aortae removed from poults fed β-aminopropionitrile (BAPN). Chick embryo heart tissue grown in organ culture produces extra-cellular fibres which give tinctorial reactions typical of elastin, and these fibres are reduced in amount in cultures which contain BAPN[14]. In the present experiments, some of which have already been briefly reported[13], the amino-acid composition of elastin from BAPN-fed chicks has been determined and the effect of BAPN on desmosine formation in aorta grown in organ culture has been investigated.

Two groups of chicks were fed a commercial chick meal for one week. Then 0·05 per cent of BAPN (3-amino-propionitrile fumarate, Aldrich Chemical Co., Milwaukee, Wisconsin) was added to the diet of one group and the feeding continued for 2 weeks. At 3 weeks of age the aortae were removed, cleaned of blood and adhering tissues and ground in a glass homogenizer in 0·9 per cent saline. The insoluble portion was dehydrated with acetone, washed with diethyl ether, and dried. Sufficient water was added to rehydrate the powder and 0·2 ml. of 90 per cent formic acid per mg of dry tissue was added. The suspension was held at 45° C for 36 h (ref. 15), then washed thoroughly with water and finally with acetone and ether. The amino-acid composition was determined by use of a Technicon 'Autoanalyser' after hydrolysis in 6 N hydrochloric acid under reflux for 72 h. For purposes of comparison, elastin prepared by 0·1 N sodium hydroxide treatment of aortae from copper-deficient and control chicks was similarly analysed (we thank Dr. J. E. Savage and Mr. D. Bird, University of Missouri, for the preparation of this material). Without correction for losses during hydrolysis, approximately 91 per cent of the nitrogen in the 'control' elastin and 96 per cent of that in the 'BAPN-fed' elastin was recovered. There was essentially no difference in the amino-acid composition of the experimental and control elastins except for lysine and the desmosines. The results are shown in Table 1. In the case of the copper-deficient elastin, the lysine content was nearly five times that of the control, while the desmosines averaged only 60 per cent of the control values. The 'BAPN-fed' elastin showed no change in desmosine and had only twice the normal lysine content. The failure to observe more significant differences was doubtlessly due to the mild nature of the lathyrism induced. Mortality was low and no gross abnormalities of the aorta were observed.

For investigations of organ cultures, aortae were removed from young ducklings, 1–7 days of age, under aseptic conditions, and placed in roller tubes containing tissue culture medium 199 supplemented with 25 units each of penicillin and streptomycin per ml. One half of the tubes contained 5 × 10^{-3} M BAPN and all contained 0·1–0·75 μc. of [U-^{14}C]-lysine per ml. during the first 2 days of culture. The cultures were then supplied with medium without labelled lysine and continued for 10–14 days with renewal of medium on alternate days. The cultures were collected, washed, dehydrated with acetone and, finally, washed with ether. Elastin was isolated by heating in 0·1 N sodium hydroxide for 1 h in a boiling water bath and repeated washing with hot water. Lysine, desmosine and isodesmosine were isolated and their specific activities determined as previously described[8]. Table 2 shows that the specific activity of the lysine isolated from the elastin was directly proportional to the concentration of the labelled lysine in the culture medium. The presence of BAPN slightly increased the specific activity of lysine, but in each experiment the specific activities of desmosine and isodesmosine were depressed to about one-tenth or less of the value found for the controls.

The results now obtained show that, as in copper deficiency, the presence of the lathyrogen results in the formation of an elastin with a high lysine content. The organ culture experiments demonstrate that BAPN inhibits the incorporation of lysine into the desmosine cross-linkages. Since the amino-acid analyses shown in Table 1 were performed on elastin which remained insoluble after extraction with hot alkali or concentrated formic acid it seems reasonable to suppose that the elastin isolated was at least partly cross-linked, and a marked fall in the content of desmosine and isodesmosine was not to be expected. However, with lathyritic elastin, as with that resulting from copper deficiency, the sum of lysine plus quarter-desmosine is markedly greater than is found with normal elastin, either from young or old animals. It is therefore apparent that there is an intermediate in desmosine synthesis which is destroyed by acid hydrolysis or has not yet been found in the hydrolysis products.

The analogy between the effects of BAPN and copper deficiency on the chemical and physical properties of elastin suggests that an enzyme system concerned in the condensation of lysine side-chains to desmosine requires

Table 1. Effect of Copper Deficiency and BAPN Feeding on the Composition of Purified Chick Aorta Elastin

Sample	Control (1)	Copper-deficient (1)	Control (2)	BAPN-fed (2)
Purification procedure	0·1 N NaOH at 98°	0·1 NaOH at 98°	Formic acid at 45°	Formic acid at 45°
N % (ash and moisture free)	16·3	16·6	16·7	17·1
	(Amino-acid residues per 1,000 residues)			
Hydroxy-proline	11·2	12·5	10·7	9·5
Isodesmosine	1·1	0·7	0·8	0·9
Desmosine	1·1	0·8	0·9	0·9
Lysine	3·6	17·4	5·3	10·5
Quarter-desmosines	10·9	6·0	6·8	7·2
Lysine + ¼-desmosines	13·6	23·4	12·1	17·7

Table 2. Effect of BAPN on the Incorporation of Lysine into the Desmosines of Elastin Isolated from Aortae Grown in Organ Culture

Exp. No	Culture conditions: ^{14}C-lys. per ml. (μc.)	Culture conditions: Days† in culture	Specific activity of compounds isolated*: Lysine Control	Lysine BAPN	Desmosine Control	Desmosine BAPN	Isodesmosine Control	Isodesmosine BAPN
1‡	0·1	8/3	950	1,240	600	0	950	0
2	0·25	2/14	2,450	2,720	1,580	220	1,680	90
3	0·75	2/13	6,680	7,760	6,910	700	7,810	410

* C p m per μmole.
† Days in presence of ^{14}C-lysine/days without ^{14}C-lysine
‡ Whole aortae from 7-day-old ducklings were used in Exp. 1 and from 2-day-old ducklings in Exp. 2. Chopped aortae (2–3 mm pieces) from day-old ducklings used in Exp. 3.

copper and that BAPN inhibits at least one step in the reaction chain. Although collagen and elastin have very different compositions and structure, it is of interest to note the apparent parallelism displayed in the effect of BAPN on the processes by which primary peptide chains are organized into a coherent fabric.

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Rate of Protein Biosynthesis in Hepatoma 223

Hepatoma 223, unlike the liver of an adult rat, rapidly increases its protein content. It might, therefore, be expected that its protein-synthesizing activity would be greater than that of liver so the efficiency of the *in vitro* systems from the two tissues were compared.

The cell sap and ribosome fractions of liver were obtained by methods previously described[1]. The cell sap was passed through a column of 'Sephadex *G*-25' equilibrated with medium *B* (48 mM sodium chloride, 100 mM potassium chloride, 10 mM magnesium chloride and 50 mM Trizma buffer, *p*H 7·6) to remove low molecular weight inhibitory substances[2]. The ribosomes and cell sap of the tumour were isolated by an essentially similar procedure. Ribosomes and excess of the cell sap fraction were incubated together in medium *B* containing 5 mM adenosine triphosphate, 0·05 mM guanosine triphosphate, 10 μg/ml. of an amino-acid mixture (lacking leucine) and 0·1 μc./ml. DL-[1-^{14}C] leucine. The reaction was stopped by addition of 0·5 N perchloric acid and the proteins isolated and prepared for counting as previously described[1].

It was found in a number of cross-over experiments in which ribosomes of tumour were incubated with tumour or liver sap that the efficiency of the ribosomal fraction of the tumour did not differ significantly from that of liver (96 per cent ± 8 per cent). It was found that the efficiency of the cell sap fractions from the two tissues were also very similar provided that their concentrations were not limiting.

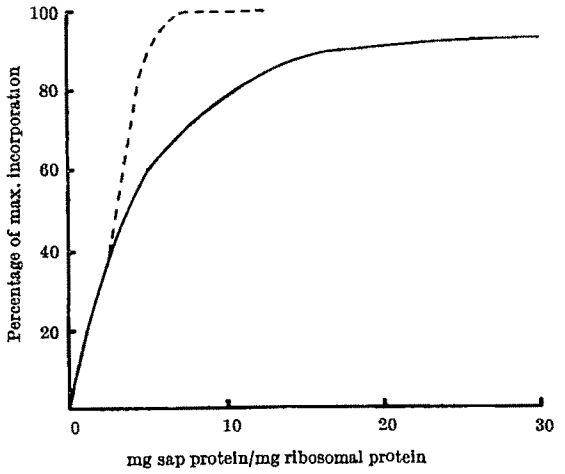


Fig. 1. The dependence of the incorporation of amino-acids into protein by the cell-free system, on the concentration of cell sap protein. The conditions of incubation are given in the text. 100 per cent represents about 3,000–5,000 c.p.m./mg ribosomal protein. —, Liver, . . . , tumour

The extent of amino-acid incorporation into protein in the ribosome cell-free system is, however, greatly affected by the amount of cell sap protein added (Fig. 1). It will be seen that, in the case of liver, a ratio of about 20 : 1 of cell sap protein to ribosomal protein is needed to ensure that enough sap constituents are present. It is also clear that the tumour cell sap saturates at a lower protein concentration than does that of liver. For example, the amount of protein required to give 80 per cent saturation in the liver system is about 3·5 times as much as in the tumour system. The higher efficiency of the cell sap protein from the tumour seems to reflect a higher concentration of soluble components of the protein synthesizing mechanism relative to other proteins in the cell sap fraction. We have noted, for example, that the tumour sap contains twice as much *s*RNA as liver sap per gram wet weight of tissue (that is, four times as much *s*RNA per mg cell sap protein) and this may account for the difference.

Table 1. Composition of Tumour and Liver (mg/g Wet Weight Tissue)

	Liver	Tumour
RNA	11	6·3
Protein	225	110
Ribosomes	13	7 5
Soluble fraction	90	44

RNA was estimated by the method described in ref. 1.
For the calculation of the quantity of ribosomes it is assumed that 50 per cent of the RNA of the cell is associated with ribosomes (ref. 3) and that they are 42 per cent RNA. It was also assumed that the amount of soluble fraction protein is 40 per cent of the protein of the whole tissue (ref. 3).

Table 1 shows the experimentally determined RNA and protein contents of liver and tumour and the calculated concentrations of ribosomes and cell sap. The tumour contains about half the ribosomal and cell sap content of liver (per g of tissue). The cell sap of tumour is several times as active as that of liver so it appears to be unlikely that cell sap activity is rate limiting. Since the tumour has half the number of ribosomes of liver and each has about the same activity as those of liver, the rate of protein synthesis of the tumour must be about half that of liver. Nevertheless, the tumour accumulates protein. This must arise because of some mechanism other than an increased rate of protein synthesis. It could be explained by differences between liver and tumour in the proportion of the protein produced which is retained by the tissue, or by differences in the type and stability of proteins made, and by other factors.

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Presence of Sodium–Potassium-stimulated ATPase in Boar Epididymal Spermatozoon

SINCE Skou[1] reported that an adenosine triphosphatase (ATPase) stimulated by sodium and potassium in the presence of magnesium (Na–K ATPase) is present in submicroscopic fraction of crab nerve, many investigators have reported the presence of the same ATPase in various tissues[2–6] (see also Hokin and Hokin's recent review[7]), and it was suggested that it plays an important part in the process of active transport of potassium and sodium ions. However, information concerning the presence of such an ATPase in spermatozoa is not, as yet, available. It seems very likely that active ion transport mechanism is also present in the spermatozoon, as its motility is affected by ionic environment. This communication reports our detection of Na–K ATPase activity in boar epididymal spermatozoon.

Spermatozoa obtained from fresh epididymal tissues of a boar were washed twice in 0·25 M sucrose solution and re-suspended in a 0·25 M sucrose solution containing 35 mM *tris*-hydrochloric acid buffer (*p*H 7·4). ATP was rendered free of sodium and potassium by passing it through a 'Dowex 50' cation-exchange resin column in the hydrogen form. The free acid was brought to *p*H 6·8 with 1 M *tris* solution. The reaction mixture contained 3 mM ATP, 3 mM magnesium ions, 35 mM *tris*-HCl buffer (*p*H 7·4) and various concentrations of sodium and potassium ions. Ouabain was added to the medium as required. The total volume of the reaction mixture was kept constant at 2·0 ml. After incubation for 30 min at 37° C, the reaction was stopped by the addition of 1 ml. of 30 per cent trichloroacetic acid, and liberated inorganic phosphorus (P_i) was determined by the method of Fiske and SubbaRow. Activity was expressed as μmoles of inorganic phosphorus/10^{10} sperm/h. The ATPase activity of intact spermatozoa is shown in Fig. 1.

As shown in Fig. 1, ATPase activity of intact spermatozoa increased in the presence of 3 mM of magnesium, and it was stimulated further by sodium ions but not by potassium ions alone. The activity was greatly enhanced by the addition of both sodium and potassium ions in the presence of magnesium ions. Fig. 1 shows also that the ATPase activity in the complete system is inhibited by the addition of 5×10^{-4} M ouabain. The presence of such an ATPase activity in intact spermatozoa suggests that spermatozoa may have a function of active transport of potassium and sodium ions. The following experiments were undertaken to investigate the distribution of Na–K ATPase activity in the spermatozoa.

Washed spermatozoa were suspended in 0·25 M sucrose solution containing 0·2 per cent sodium deoxycholate, 5 mM EDTA, 30 mM histidine–HCl, 30 mM 2-amino-2-methyl-1,3-propanediol, at *p*H 7·0, and subjected to ultrasonic treatment at 10 kc/s for 5 min. The sonicate was homogenized using a 'Teflon' pestle-glass homogenizer at 1,000 r.p.m. for 3 min, and the homogenized sonicate was fractionated by differential centrifugation into four fractions, namely, head, tail, Fraction 1 (which was collected by centrifugation of the supernatant at 10,000*g* for 10 min) and Fraction 2 (which was collected by centrifugation of the supernatant of Fraction 1 at 105,000*g* for 60 min). Each fraction was washed twice in 0·25 M sucrose solution and tested for the Na–K ATPase activity. Typical results of an experiment are shown in Table 1.

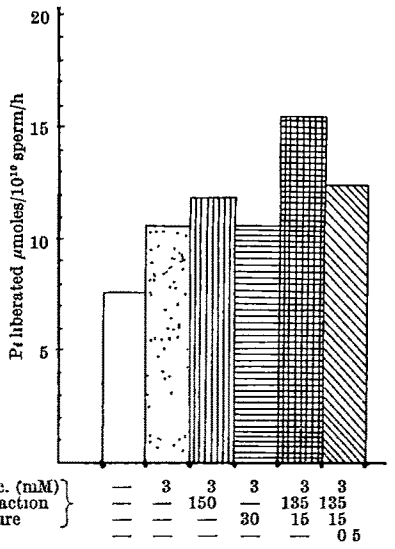


Fig. 1. ATPase activity of intact boar epididymal spermatozoa. The incubation medium consisted of 3 mM ATP, various cations as described above, and 35 mM *tris*-HCl buffer (*p*H 7·4)

Table 1 ATPASE ACTIVITY OF FRAGMENTED BOAR EPIDIDYMAL SPERMATOZOON

Fragmented spermatozoon	Activity (P_i, μmoles/N mg/h) Mg	Mg+Na+K	Mg+Na+K/Mg
Head	2·07	2 12	1·02
Tail	17·72	18·90	1·07
Fraction 1	5·53	6 90	1·25
Fraction 2	6·68	8·70	1 30

The incubation medium consisted of 3 mM ATP, 3 mM magnesium chloride, 105 mM sodium chloride, 30 mM potassium chloride, 35 mM *tris*-HCl buffer (*p*H 7·4). The enzyme suspension was added after 5 min temperature equilibration at 30° C, and incubation was continued for a further 30 min. The reaction was stopped by the addition of 1 ml. of cold 30 per cent trichloroacetic acid and aliquots were assayed for inorganic phosphate by the method of Fiske and SubbaRow.

It will be seen from these results that in the presence of magnesium alone ATPase was highest in the tail—which agrees with the results obtained by Nelson[8] for fragmented bull spermatozoa. On the other hand, Na–K ATPase activity was highest in Fraction 2, which contains microsomal sediment, and rather low in other fractions. In other experiments, Na–K ATPase activity of Fraction 2 was inhibited by addition of 10^{-5} M ouabain. Consequently it seems likely that Na–K ATPase activity in intact spermatozoon did not originate from the tail fraction, which consists of a myosine-like substance, but chiefly from Fractions 1 and 2, namely, membranes, mitochondrial and microsomal fractions. It is probable that this sodium–potassium-activated ATPase plays an important part in the enzymatic mechanism of active ion transport involved in the excitation and movement of the spermatozoon.

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Sub-cellular Distribution of Serotonin in the Developing Rat Brain

It is well known that the neonatal rat brain contains relatively little serotonin[1,2] and that the level of this amine increases slowly with age. Karki *et al.*[3] have shown that the enzymes mediating the synthesis and oxidation of serotonin are also low in the immature rat brain, and increase thereafter with growth of the animal. The aforementioned authors suggest that the low levels of indolamines in the rat brain at birth can be attributed to a deficiency of the binding or storage system, and are not a reflexion of the insufficiency of the enzyme systems. Schanberg and Giarman[4,5] have shown that endogenous brain serotonin distributes itself between the particulate matter of brain homogenates and the supernatants, and that this relationship is susceptible to alteration by drugs. It was, therefore, of interest to investigate the sub-cellular distribution of serotonin in the developing rat brain and to determine whether it will parallel other maturational changes.

Rats of the Wistar strain, bred in our colony under standard conditions of management and nutrition, were used in this investigation, and were selected at various ages after birth. Animals were decapitated rapidly, the brains minus cerebella removed, weighed and homogenized in unbuffered 0·3 M sucrose containing tranylcypramine (1 μmole). All procedures were carried out at 4° C. Sufficient brains were pooled to give a homogenate dilution of 20 per cent (w/v). Duplicate aliquots of the homogenates were centrifuged for 30 min in a No. 40 rotor of the model *L* Spinco ultracentrifuge. The high-speed residue and supernatant were extracted separately for their serotonin content by the method of Shore and Olin[6], as modified by Mead and Finger[7] and assayed in concentrated hydrochloric acid as described by Bogdanski *et al.*[8]. All results are averages of six or more individual determinations, and represent 10–30 animals, depending on the age group.

Table 1. Wet Weights and Serotonin Content of the Developing Rat Brain

Age (days)	Brain wet weight–cerebellum (g)	Percentage of adult brain wet weight–cerebellum	5-HT (γ/g)	Brain 5-HT γ/gm as per cent of adult levels	Percentage of total 5-HT in residue	Ratio brain wt. 5-HT content
1	0·26	17·1	0 26	39·3	72·4	1·0
2	0·26	17·2	0·27	41·6	72 0	1·05
4	0·43	28·6	0·31	47 3	73 5	1·4
10	0·68	45·5	0 30	46·4	77·8	2·3
12	0·85	56·3	0 36	55·3	77·5	2·4
22	1·03	68 6	0·37	56 9	73 2	2·8
31	1 13	75·3	0·50	76·9	73 4	2·3
33	1 32	88·0	0·51	78·9	72 0	2·5
Adult	1 5	—	0·65	—	74·5	2·3

The weight of brains and the amine content of rat brains at various stages of development (Table 1) were obtained without the cerebellum. It is apparent that the rat brain at one day of age contains 39 per cent of the adult amine content, but only 17 per cent of the adult weight. The values for 5-HT content in the developing brain are in agreement with those quoted by Karki *et al.*[3]. At 1 day post-natally, the ratio of brain weight to serotonin is unity, and increases to 2·3 by 10 days. Thereafter, this ratio is approximately constant, suggesting that both wet weight and serotonin-levels are maturing at equal rates.

A number of investigators[4,9] have consistently observed that the bulk of endogenous brain serotonin (75 per cent) is associated with the high-speed residue. Our results show that homogenates prepared from rat brains at all ages from 1 day post-natally yield essentially an adult subcellular distribution, in that in excess of 70 per cent is recovered in the particulate fraction. The concentration of a number of enzymes, such as succinic dehydrogenase[10], cholinesterase[11], and glutamic acid decarboxylase[12,13], is low in the immature rat brain and increases slowly to adult levels. Typical adult electrical activity can only be recorded from the rat cortex at 2 weeks of age[14], and it has been suggested that mature bioelectric activity depends on completion of synaptic connexions in the cortex[15,16].

The suggestion has been made by Karki[3] that the immature rat brain cannot bind serotonin as efficiently as it does at maturity, and a similar phenomenon has been observed by Glowinski *et al.*[17] for the binding of circulating norepinephrine by the developing heart. Though the immature brain may be deficient in its ability to store serotonin, such differences are not evident in the present results on the sub-cellular distribution. It can only be suggested that if specific cellular components are responsible for the binding of small quantities of endogenous amine, then these components do not exhibit maturational changes which are qualitative in nature. It is tempting to speculate that the *in vivo* binding of serotonin might correlate with the maturation of synaptic vesicles, and also possibly to the biosynthesis of gangliosides. Possibly the cellular components responsible for the binding of serotonin are present at birth in the rat brain and mature at the same rates as the wet weight, and the total amine storage capacity, thus resulting in an essentially constant sub-cellular distribution.

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Oscillation in Enzyme Reactions

The widespread existence of periodic phenomena in living organisms is well known. These events, like all other material phenomena in living systems, should ultimately be considered in terms of the individual chemical reactions occurring during the life process. Since chemical reactions occurring in biological systems are catalysed by enzymes, it was the purpose of our investigation to examine the possibility of oscillatory behaviour in enzyme-catalysed reactions.

The mathematical treatment of the differential equations arising in chemical kinetic problems is often impeded by the fact that the exact analytic solution of the equations is difficult, if not impossible, to obtain. Nevertheless, Lotka[1] in 1910 proposed an autocatalytic system wherein periodicity is clearly possible, and in 1921 Bray[2] illustrated experimentally a chemical system wherein periodicity is actually observed.

Difficulties attending the mathematical treatment of the differential equations arising in enzyme kinetic problems have served as a severe impedance to the development of enzyme kinetic theory. However, Christiansen[3] and Ozawa[4] obtained analytical results indicating that oscillations could occur in certain types of enzyme-catalysed reactions, provided certain conditions are met. Since then, Anderson[5], Chance[6] and Shnoll[7] have

Table 1. MECHANISMS INVESTIGATED FOR POSSIBLE OSCILLATORY CHARACTER

Mechanism		I	II	III
	$X_0 + S \rightleftharpoons X_1 \rightleftharpoons X_2 \rightleftharpoons \ldots \rightleftharpoons X_n \rightleftharpoons X_0 + P$	$n = 1$	$n = 2$	$n = 3$
Mechanism IV	$X_0 + S_1 \rightleftharpoons X_1 + P_1$	$X_1 + S_2 \rightleftharpoons X_0 + P_2$		
Mechanism	$X_0 + S_1 \rightleftharpoons X_1 + P_1$ $X_1 + S_2 \rightleftharpoons X_2 + P_2$ $X_2 + S_3 \rightleftharpoons X_0 + P_3$	V P_1,S_3 absent	VI P_2,S_3 absent	VII P_1,S_2 absent
Mechanism	$X_0 + S_1 \rightleftharpoons X_1 + P_1;\ X_1 + S_2 \rightleftharpoons X_2 + P_2,$ $X_2 + S_3 \rightleftharpoons X_3 + P_3;\ X_3 + S_4 \rightleftharpoons X_0 + P_4$	VIII P_1,P_2,S_3,S_4 absent		IX P_1,S_2,P_3,S_4 absent
Mechanism X	$X_0 + S_1 \rightleftharpoons X_1$ $X_0 + S_2 \rightleftharpoons X_2$ $X_1 + S_2 \rightleftharpoons X_3$ $X_2 + S_1 \rightleftharpoons X_3 \rightleftharpoons X_4 \rightleftharpoons X_5 + P_1$ $X_4 \rightleftharpoons X_6 + P_2$ $X_5 \rightleftharpoons X_0 + P_2$ $X_6 \rightleftharpoons X_0 + P_1$			

each obtained experimental results wherein periodicity is experimentally observed in enzyme systems.

The present investigation involves the examination of the behaviour of analogue computer simulations of each of the ten commonly encountered enzyme mechanisms listed in Table 1. The simulations were carried out on a *TR*-48 (solid state) computer, and the resultant concentrations displayed on an oscilloscope with time as the other axis. Hopefully, observations made for a large number and range of initial conditions and individual rate constants enable one to ascertain whether or not oscillatory behaviour should be expected for any of the mechanisms. The details of the procedure used for drawing inferences from this type of investigation are discussed elsewhere[8,9]. Briefly, negative results from this type of procedure are, strictly speaking, useless, but positive results are quite meaningful.

No evidence of oscillatory behaviour was found for any substrate or product (S or P) for any of the mechanisms listed in Table 1. Furthermore, no evidence of oscillatory behaviour for any form of the enzyme (X_i) was found for mechanisms I–IV in Table 1. On the other hand, the two-substrate, two-product mechanisms wherein more than one enzyme intermediate occurs (mechanisms V–X, Table 1) can experience up to three identifiable occasions when the intermediates pass through maximum or minimum concentrations. A typical example of the oscillatory behaviour is illustrated in Fig. 1.

Many other simulations were obtained wherein oscillatory behaviour occurred. We now define:

$$T = (t_2 - t_1)/t_{\frac{1}{2}}$$

where t_1 is the instant the first maximum occurs, t_2 is the instant the second maximum occurs, and $t_{\frac{1}{2}}$ is the time required for the products to grow from their initial concentrations (zero) to half their equilibrium concentrations, the equilibrium concentrations being defined in the absence of the enzyme. The general range of values found for T for mechanisms V–X, Table 1, is between 0·1 and 0·5. This range, however, can be extended in either direction by proper choice of the individual rate constants.

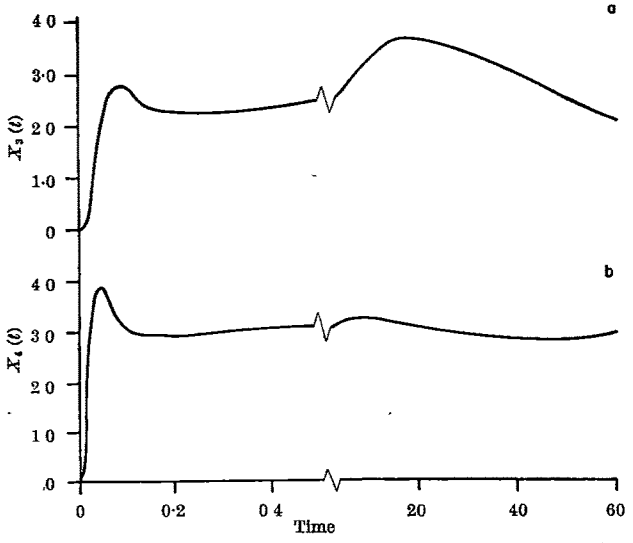


Fig. 1. *a*, A typical plot of $X_3(t)$ for mechanism X, Table 1. *b*, A typical plot of $X_4(t)$ for mechanism X, Table 1

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Highly Damped Oscillations in a Peroxidase-like Enzyme Reaction

RHYTHMIC biological processes are known to occur in most living organisms[1]. Even though these rhythms have been extensively investigated, little is known about their enzymatic or physical basis. Damped oscillations of glycolytic intermediates have been reported to occur in anaerobic yeast[2–5]. These findings heightened interest in oscillatory phenomena because the basis for these oscillations is more readily examined in this system. Indeed, simulation studies by Higgins[6] indicated that an activation of phosphofructokinase by fructose-1,6-diphosphate could produce oscillations similar to those experimentally observed. Another oscillatory system based on an inactive enzyme has been described by Spangler and Snell[7].

It is of fundamental importance to determine whether oscillations can only occur in enzyme systems having inhibition or activation of enzymes by other reactants, or whether most enzymatic reactions can oscillate. The present investigation indicated that any reaction involving two or more enzyme-substrate complexes has kinetic equations characteristic of damped oscillations, if the kinetic constants and substrate concentration satisfy certain conditions.

As a specific illustration, consider the peroxidase-like mechanism as follows:

$$\underset{(\varepsilon - p_1 - p_2)}{E} + \underset{x}{S} \xrightarrow{k_1} \underset{p_1}{E{\cdot}S_{\mathrm{I}}} \qquad (1)$$

$$\underset{p_1}{E{\cdot}S_1} + \underset{a}{AH} \xrightarrow{k_2} \underset{p_2}{E{\cdot}S_{\mathrm{II}}} + A \qquad (2)$$

$$\underset{p_2}{E{\cdot}S_{\mathrm{II}}} + \underset{a}{AH} \xrightarrow{k_3} E + A + \underset{p_3}{\text{product}} \qquad (3)$$

The pertinent kinetic equations are:

$$dp_1/dt = k_1x(\varepsilon - p_1 - p_2) - k_2ap_1 \quad (4)$$

$$dp_2/dt = k_2ap_1 - k_3ap_2 \quad (5)$$

$$dp_3/dt = k_3ap_2 \quad (6)$$

The solutions of these simultaneous non-linear differential equations do not exist in closed form. Fortunately, however, the general solution is not needed to demonstrate that damped oscillations can occur. Consider an open system in which the reaction vessel has sensors and a control unit to maintain the substrate concentration (x) and donor concentration (a) at constant levels after the reactants have been mixed. As a further simplification, assume that the two enzyme-substrate complexes and the product initially have zero concentration. Under these conditions, equations (4), (5), and (6) can be solved explicitly for p_1, p_2, and p_3 by using Laplace transforms[8]:

$$p_1 = \beta_1(1 - e^{-\gamma t}\cos\omega t) + \lambda_1 e^{-\gamma t}\sin\omega t \quad (7)$$

$$p_2 = \beta_2(1 - e^{-\gamma t}\cos\omega t) + \lambda_2 e^{-\gamma t}\sin\omega t \quad (8)$$

$$p_3 = k_3a\beta_2 t + \frac{k_3a}{\gamma^2 + \omega^2}\{(\omega\lambda_2 - \gamma\beta_2)(1 - e^{-\gamma t}\cos\omega t) - (\omega\beta_2 + \gamma\lambda_2)e^{-\gamma t}\sin\omega t\} \quad (9)$$

where

$$\beta_1 = \frac{k_1k_3\varepsilon ax}{\gamma^2 + \omega^2}, \text{ steady-state level of } p_1$$

$$\beta_2 = \frac{k_1k_2\varepsilon ax}{\gamma^2 + \omega^2}, \text{ steady-state level of } p_2$$

$$\omega = \sqrt{\Phi - \gamma^2},\ \Phi > \gamma^2$$

$$\gamma = 1/2\,(k_1x + (k_2 + k_3)\,a)$$

$$\Phi = k_3a\,(k_1x + k_2a) + k_1k_2ax$$

$$\lambda_1 = \frac{k_1\varepsilon x}{\omega}\left[1 - \frac{k_3\gamma a}{\gamma^2 + \omega^2}\right]$$

$$\lambda_2 = -\frac{k_1k_2\gamma\varepsilon ax}{\omega(\gamma^2 + \omega^2)}$$

t = time of reaction

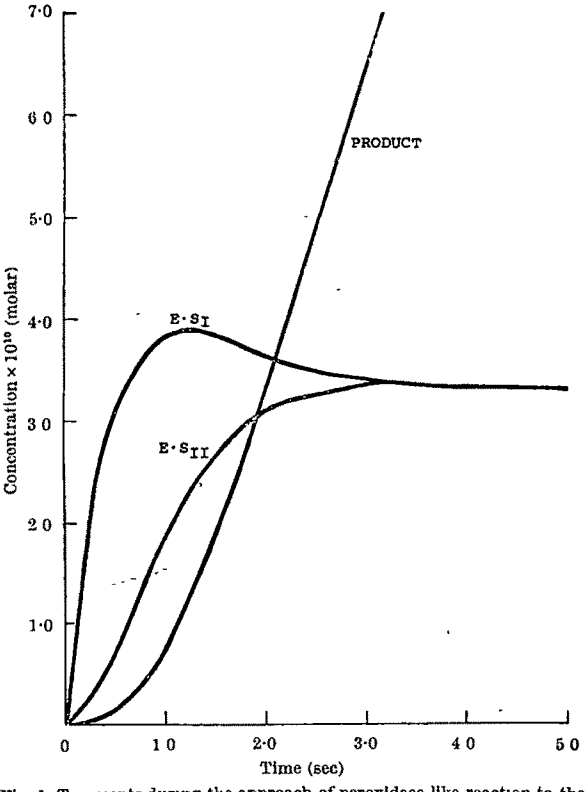


Fig. 1 Transients during the approach of peroxidase-like reaction to the steady state. $k_1x = k_2a = k_3a = 1{\cdot}0\ \text{sec}^{-1}$, $\gamma/\omega = \sqrt{3}$, $\varepsilon = 1{\cdot}0 \times 10^{-9}$ M

The sine- and cosine-exponential terms in equations (7), (8), and (9) indicate that the concentrations of the enzyme-intermediates will show damped oscillations. This condition will exist when Φ is greater than γ^2. The extent of damping is related to the ratio of γ to ω and is determined by the kinetic constants and substrate concentrations. Analysis showed that the oscillations will be least damped when the following conditions hold: $k_1x = k_2a = k_3a$. Even under these conditions, however, the oscillations damp out rapidly (Fig. 1). The oscillations are so small that an accuracy of one part in ten thousand is necessary to observe them. Thus, it is understandable that the analogue computer investigations[9] and also experimental measurements[10,11] of peroxidase kinetics have failed to reveal these damped oscillations, because the errors in these systems approach ± 10 per cent.

In summary, it appears that the oscillations in a peroxidase-like reaction are too highly damped to be observed experimentally with present techniques. Thus it appears that enzymatic reactions of systems such as peroxidase will have measurable oscillations only if some type of enzyme activation or inhibition is involved in a metabolic pathway.

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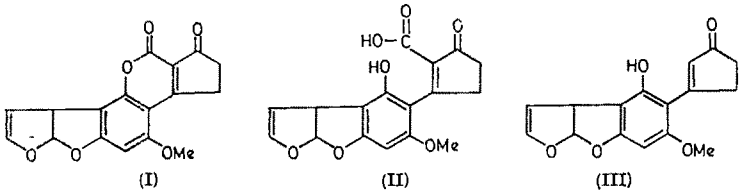
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Experimental Detoxification of Groundnut Meals containing Aflatoxin

During work involving sterilization by autoclaving of groundnut meals containing aflatoxin, the hepatotoxic metabolites of *Aspergillus flavus*, Link, ex-Fries[1], it was noted that an apparent reduction in the aflatoxin *B*1 content was induced when the normal methods of fluorescence assay[2] were afterwards applied to the sterile meal.

We have now demonstrated that sterilization by autoclaving at 15 lb./in.^{2} (120° C) of wet toxic groundnut meals resulted in a progressive reduction in their toxicity, due to aflatoxin *B*1, with time. Similar experiments involving treatment of pure aflatoxins (either produced by artificial culture or isolated from toxic extracts by preparative-scale thin-layer chromatography) resulted in the recovery of non-fluorescent products in which the characteristic ultra-violet absorption band at 363 mμ (ref. 3) had disappeared.

In a typical experiment, duplicate 20-g quantities of toxic groundnut meals (having moisture-levels of 10 per



(I) (II) (III)

cent and 60 per cent, respectively), shown by thin-layer chromatographic assay[2] to contain 7,000 μg/kg (7·0 p.p.m.) aflatoxin *B*1, were autoclaved at 15 lb./in.² (120° C) for 0·5, 2·0 and 4·0 h, respectively. After this treatment the samples were re-assayed, with the results shown in Table 1. The individual replicates in the two moisture-level series showed no significant differences.

Table 1. Effect of Autoclaving on Aflatoxin *B*1 Content of Toxic Groundnut Meals

Autoclaving time (h)	Moisture content (per cent)	Aflatoxin *B*1 content (μg/kg)
0 0	10	7,000
0 0	60	7,000
0·5	10	5,000
0 5	60	5,000
2·0	10	2,300
2 0	60	2,000
4 0	10	370
4 0	60	340

In order to ascertain whether autoclaving of toxic meals resulted in any significant alteration in their hepatotoxic effects, a sample (60 per cent moisture) containing 7,000 μg/kg aflatoxin *B*1 was autoclaved for 4 h and submitted, after re-drying, together with a similar quantity of untreated material, to the Central Veterinary Laboratory, Weybridge, for biological testing. Feeding trials were carried out using day-old Khaki-Campbell ducklings, both the original and the autoclaved meals being administered at two levels, representing 5 per cent and 10 per cent dietary incorporations, respectively, to matched groups of four ducklings. The category of toxicity of each prepared diet was assessed by histopathological examination of the liver tissue of both dead and surviving ducklings. Results are shown in Table 2.

Table 2. Effect of Feeding Autoclaved Toxic Groundnut Meal

Toxic groundnut meal	Level in diet (per cent)	Duckling deaths	Category of toxicity of diet
Untreated	5	1/4	High
(7,000 μg/kg *B*1)	10	4/4	Very high
Autoclaved	5	0/4	Very slight
(1,000 μg/kg *B*1)	10	0/4	Slight

From these results it was clear that autoclaving a moist toxic groundnut meal reduced its toxicity very considerably.

Autoclaving of pure aflatoxin *B*1 at 15 lb./in.² (120° C) for 4 h gave methanol-soluble, non-fluorescent reaction products in which no traces of starting material could be detected by thin-layer chromatography. Examination of the ultra-violet absorption spectrum of this solution showed that the characteristic peaks at 265 mμ and 363 mμ of pure aflatoxin *B*1 had disappeared to be replaced by a broad band at 258 mμ. Addition of alkali to this solution induced a bathochromic shift of 10 mμ to 268 mμ. Similar results were obtained using aflatoxin *G*1. The autoclaving treatment was applied also to a concentrate of aflatoxin *B*1 prepared from a toxic groundnut meal by separating and purifying the band formed on thin layers of Kieselgel '*G*' (Merck). (Three successive developments with 2 per cent methanol in chloroform and final desorption by methanol[4].) A similar result was again obtained.

Finally, 20 μg pure aflatoxin *B*1 was refluxed with water for 10 h. Extraction of the reaction products with chloroform, followed by thin-layer chromatography on Kieselgel '*G*' (Merck) as previously described[2], gave a blue fluorescent compound (R_F 0·32) not identical with aflatoxin *B*1 (R_F 0·50). The ultra-violet spectrum of this compound in methanol gave a well-defined band at 324 mμ, subsequent addition of alkali producing a bathochromic shift to 360 mμ. The spectrum of pure *o*-coumaric acid also showed a characteristic band at 324 mμ, alkali likewise inducing a bathochromic shift of 36 mμ. Aflatoxin *G*1 behaved somewhat similarly under these conditions.

In view of the known heat stability of the (anhydrous) aflatoxins it does not appear that the detoxification of meal by prolonged autoclaving can be explained by simple thermal degradation. As the presence of moisture is necessary it is likely that the first change is the hydrolytic opening of the lactone ring of aflatoxin *B*1 (I) to form a substituted *o*-coumaric acid (II). The aqueous hydrolysis experiment already described gives support to this, since one of the coumaric acid-type spectral bands was observed. However, after the autoclaving treatment this characteristic band was not seen, suggesting that more drastic changes ensued: for example, decarboxylation of the acid might occur (III), but in any event the way would then be open to further decomposition, leading to non-toxic breakdown products.

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PHYSIOLOGY

Evidence for a Humoral Natriuretic Factor released by Blood Volume Expansion

Following the demonstration that diuresis and natriuresis, caused by blood volume expansion with iso-oncotic infusions in dogs, are similar in denervated and innervated kidneys[1], it was logical to attempt the direct demonstration by crossed perfusion of a humoral effector mechanism. This undertaking was preceded by the report of Mills *et al.*[2] that diuresis and natriuresis produced by iso-oncotic infusions in dogs could not be prevented by pitressin and mineralocorticoid administration. In the description by de Wardener *et al.*[3] of complicated cross-perfused whole-animal preparations, as well as of isolated kidney perfusions, evidence was advanced for the operation of a natriuretic material following large saline infusions in dogs. Cort and Lichardus[4], using carotid occlusion in the cat, have shown that a resulting natriuresis, presumed to be due to blood volume redistribution, could be elicited in a second cat by transferred blood. This experiment offered direct evidence of a natriuretic substance by a bioassay procedure in which the stimulus itself could not have influenced the test animal. A large amount of early work led Homer Smith[5] to deduce the existence of a natriuretic system other than the mineralocorticoid mechanism for regulation of sodium metabolism. Recently, evidence has been accumulating, in particular from the work of Levinsky, Rector and Berliner *et al.*[6–8], that expansion of the extracellular fluid volume with saline can decrease renal tubular sodium reabsorption in circumstances where neither change in mineralocorticoid levels nor in glomerular filtration rate can explain the effect.

In the crossed renal perfusions to be reported, carried out on pairs of hydrated anaesthetized dogs, we have observed transfer of a humoral effect increasing sodium

excretion and urine flow following modest infusions (30 per cent of estimated blood volume) of iso-oncotic solution or 'artificial blood'; the latter was previously shown by exchange transfusion to cause a renal response attributed almost entirely to the stimulus of intravascular expansion[9]. The results of the crossed renal perfusion have already been communicated briefly[10]. Animals were prepared for crossed perfusion from the femoral artery of the heparinized donor to an isolated aortic sac of the recipient connected to the left renal artery—the right renal artery being tied off. The renal venous return from an inferior vena cava sac was collected at constant pressure into a reservoir from which it was returned with a sigmamotor pump to the femoral vein of the donor. The connexion procedure involved no more than a few seconds of renal ischaemia. Arterial perfusion pressure was maintained constant (or deliberately altered) with a clamp on the perfusion line. Renal blood flow was measured directly by intermittent diversion of the venous return into a graduated cylinder, and filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated by measuring exogenous creatinine and *para*-aminohippurate clearance, respectively. After control periods, the blood volume of the donor animal was expanded by infusion of 'artificial blood' consisting of washed canine erythrocytes resuspended in 6 per cent bovine albumin in Ringer–Locke solution. In other experiments the bovine albumin solution alone or the Ringer–Locke solution alone was used. Fig. 1 shows a

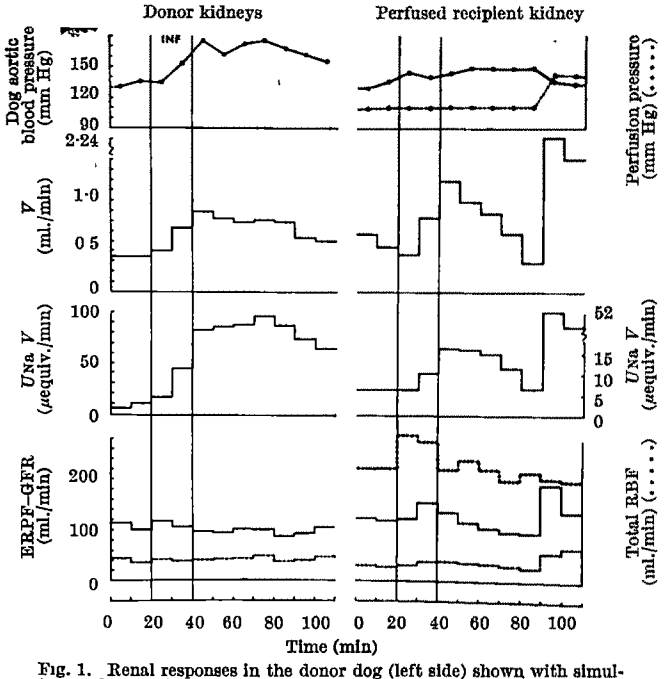


Fig. 1. Renal responses in the donor dog (left side) shown with simultaneously recorded responses in the perfused recipient dog kidney (right side) following infusion (INF) of artificial blood to the donor animal. The values for the perfused organ have been doubled. During the last two experimental periods, the clamp was removed from the arterial perfusion line

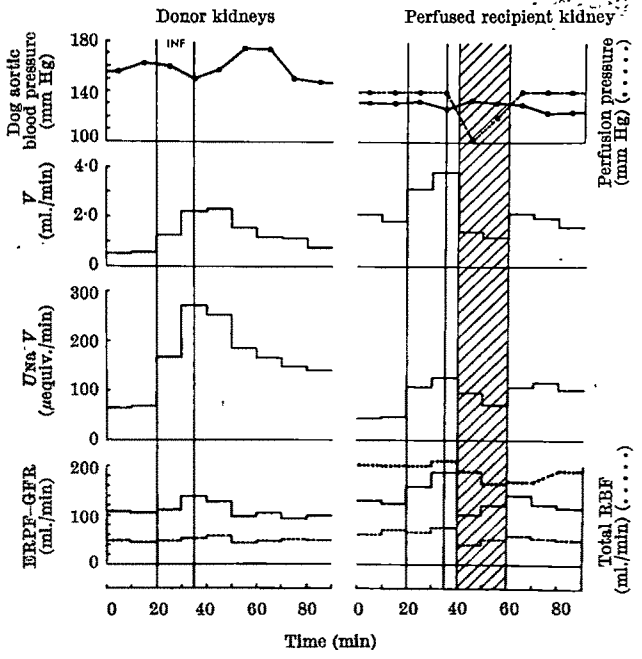


Fig. 2. Renal responses in donor dog and in perfused recipient dog kidney following infusion of Ringer–Locke's solution to donor animal. During the periods included in the hatched column the perfusion pressure was deliberately lowered; increased sodium excretion continues in spite of reduced GFR in the perfused organ

good response in the perfused kidney, with changes in some renal parameters representative of eight experiments with artificial blood as the infusate. Averaged data are given in Table 1.

The perfusion pressure was held constant at a somewhat reduced level (range of difference from donor blood pressure, 17–72 mm mercury, the average being 37) to enable regulation in spite of fluctuations and decline to normal of donor pressure after the infusion; that this contributed to the usually low output of the perfused organ is supported by the recorded effects of abrupt increase in perfusion pressure (Fig. 1). Although perfused at constant pressure, the recipient kidney consistently exhibited an increase in renal blood flow (RBF), attributable to vasodilation, during and immediately following the infusion to the donor. This change did not persist and was outlasted by increase in water and sodium excretion. The patterns of the latter in donor and perfused kidneys were not usually parallel; the peak natriuresis, on the average, was delayed in the recipient kidney as was the peak diuresis. Haemodynamic alterations, consisting of increasing values of GFR and ERPF in the perfused kidney, became stable or decreased in trend while the sodium and water excretion continued to increase. This observation, coupled with the demonstration (Fig. 2) in five experiments (using 'artificial blood' and other infusates) of persisting increase in sodium output above initial levels during deliberate reduction in GFR below control values (achieved by clamping the arterial perfusion

Table 1. Comparison of Percentage Changes in Renal Parameters of Donor (*D*) and Perfused Recipient Kidneys (*R*) following Blood Volume Expansion (Infusion) in the Donor

Periods (min)		Control 0–20	Infusion 30	Infusion 40	Following infusion 50	Following infusion 60	Following infusion 70	Following infusion 80
V	*D*	100 ± 3	*120 ± 16	*203 ± 67	*278 ± 103	*253 ± 104	*233 ± 94	*228 ± 87
	R	100 ± 6	109 ± 17	*136 ± 25	*163 ± 41	*170 ± 44	*161 ± 40	*138 ± 29
$U_{Na}V$	*D*	100 ± 6	*129 ± 21	*236 ± 91	*362 ± 178	*332 ± 174	*347 ± 183	*358 ± 196
	R	100 ± 6	*125 ± 26	*150 ± 30	*170 ± 44	*184 ± 55	*197 ± 64	*162 ± 42
ERPF	*D*	100 ± 6	103 ± 12	105 ± 10	95 ± 14	96 ± 13	103 ± 14	112 ± 14
	R	100 ± 11	115 ± 17	*141 ± 11	*136 ± 10	131 ± 11	128 ± 7	123 ± 21
RBF	*R*	100 ± 1	105 ± 6	*114 ± 8	111 ± 9	105 ± 5	101 ± 2	101 ± 6
GFR	*D*	100 ± 5	100 ± 12	98 ± 10	79 ± 28	89 ± 13	89 ± 17	108 ± 22
	R	100 ± 5	116 ± 15	117 ± 15	121 ± 17	113 ± 15	122 ± 10	115 ± 21

* Changes from control values significant at $P < 0{\cdot}01$; variation given is *S.D.*; $n = 8$.

line), suggests that reduced tubular sodium reabsorption had also occurred.

It would appear that a humoral influence operated on these cross-perfused kidneys to produce natriuresis and diuresis related to the same changes in the donor animals. In view of the constant perfusion pressures, the associated increases in renal haemodynamic parameters were also of humoral origin. They may have contributed to, but are unlikely to account for, the alteration in output. The sub-normal output of the perfused kidney in the majority of our experiments may not be explicable entirely as an effect of reduced perfusion pressure and is the subject of further investigations. This shortcoming does not, however, in our view, invalidate the conclusion that a natriuretic substance (or inhibitor of an anti-natriuretic substance as discussed by Cort and Lichardus[4]) operated on renal excretion of sodium in response to the stimulus of blood volume expansion.

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Effect of Glucagon on the Alkaline Phosphatase Activity in the Pancreatic Islets of Rats

ALKALINE phosphatases have been demonstrated by histochemical staining methods in the pancreatic islet cells of different species[1–4]. In the islets of adult mammals a positive reaction for this enzyme has so far been found only in the α cells[1,2], while in birds the reaction is present mainly in the β cells[3,4]. Since at least in mammals the islet α cells represent the site of glucagon production[5], it is noteworthy that Verne and Petkov[6] reported a diminished staining intensity for alkaline phosphatases in these cells after administration of glucagon to rats. In the present investigation the effect of glucagon on the alkaline phosphatase activity of the islet tissue has been re-evaluated by the application of quantitative chemical micro-methods to isolated pancreatic islets of rats.

Male Sprague–Dawley rats, weighing about 95 g and allowed free access to food and water, were separated into two groups: (1) Ten rats received subcutaneous injections of glucagon (Eli Lilly and Co.) dissolved in glycine–sodium hydroxide–sodium chloride buffer (0·1 M, *p*H 9·6), each injection corresponding to 0·3 mg glucagon per kg body-weight. The injections were given at intervals of 8 h until a total amount of 3·0 mg glucagon per kg body-weight had been administered to each animal. (2) Ten control rats received corresponding injections of buffer solution only. Three hours after the final injection the animals were killed by a blow on the neck and the pancreas rapidly excised and immersed in 0·25 M sucrose solution maintained at + 2° C. About 25 islets from each animal were isolated by free-hand dissection[7] and homogenized in 100–200 μl. ice-cold bi-distilled water. Homogenates of exocrine pancreatic parenchyma and of the liver were also prepared. The alkaline phosphatase activity was assayed in triplicate samples (10 μl. each) of homogenate at *p*H 10·0 as described by Bessey *et al.*[8]. The protein concentration of the homogenates was measured in triplicate samples (10 μl. each) according to Lowry *et al.*[9], and the enzyme activity was expressed as moles of substrate split per kg dry weight of a standard serum albumin reference (Armour Pharmaceutical Co., Lot 0370) and hour. It was ascertained that the substrate cleavage constituted a linear function of the incubation time and the enzyme concentration. For histochemical staining purposes small pieces of fresh pancreas were frozen at −70° C, and cryostat sections, 10μ thick, attached to glass microscope slides. The fresh sections were treated according to Gomori[10] using sodium-β-glycerophosphate (0·02 M) as substrate at *p*H 9·4. Incubations were carried out for 1–2 h at +37° C, control experiments being performed by incubating sections in a medium lacking the substrate.

There was a marked retardation of growth in the glucagon-treated animals, the body-weights of which had decreased no less than 18 ± 2 g at the end of the experiment. By contrast, the weights of the controls increased 16 ± 1 g. As can be seen in Table 1, the alkaline phosphatase activity of the islet tissue in the control animals was more pronounced than that of the exocrine parenchyma ($P < 0·01$), while it appeared less intense than in the liver ($P > 0·05$). Administration of glucagon brought about a marked increase of the enzyme activity in all the three tissues, the differences between control and experimental animals being significant at the 3 per cent level for the islets and at the 1 per cent level for the acinar tissue and the liver. A positive histochemical staining reaction was noted in the islets of both control and glucagon-treated rats, the staining being distinctly localized to cells at the periphery of the islets and to capillary walls. There was no obvious difference in the staining intensity between the two groups of animals.

Table 1

Animals	Islets	Acini	Liver
Control rats (10)	0·56 ± 0·09	0·24 ± 0·03	0·81 ± 0·14
Glucagon rats (10)	0·94 ± 0·13	0·46 ± 0·06	1·54 ± 0·20

Alkaline phosphatase activity in the endocrine and exocrine pancreas and the liver of controls and glucagon-treated rats. The figures denote mean value and standard error of the mean expressed as moles of substrate split per kg dry weight of standard albumin reference and hour. The number of animals analysed is given within parentheses.

There is much evidence to indicate that administration of glucagon brings about a marked functional depression of the islet α cells. Degranulation and atrophy of these cells were demonstrated in guinea-pigs[11] and rabbits[12] after prolonged courses of glucagon injection, and in rats a few days' treatment with this hormone exerted a significant reduction of the mean nuclear size of the α cells[13]. More recently it has been shown that morphological signs of a depressed α cell function actually are confined to the silver negative α_2 cells, supporting the view that glucagon production is a special feature of this type of α cell[14,15]. In addition to the regressive morphological changes, histochemical signs of a decreased α cell function have been demonstrated after administration of glucagon. Disappearance of zinc from the rabbit α cells was reported by Logothetopoulos and Salter[12], and Petersson and Hellman[15] observed a reduced staining for tryptophan in the α_2 cells of guinea-pigs. As mentioned here, reduction of the histochemically demonstrable alkaline phosphatase activity after glucagon injections has been reported in rats[6].

The present histochemical results agree well with the concept that non-specific alkaline phosphatases are distinctly localized to the α cells of normal adult rats. The positive staining reaction in these cells is in line with our quantitative data indicating a significantly higher enzyme

activity in the endocrine than in the exocrine pancreas. The previous observation of a diminished staining for alkaline phosphatases in the islets of glucagon-treated rats[6] was not supported by the present investigation. On the contrary, the quantitative chemical microassay clearly revealed that glucagon administration induced a marked rise in the enzyme activity level when expressed per unit protein. It seems, however, difficult to relate these results specifically to a depressed hormone secretion from the islet α cells, since the alkaline phosphatase activity was elevated also in the exocrine parenchyma and in the liver.

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PHARMACOLOGY

Effect of Chlorothiazide on Water Consumption in the Rat

Chlorothiazide has been in use for several years as a diuretic drug. Its diuretic effect is usually ascribed to inhibition of sodium reabsorption in tubular cells[1]. In recent years chlorothiazide has been reported to decrease urine volume in diabetes insipidus[2]. The antidiuretic effect is accompanied, however, by saluresis, that is, increased sodium excretion[3]. To explain the paradoxical action of chlorothiazide it has been suggested that the drug could also affect thirst, by decreasing plasma osmolarity through increased urinary sodium loss[4,5]. We have recently reported that chlorothiazide reduced water intake in bilaterally nephrectomized rats, thus demonstrating an extra-renal effect[6]. Osmotic receptors related to thirst regulation have been postulated to be located in the hypothalamus[7,8]. Stein and Seifter have recently reported chemical excitation of thirst by deposition of acetylcholine crystals through a cannula into a certain area in the hypothalamus[9]. Applying their technique, we have deposited chlorothiazide crystals in the same area of the hypothalamus of rats, weighing 350–400 g, using the co-ordinates of Stein and Seifter[8]. In control animals either calcium carbonate or talc was deposited in the same location. Food and water were given freely and daily consumption was recorded. Water intake was found decreased in rats with hypothalamic chlorothiazide deposit compared with the control animals (Fig. 1). No difference in food consumption was noted between the two groups and the difference in water intake persisted also after withdrawal of the food. That chlorothiazide was not absorbed to any appreciable extent into the circulation was suggested by the lower urinary volume in the chlorothiazide rats relative to the control animals (20–26 per cent less). Furthermore, the potassium/sodium ratio in the urine was the same in the chlorothiazide and the control groups.

The effect of hypothalamic chlorothiazide deposition on water consumption was accentuated when hypertonic sodium chloride solution was injected subcutaneously to increase thirst. Animals, prepared as described here, received on alternate days 5 ml./kg of 4·5 per cent sodium chloride solution subcutaneously. The mean daily water intake during three days with hypertonic saline injection was compared with that during three days without such treatment. Four experiments were performed and the results are shown in Table 1. The results are expressed as the ratio of water consumption by the hypothalamic chlorothiazide group to the consumption by the control rats in each experiment. In all four experiments the depression in water intake by intrahypothalamic chlorothiazide was larger when hypertonic saline was injected.

Table 1. Comparison of the Effect of Intrahypothalamic Chlorothiazide on Water Consumption with and without Subcutaneous Injection of Hypertonic Sodium Chloride

	Relative water consumption* (per cent)			
Experiment No †	1	2	3	4
Without sodium chloride‡	93	74	54	92
With sodium chloride§	57	50	34	79

* Expressed as $\frac{\text{Water consumption of chlorothiazide group}}{\text{Water consumption of control group}} \times 100$

† In each experiment chlorothiazide was deposited into the hypothalamus in four rats and calcium carbonate was deposited in the corresponding area in four other rats (control group).

‡ Without sodium chloride—relative water consumption when no sodium chloride was administered

§ With sodium chloride—relative water consumption when 4·5 per cent sodium chloride was injected subcutaneously

In a further attempt to detect possible extra-renal effects of chlorothiazide the following experiment was performed. Rats deprived of water for four days were given 4·5 per cent sodium chloride solution, 5 ml./kg subcutaneously. This pretreatment was intended to increase the osmotic drive for drinking to the extreme. Thirty minutes later the rats were divided into two groups, four rats in each. One group was injected subcutaneously with chlorothiazide, 200 mg/kg, dissolved in dilute sodium hydroxide; the other group was injected with an equivalent volume of the solvent alone. Water was then given freely to both groups of animals. Within the next 5–10 h water consumption was found to be smaller in the chloro-

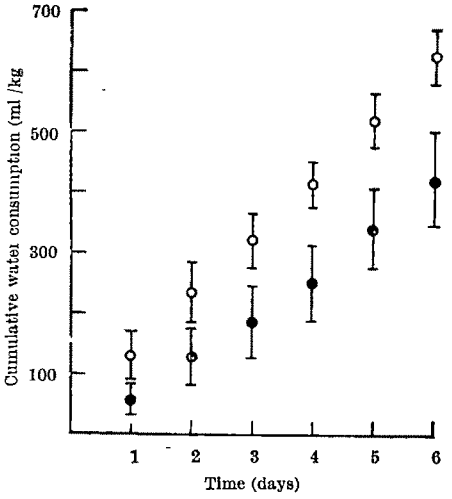


Fig. 1 Effect of intrahypothalamic chlorothiazide deposit on water consumption in rats. ○, Control group (18 animals); ●, experimental group (18 animals) Bars indicate *S.D.* For further details see text

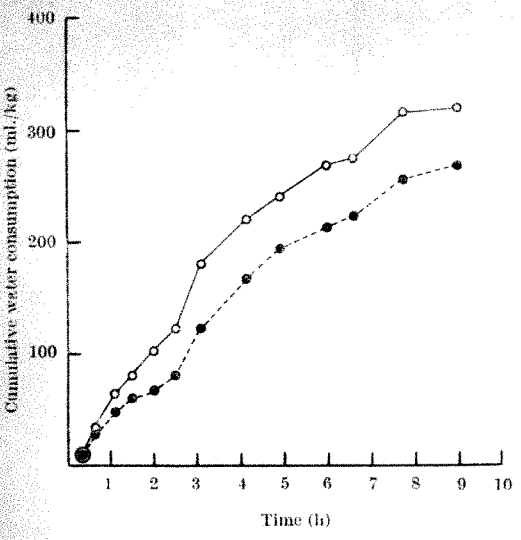


Fig. 2. Effect of subcutaneous chlorothiazide on water consumption in dehydrated rats. ○, Control group (four rats); ●, experimental group (four rats), injected with chlorothiazide. For further details see text

thiazide-treated rats than in the control rats (Fig. 2). This effect was found in four out of six experiments; in two experiments no significant difference was observed. (A possible explanation could be variation in intensity of thirst due to differences in humidity and ambient room temperature from one experiment to another.) The depression of water consumption by chlorothiazide in severely dehydrated, thirsty rats contrasted with the lack of effect or increased water intake induced by the drug in control experiments with hydrated rats. Dicker and Eggleton have recently reported decreased urine volume induced by hydrochlorothiazide in water-loaded rats[10], which seems to conflict with the results presented here. Hydration in their experiments, however, consisted of intragastric administration of a water load immediately after application of the drug. The possibility of delayed water reabsorption from the intestines caused by the drug has not been explored.

The experiments reported in this communication are consistent with the hypothesis put forward by Robson and Lambie[4] and Skadhauge[5] that the antidiuretic action of chlorothiazide in diabetes insipidus is through an extra-renal mechanism. However, these authors have assumed that sodium loss, caused by the drug, resulted in haemodilution, thus relieving thirst. This explanation has been recently challenged by Kennedy *et al.* in a thorough investigation on rats with diabetes insipidus[11]. They have shown that haemodilution induced by chlorothiazide in normal and obese rats failed to decrease water intake. They have also correctly argued that if decreased water intake were the only mechanism of action of chlorothiazide, there is no explanation why water deprivation does not cure diabetes insipidus. It seems that a combination of renal and extra-renal effects of the drug may offer better comprehension of the paradoxical actions of chlorothiazide. The renal action results in increased sodium loss (leading also to haemodilution). In addition, the drug may decrease the urge to drink by some action on the hypothalamic 'thirst centres', presumably through some effect on sodium transport in the osmotic receptors (which may be not dissimilar from the effect on kidney tubular cells). This, in turn, would result in decreased water intake and decreased urine output. Thus, increased solute loss through a renal mechanism combined with decreased water intake by inhibiting a central thirst mechanism could produce the effect of chlorothiazide observed in diabetes insipidus. This would eliminate the necessity of assuming chlorothiazide to inhibit sodium reabsorption and, at the same time, to increase water reabsorption by the kidney, which is required if a pure renal action is accepted[12].

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HAEMATOLOGY

Xg Blood Groups of American Indians

ONE hundred and ninety-five Indians, mostly Navajo, from the area surrounding Santa Fe, New Mexico, were tested for the Xg^a blood group antigen with the following results:

	Xg (a+)	Xg (a−)	Total
Males	70	18	88
Females	100	7	107

The data were tested for mutual concordance of the gene frequency between sexes by the method of Haldane[1]. This method gave a normal unit variance test statistic with the value of approximately unity, so that there is no evidence of discordance between the sexes in this relatively small material.

The gene frequency estimate derived from the data is $q(Xg) = 0{\cdot}2636$, $p(Xg^a) = 0{\cdot}7364$. The information about the gene frequency, from Haldane's formula, is 998·81 units, corresponding to a standard error of the estimate of 0·0316. A 95 per cent confidence interval surrounding this estimate is:

$$Pr\ (0{\cdot}2017 < q < 0{\cdot}3255) = 0{\cdot}95$$

This estimate is significantly different from that found by Gavin *et al.*[2] for Caucasians, and is the lowest frequency so far reported for this gene in any population.

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Hypothetical Significance of Disturbances of Zinc and Protoporphyrin Metabolism in Leukaemic Cells

ELSEWHERE[1,2] I have demonstrated a significant decrease in the zinc content of bone marrow and blood granulocytes in patients with acute and chronic myelocytic leukaemias, plasmocytoma and Hodgkin's disease, and in various patients with malignant neoplasms. Vallee and Hoch[3] isolated a zinc-protein containing about 0·3 per cent zinc from normal granulocytes. This protein contains almost all the water-soluble zinc in the granulo-

cytes[3]. In their opinion, this protein is an enzyme of unknown specificity. It is conceivable that the amount of this zinc-protein in granulocytes is decreased in leukaemic states. The results of Gordon[4], Saillen[5], Vanotti[6] and Walters[7], on the disturbances in haem and protoporphyrin synthesis in leukaemic cells, are also of interest. Free protoporphyrin can be demonstrated in these cells, but it cannot be shown in normal granulocytes. This means that the synthesis of an unknown protein with a protoporphyrin prosthetic group is inhibited in leukaemic cells.

The decrease in the content of zinc and the increase of free protoporphyrin appear to be closely related disturbances in leukaemic cells; a finding which may be important in the pathogenesis of leukaemia.

Craddock[8] suggests that a feedback mechanism is involved in the maturation of granulocytes, and has advanced the hypothesis of the presence of a protein in mature granulocytes which regulates the maturation of these cells. The humoral regulation of cell maturation is also suggested by Szent-Györgyi[9,10], who notes the lack of a protein regulating maturation in cancer and leukaemic cells.

In view of the aforementioned disturbances in zinc and protoporphyrin metabolism in leukaemic granulocytes, it is possible that the protein which regulates the maturation of granulocytes contains a zinc-protoporphyrin prosthetic group. Zinc-protoporphyrin proteins are known in animals and micro-organisms, and bivalent zinc may react with a protoporphyrin in the same way as does iron in haemoglobin or peroxidases, magnesium in chlorophyll, or cobalt in cyanocobalamine.

The treatment of leukaemic granulocytes with zinc-protoporphyrin compounds would be an investigation of great interest, but such compounds seem to be difficult to obtain.

One method of solving this problem may be to use micro-organisms to synthesize such a compound. Many bacteria are able to synthesize the zinc-porphyrin group and this may be why staphylococcal infection sometimes causes a remission in acute leukaemia.

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Histochemical Demonstration of Sodium–Potassium-activated Adenosinetriphosphatase in Normal and Leukaemic Granulocytes

Sodium–potassium-activated adenosinetriphosphatase (Na–K-ATPase) is an enzyme regulating the active cation transport through the cell wall[1]. This enzyme differs from other ATPases in its great sensitivity to cardiac glycosides, and is inhibited by ouabain (strophantine *G*) at a 10^{-5} M concentration[2]. Na–K-ATPase systems have been demonstrated in various animal and human tissues[1,3,4]. In 1964 Block and Bonting[5] found Na–K-ATPase activity in leucocyte suspensions isolated from human blood. The close relation of this enzyme to the active transport of sodium and potassium in granulocytes was demonstrated. Block and Bonting also observed a significant increase in ATPase activity in leucocyte suspensions taken from cases of acute and chronic myelocytic leukaemia and chronic lymphocytic leukaemia[5].

In 1964, McChurkin[6] published a histochemical method for demonstrating Na–K-ATPase activity in tissue slices and confirmed the activity of this enzyme in muscle, kidney, brain and liver.

In the present work, McChurkin's procedure[6] was modified for an investigation of bone marrow and blood slides. The technique used here differs from the original technique in the method of fixation and the period of incubation. The slides were fixed for 10 min in cold (4° C) formalin vapour and then incubated for 3 h in a solution containing ATP (calcium salt), magnesium, sodium and potassium ions and *tris*-buffer (*p*H 7·8) in the proportions given in the original technique. The control reactions for other ATPases and non-specific alkaline phosphatase were carried out, and the inhibition of Na–K-ATPase with ouabain (10^{-5} M) was also performed. The percentage of cells containing Na–K-ATPase was measured using the 'score' method (range 0–4), as in the investigations of alkaline phosphatase activity in blood cells[7].

Ten healthy adults, five cases of acute myeloblastic leukaemia, three cases of chronic granulocytic leukaemia and four of chronic lymphocytic leukaemia were investigated.

The results are summarized in Tables 1 and 2, where the average values of Na–K-ATPase and ATPase activity are given.

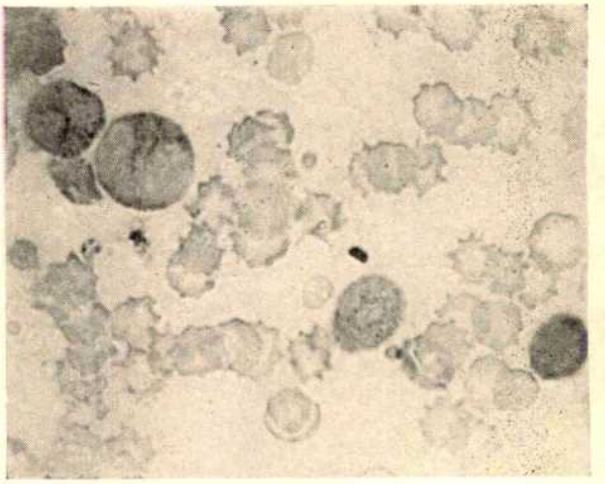


Fig. 1. Na–K-ATPase activity in normal granulocytes. Bone marrow. (×750)

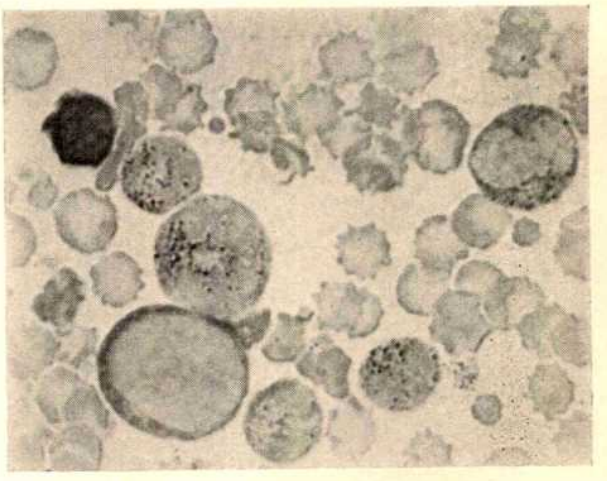


Fig. 2. Chronic myelocytic leukaemia. Bone marrow. Increased Na–K-ATPase activity in granulocytes. (×750)

Table 1. Na–K-ATPase and ATPase Activity in Bone Marrow and Blood Cells

	Normal cells	
	Na–K-ATPase	ATPase
Erythroblasts	–	–
Myeloblasts	±	–
Promyelocytes	+	–
Myelocytes	+	+
Metamyelocytes	+ +	+
Young granulocytes	+	+
Mature granulocytes	34·6% cells 42·1 score	12·8% cells 18·3 score
Lymphocytes	–	–
Reticulum cells	+	+
Blood cells:		
Granulocytes	26·4% cells 31·2 score	8·6 % cells 10·2 score
Lymphocytes	–	–

Table 2. Na–K-ATPase and ATPase Activity in Blood Granulocytes in Normal Adults and in Leukaemic Cases

	Na–K-ATPase	ATPase
Normal adults	26·4% cells, 31·2 score	8·6% cells, 10·2 score
Acute myeloblastic leukaemia	94·1% cells, 144·6 score	12·6% cells, 14·7 score
Chronic myelocytic leukaemia	63·6% cells, 98·7 score	14·1% cells, 18·2 score
Chronic lymphocytic leukaemia	38·2% cells, 49·3 score	10·6% cells, 12·6 score

Although these investigations were carried out on a small sample, the results show the differences between the activity of Na–K-ATPase and ATPase in granulocytes. The marked increase in Na–K-ATPase activity in leukaemic granulocytes (Fig. 2) is shown by comparison with the activity of this enzyme in normal granulocytes (Fig. 1). These results are similar to those obtained with the use of biochemical methods by Bonting and Block[5], but no Na–K-ATPase activity was demonstrated by histochemical methods in lymphocytes and no marked increase in activity was established in chronic lymphocytic leukaemia. The histochemical demonstration of Na–K-ATPase activity in blood cells seems to be a valuable complement to the biochemical technique, since it makes possible the cytological localization of enzyme activity.

The role of this increased activity of Na–K-ATPase in leukaemic cells in the pathogenesis of leukaemia is still not clear, but it indicates that the active transport of cations is disturbed in leukaemic cells.

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PATHOLOGY

Experimentally produced Cerebral Status Spongiosus and Continuous Pseudorhythmic Electroencephalographic Discharges with a Membrane-ATPase Inhibitor in the Rat

In an experimental investigation of sub-acute spongiform encephalopathy[1], which, according to some authors[2,3], is a variety of Jakob-Creutzfeld disease, ouabain was intracranially injected into the rat.

The possibility was considered that the disease was due to some defect of membrane-enzymes, resulting in disturbances of water and electrolyte metabolism and possibly in status spongiosus. It is believed that in the cerebral grey matter there is an extremely limited extracellular space, and that glial cells are the pathway by which fluid, electrolytes and colloids are transported to and from neurones. Phosphatase activity has been implicated in the regulation of membrane permeability; ouabain is a powerful inhibitor of membrane-ATPase activity[4].

In rats injected intracranially with ouabain (0·3–0·6 mg in 0·25 m.c.c. of isotonic saline solution), a stereotyped response was observed. Shortly after recovery from ether anaesthesia, the rats showed striking motor activity; they ran swiftly through the laboratory, apparently unaware of obstacles, circling and jumping. Bursts of motor activity alternated with periods in which the rats lay quietly. This lasted for about half an hour and then generalized convulsions appeared; these became very frequent until one fit followed another with short intervals. After 2–3 h the convulsions became rarer; at this stage the rats lay in a coma but with their limbs almost continuously jerking. The survival time in this condition was variable; some rats died 3–4 h after the injection, others were still alive after 2–3 days and were killed with ether. For electroencephalographic recording two electrodes were implanted in the parietal bones before the intracranial injection; a third electrode was inserted in the nose to record rhinencephalic activity. The electroencephalogram was normal until the convulsions appeared; then there were multiple spikes in rapid rhythm alternating with high-voltage waves and, rarely, with wave-and-spike pattern. In the interval between the attacks slow waves of different type and pattern were recorded.

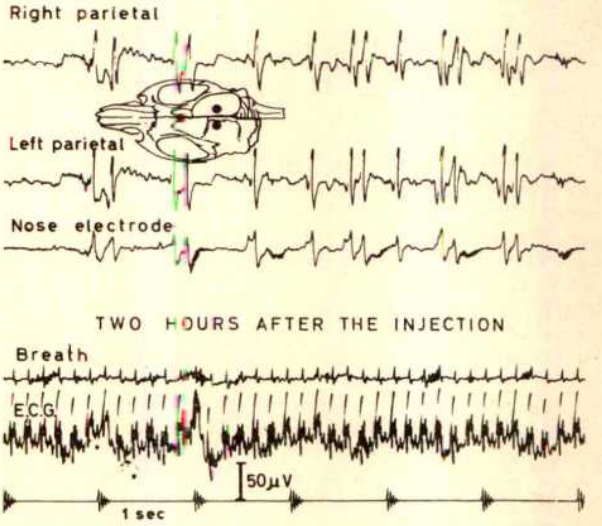


Fig. 1. Electroencephalogram 2 h after the injection. For interpretation see text

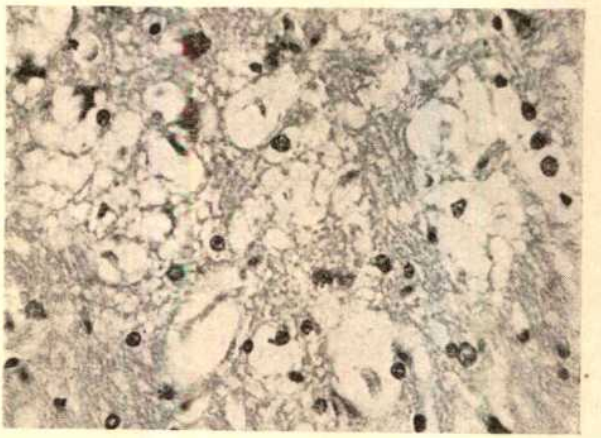


Fig. 2. Status spongiosus of the thalamus (65-h survival). (Haematoxylin and eosin, × *c.* 200)

When the convulsions had ceased and the rats lay motionless in a coma (the limbs were tied) the electroencephalogram showed a highly characteristic pattern constituted by long runs of regular, bilaterally synchronous high-voltage bi- or tri-phasic complexes at 50–70 per min. High-voltage slow waves were sometimes superimposed on this pattern.

Histological examination of the brain was carried out in two rats which survived for 55 h and 65 h after the injection. The most striking abnormality was severe cell loss and vacuolation of the 'ground substance' in the cerebral cortex and thalamus, amounting to true status spongiosus in some places.

We believe that this experiment lends support to the hypothesis that spongiform encephalopathy is due to some defect of membrane-enzymes. We should like to emphasize the similarity of the electroencephalogram findings, which are considered pathognomonic of the disease.

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Enhancement of Transplantability of 3,4,9,10-Dibenzpyrene-induced Fibrosarcoma in Random-bred Mice

Three polycyclic hydrocarbons—3-methylcholanthrene (3-MCA) (refs. 1–3), dibenz (*a*, *h*) anthracene (DBA) (ref. 4) and dibenzo (*a*, *i*) pyrene (3,4,9,10-dibenzpyrene; DBP) (ref. 5)—have so far been shown to induce tumours antigenic to their isogeneic and/or their autologous host. The immunity produced is weak and can usually be overcome by a sufficiently large inoculum of tumour cells. Thus, tumours induced in inbred strains with these carcinogens take successfully on implantation into isogeneic mice, but give negative results in the allogeneic or random-bred strains[6–9]. Our investigations on the transplantability of the DBP-induced tumour into isogeneic and allogeneic mice agree with these findings. DBP-induced fibrosarcomata originally induced in strain *A*/Heston mice or *BALB*/*c* mice and transplanted into isogeneic hosts have been successfully transplanted, whereas the random-bred *CRCD*♀ mouse was resistant to allografts of the induced tumour and we concluded that growth failure was due to an allograft response.

To determine under what conditions allografts of DBP-induced fibrosarcoma would grow successfully in the random-bred mouse, Caesarean-derived *ICR*/*Ha* random-bred albino female weanlings (*CRCD*♀) obtained from the Charles River Mouse Farm, Wilmington, Massachusetts, were given a single subcutaneous injection of DBP (0·5 mg/mouse in 0·2 ml. tricaprylin) at the nape of the neck. When the induced subcutaneous tumours measured 1·0–2·0 cm in diameter they were excised aseptically; fragments were implanted by trochar subcutaneously into random-bred mice. The mice were housed in plastic or 'Fiberglas' cages, ten mice per cage, with a standard laboratory diet and tap water available *ad libitum*.

In our first series of experiments, 324 random-bred *CRCD*♀ females weighing 18–22 g were challenged subcutaneously with a 2 × 2 mm fragment of a primary DBP-induced fibrosarcoma. The pooled results (Fig. 1, Series I) revealed that the tumour graft had regressed in 310 mice and that 14 (4 per cent) of the mice showed progressive tumour growth lethal to the recipients. Forty-six mice in which the first transplant had regressed were later rechallenged with a second primary tumour fragment and only one mouse had a progressively growing tumour. The second grafts regressed more rapidly than the first grafts.

In a second series of experiments, when the DBP-induced fibrosarcoma had reached a diameter of 1·0–2·0 cm, thirty-two mice were auto-transplanted with their own surgically excised tumour. In each case a residuum of primary tumour was present. The results showed that the autograft had taken successfully in fifteen mice (43 per cent), demonstrating enhanced acceptance. To determine whether the host resistance could have been depressed by the presence of primary tumour, allografts of DBP-induced fibrosarcoma were transplanted into fifty random-bred mice bearing primary DBP-induced tumours, and thirty-six (72 per cent) of the allografts grew (Fig. 1, Series II). On the basis of these results, it is probable that acceptance of tumour autografts and allografts in random-bred mice bearing dibenzpyrene-induced sarcomata was due to a depression of the host response.

During the course of our experiments, Matsuyama and Nakamura[10] reported that a urethane-induced fibrosarcoma grew better in homologous mice bearing methylcholanthrene-induced tumours, whereas the tumours regressed completely in the normal homologous hosts, and concluded that the resistance was reduced in the mice bearing methylcholanthrene-induced sarcomata.

Previous investigations[3,11,12] have shown enhanced acceptance of chemically induced neoplasms in the second and subsequent transplant generations. In our third series of experiments, twenty-five mice were allografted with the primary tumour and twenty-five mice with a second generation transplant from mice bearing a primary and first transplant generation. Our results (Fig. 1, Series III) showed the typical allograft response to the primary tumour, whereas the second generation allograft grew in eighteen out of twenty-five mice (72 per cent) and was evidence that the antigenicity of the tumour had been lowered after the first transplant generation.

Thus, the random-bred mouse with its heterozygosity of genetic factors behaves similarly to homologous inbred strains with respect to selective pressures which enhance the acceptance of tumour allografts.

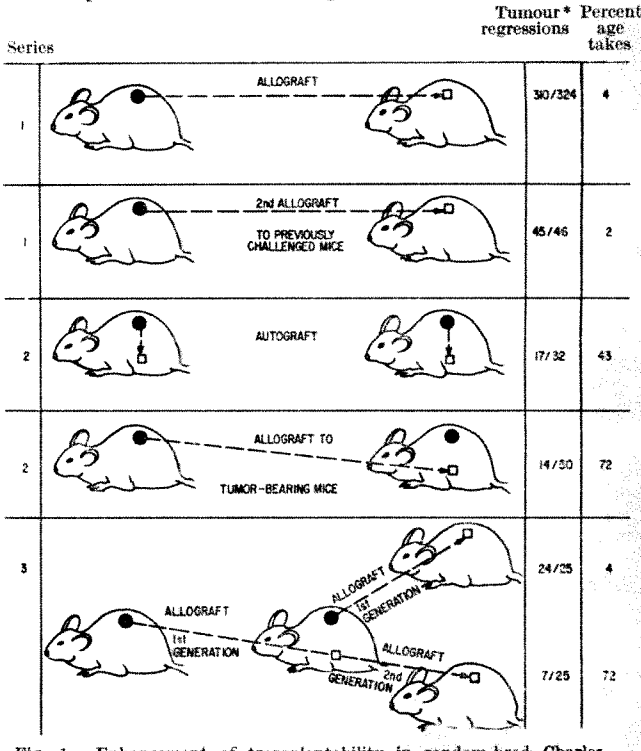


Fig. 1. Enhancement of transplantability in random-bred Charles River *CD*♀ mice. ●, Primary tumour; □, transplanted tumour; *, No. of regressions/No. of mice inoculated

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Differential Heat Sensitivity of Cells in Tissue Culture

IN the search for a specific fibroblast inhibitor that would not affect epithelial cells, a small but definite difference in heat sensitivity between these two cell types was noted.

Human fibroblasts were obtained from a skin biopsy and epithelial cells from carcinoma lines *C*-4 and *C*-27 [1]. Cells of either type were grown as pure cultures as well as mixed, in Leighton tubes with 11 × 55 mm windows. They were fed Waymouth's medium *MB* 752/1, supplemented with 10 per cent foetal calf serum.

The effects of conducted heat and of radiant heat were examined. Conducted heat was supplied by total immersion of culture tubes in water at temperatures ranging from 44° to 48° C. The source of radiant heat was four 3,200° K, 500-W studio flood lamps, separated from the cultures by a Corning infra-red filter No. *CS* 7-56. Inverted culture tubes were placed at a distance of 28 cm from the heat source. After heating by either method, cultures were cooled immediately, given fresh medium and observed for damage over several days. Experiments were performed on series of identical cultures, divided into groups of three tubes.

In one series of experiments, the proportion of surviving cells was determined by vital staining with neutral red and

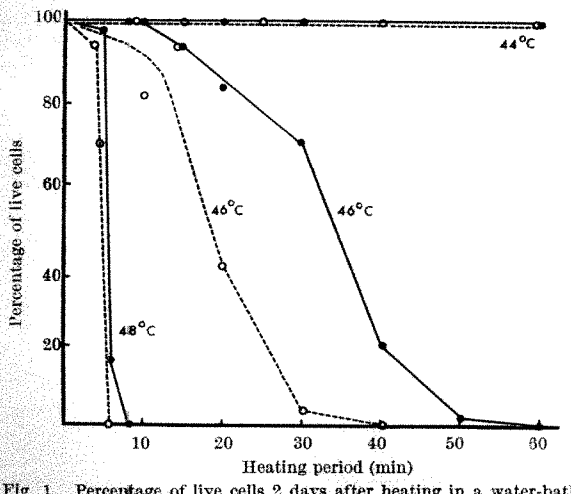


Fig. 1. Percentage of live cells 2 days after heating in a water-bath. ----, Fibroblasts; ——, epithelial cells

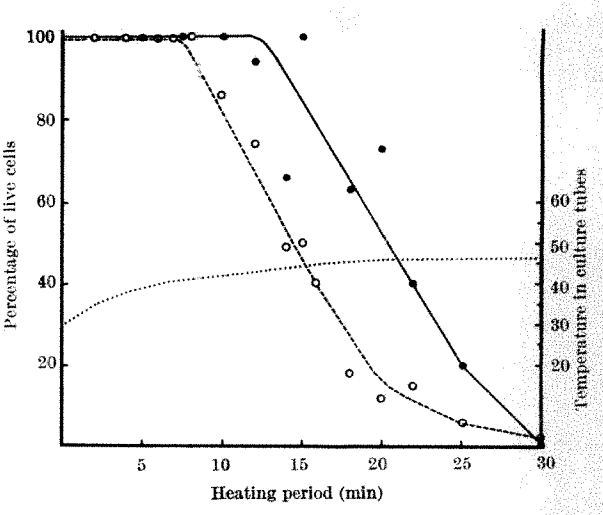


Fig. 2. Percentage of live cells 2 days after infra-red irradiation. ----, Fibroblasts; ——, epithelial cells. .....,, Temperature changes inside culture tubes, measured separately

counterstaining with trypan blue. There was little or no cell damage right after heating, but rather it reached its maximum after 2–3 days; then it often became obscured by new cell growth. Figs. 1 and 2 show the mean percentage of live cells 2 days after heating: fibroblasts were more sensitive to conducted heat as well as to infra-red rays. The figures are based on 109 and 180 observations respectively. While there was a definite differential effect of conducted heat at 46° C, both cell types responded similarly to 44° C and 48° C. In contrast to conducted heat, infra-red radiation killed 50 per cent of fibroblasts in 14 min, when the temperature in the culture tubes was only 44° C. 50 per cent of epithelial cells were killed by 20 min of irradiation, during which time the temperature had reached 46° C.

In an additional series of experiments, cultures were heated in a waterbath at 46° C for varying periods of time, and then cell protein per culture was determined at intervals on groups of tubes by the method of Oyama and Eagle[2]. As with results by vital staining, there was no difference between heated and control cultures on the day of heating; however, as shown in Table 1, 20 min at 46° C prevented any further increase in fibroblasts for the duration of the experiment, while epithelial cells resumed growth after a brief lag period. 46° C for 30 min prevented further growth in all cultures, and was followed by a gradual loss of protein due to the release of dead or injured cells from the glass. If the values in Fig. 1 are compared with Table 1, it becomes evident that many cells contributing to the protein value on day 2 were severely damaged or dead, and therefore further changes in protein content were probably the result of simultaneous dying and multiplication of cells with varying degrees of injury.

Figs. 3, 4 and 5 are examples of mixed cultures 2 days after exposure to infra-red irradiation for 0, 14 and 25 min respectively. The differential effect is illustrated in Fig. 4, with almost total loss of fibroblasts but no noticeable damage to epithelial cells.

These observations suggest that fibroblasts in culture are more heat-sensitive than epithelial cells, that cell

Table 1. EFFECT OF HEATING AT 46° C ON FIBROBLASTS AND EPITHELIAL CELLS IN CULTURE

Days after heating	Fibroblasts Heating period (min) 0	10	20	30	Epithelial cells Heating period (min) 0	10	20	30
0	100	100	100	100	100	100	100	100
2	145	160	111	99	182	163	101	72
4	240	200	100	85	314	244	155	70
7	262	216	103	35	423	320	178	50
11	394	287	94	17	588	607	317	51

Means of cell protein determinations on triplicate cultures, expressed as percentages of values on day 0.

death by this method is mainly delayed rather than immediate, and that radiant heat kills fibroblasts at lower environmental temperatures than does conducted heat. Exposure to 46° C for 20 min is being considered as a means to reduce fibroblast contamination in primary epithelial cultures.

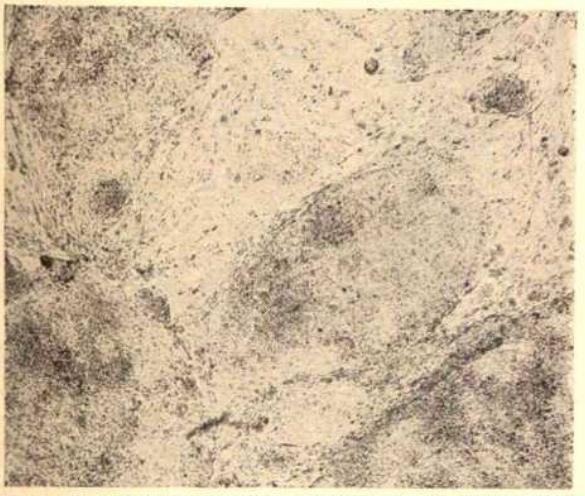


Fig. 3. Control to Figs. 4 and 5. Mixed culture. Neutral red. (×88)

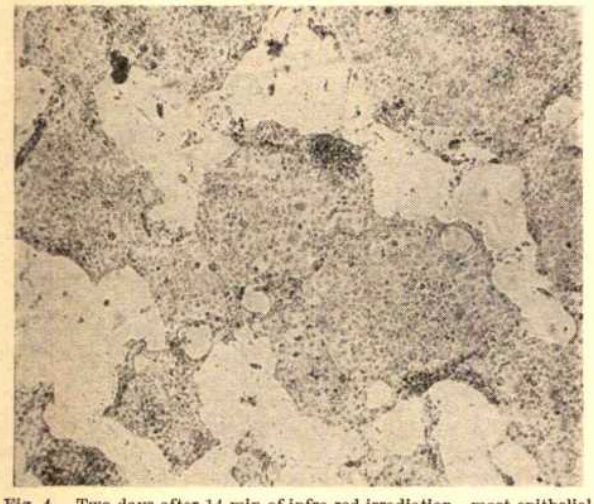


Fig. 4. Two days after 14 min of infra-red irradiation—most epithelial cells survived, but only few fibroblasts remain. Neutral red. (×88)

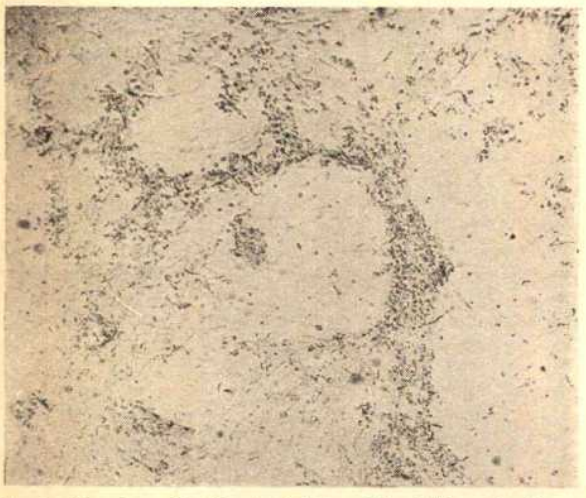


Fig. 5. Two days after 25 min of infra-red irradiation—all cells are dead. Neutral red. (×88)

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IMMUNOLOGY

Immunological Tolerance as the Result of Terminal Differentiation of Immunologically Competent Cells

The nature of immunological tolerance, the unresponsiveness evoked by the administration of large doses of an antigen to newborns[1,2], is usually explained on the basis of the views of individual authors on the mechanism of antibody formation[3–6]. The solution of the problem has been transferred to the cellular level. Burnet[7], on the basis of the clonal selection theory, explained acquired tolerance as allergic damage and elimination of the predetermined cell after contact with the corresponding antigen. Other authors[8] assumed formation of the tolerant cell at various stages of its differentiation. Medawar[9] and Lengerová[10] suggested that the antigen might prevent normal maturation of reactive cells at the stage of the stem cell and that this induces the state of the immunological tolerance (heterogeneous model). On the other hand, some authors[11], on the basis of experimental data, assume that the onset of tolerance is possible at all stages of cell differentiation when antigen is present in excess (homogeneous model).

This communication presents experimental data supporting our former view[12] "that each specific immune inhibition develops on the basis of already existing or developing specific functions". This aspect was based on earlier experimental results[13]: new-born rabbits shortly after birth were injected with an excessive dose of antigen S. para *B* which is quickly deposited in tissues from blood circulation. The excessive dose accelerated and increased the antibody response in the new-born. After 4 months, these rabbits were injected with the same antigen and a significant inhibition of antibody formation was found in comparison with control animals or with animals injected shortly after birth with a smaller amount of the antigen. Thus the dynamics of the onset of immunological unresponsiveness found in new-borns was of the same character as the development of the immune inhibition in adults which followed the active immune response only[14–16]. It is usually impossible to find antibodies after the injection of large amounts of antigen to new-borns, especially when it persists in the circulation. Therefore all theories of immunological tolerance agree that the excessive amount of an antigen induces tolerance directly, without a previous immune response.

In the experiments presented here, antibody production was detected in individual cells by the plaque technique. The results indicate that a state of unresponsiveness follows antibody formation after large doses of an antigen.

New-born rabbits reared conventionally and colostrum-free piglets reared under sterile conditions were injected with increasing doses of sheep erythrocytes. The number of antibody-forming cells was detected by the plaque technique[17] in agarose[18,19]. Plaques were developed by addition of complement only (detecting 19 *S* antibody-forming cells) or by addition of anti-γG serum against rabbit or pig γG (detecting 7 *S* antibody-forming cells), diluted 1 : 50. The anti-γG serum was added either after the incubation of antibody-forming cells 1 h before the addition of complement, or mixed directly with the

agarose, erythrocytes and cells. This procedure increases the sensitivity of detection of 7 *S* haemolytic antibodies[20] and the number of antibody-forming cells during the secondary reaction[21].

After the primary injection of the antigen, the number of antibody-forming cells in the new-born increased simultaneously with the increased amount of antigen (Tables 1 and 2). Doses of antigen inducing tolerance immediately after the primary injection (that is, lowering the number of antibody-forming cells) have not been determined. The number of cells detected after the injection of the large dose of antigen into the new-born, an average of 100 antibody-forming cells per 10^8 lymphatic cells[22], may represent the total number of immunologically competent cells present in the organism, which are differentiated into producing cells, assuming that the large quantities of antigen inhibit the proliferation of the immunologically competent cells and their descendants.

Groups of experimental animals injected with various doses of sheep erythrocytes were revaccinated after 3 weeks with different doses of the same antigen (Tables 1 and 2). The number of cells producing antibodies after the secondary stimulus was significantly lower in the animals stimulated with maximum doses of the antigen during the primary reaction, that is in those animals that produced maximum of antibody-forming cells and highest titres of antibodies during the primary reaction. Inhibition does not result from accumulation of the persisting antigen with the new dose because the dose used for the secondary stimulation is below the level of the amount which stimulates antibody formation (Table 2). However, one large dose of antigen as the primary stimulus does not evoke complete tolerance. For the development of complete tolerance, especially for the inhibition of the formation of 7*S* type antibodies, repeated doses of antigen seem to be necessary.

On the basis of the results obtained we assume that acquired immunological tolerance may be considered as the result of the process of terminal exhaustive differentiation. In the excess of antigen almost all immunologically competent cells and their activated descendants are differentiated directly (without proliferation) into short-lived antibody-producing cells (about one per 10^6 cells). After their death, only a small number of cells capable of responding to specific antigen are available (state of tolerance). In contrast, small doses of antigen activate immunologically competent cells, but not all such activated cells are directly transformed to producing cells. Most of the activated cells proliferate, increase in number and form the basis for the secondary reaction when the second dose of antigen is injected. For establishment of tolerance in this situation (that is, for the exhaustion of all activated and proliferated cells), the quantity of an antigen administered must be related to the increased number of cells. This assumption may explain spontaneous escape of tolerance, induction of tolerance in adult individuals and even in individuals primed to give a secondary response. In this hypothesis, the inhibition of immune reaction (tolerance) as well as the preparation for secondary reaction is only the expression of various processes in the differentiation of immunologically competent cells.

Table 1. Antibody Formation in Young Rabbits immunized after Birth with Different Doses of Sheep Red Blood Cells (Srbc) and revaccinated 3 Weeks after the Primary Immunization

Dose of Srbc in primary immunization	Average number of plaque-forming cells per 10^8 lymphoid cells: 8 days after primary stimulus, Developed by complement	5 days after secondary stimulus with 2×10^8 Srbc, Complement only	Anti-γG + complement
0	0	2,585	732
1 ml. 0·01% Srbc (2×10^6 cells)	0	2,735	532
1 ml. 0·1% Srbc (2×10^7 cells)	3	14,070	102,433
1 ml. 1% Srbc (2×10^8 cells)	9	1,788	7,810
1 ml. 10% Srbc (2×10^9 cells)	35	978	1,445
1 ml. conc. Srbc (2×10^{10} cells)	46	66	166

Table 2. Antibody Formation in Sterile Piglets immunized after Birth with Different Doses of Sheep Red Blood Cells (Srbc) and revaccinated 3 Weeks after the Primary Immunization

Dose of Srbc in primary immunization	Average number of plaque-forming cells per 10^8 lymphoid cells: 10 days after primary stimulus, Developed by complement	5 days after secondary stimulus with 2×10^8 Srbc, Complement only	Anti-γG + complement
0	0	0	0
10 ml. 0·01% Srbc (2×10^7 cells)	0	0	0
10 ml. 0·1% Srbc (2×10^8 cells)	0	0	0
10 ml. 1% Srbc (2×10^9 cells)	22	2,640	21,600
10 ml. 10% Srbc (2×10^{10} cells)	89	1,180	1,630
10 ml. conc. Srbc (2×10^{11} cells)	389	0	800

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Immunization with Protein–Cellulose Co-polymer (Immunosorbent)

In order to prepare an immune serum with a high level of antibodies it is usually necessary to use many injections of soluble antigen. Antigens adsorbed on aluminium hydroxide or precipitated with alumina are frequently used for this purpose. Especially good results can be obtained using antigens mixed with different water–oil emulsions usually containing Bacille Calmette-Guérin (BCG)[1].

The very intensive production of antibodies which is obtained when such adjuvants are used is apparently conditioned both by the prolonged action of the antigen and by the non-specific effect of BCG, or by other components of the emulsion.

In our experiments we attempted to discover whether it was possible to induce the intensive biosynthesis of antibodies by introducing antigen into the body in another form, namely, in the form of a durable chemical co-polymer supported on an insoluble cellulose medium[2–6].

This method eliminated such factors of the normal adjuvants as the influence of organic or inorganic oil and microbial components. At the same time, however, the

effect of protracted presence of antigen in the body would still be manifested.

In the present work a protein–cellulose immunosorbent was prepared by coupling the protein antigen to the cellulose with the help of organic residues by the method described earlier. The co-polymer was prepared in the form of a suspension and was found to be convenient for the detection and isolation of antibodies[3–5]. It seemed advisable to use a similar suspension for the immunization, bearing in mind its extremely large surface and its comparatively high content of antigen.

Six Chinchilla-strain rabbits (males) were used in the immunization experiments. A γ-globulin fraction prepared from horse serum (HGG) by ethanol precipitation was used as antigen. Three were injected subcutaneously (on both sides) with a total of 25 ml. HGG–cellulose immunosorbent suspended in 0·9 per cent sodium chloride solution. These animals each received 212 mg sorbent (dry weight). This amount of immunosorbent contained nearly 25 mg of protein. At the same time, 100,000 units of penicillin and 200,000 units of streptomycin were injected into each rabbit. Three other rabbits were used as control. In all, they received 100 mg HGG in 10 ml. of 0·9 per cent sodium chloride solution injected as before.

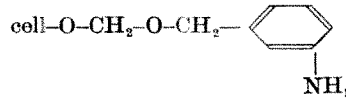
Several months after the first immunization all the rabbits were injected again, this time with soluble antigen (HGG) only. The controls were injected after 4 months, and those treated with immunosorbent were injected after 5 months and 10 days. Injections were made from behind, at four points, into the muscles of the shoulder and the hip. Each received 150 mg HGG in 0·9 per cent sodium chloride solution with antibiotics (30,000 units of penicillin and 30,000 units of streptomycin). During the course of the experiment, after the first immunization and also after the second, samples of blood were periodically taken (about 15 ml. each time). The absolute amount of antibodies in serum was determined by measuring the absolute increase in protein on the immunosorbent[3,4]. All the animals used in the present experiment remained in good health during the course of immunization.

Results obtained are summarized in Table 1.

We also found the characteristics of the curves which show the changes in the quantity of antibodies in the serum after the second immunization. As shown in Fig. 1, in which the corresponding data are given, the characteristics of these curves are, on the whole, typical of those for curves normally obtained during ordinary re-vaccination, that is, there is a maximum and then a fall in antibody level.

At the same time, it follows from Table 1 and Fig. 1 that rabbits immunized with protein–cellulose co-polymer (immunosorbent) produced very large amounts of antibodies. This happened in spite of the fact that the quantity of antigen used for first immunization was four times less (25 mg) than in controls (100 mg).

An attempt was also made to find whether the rabbits first immunized with immunosorbent would produce not only the antibody against protein, but also the antibody against the insoluble basis of immunosorbent. To examine this question, rabbits Nos. 176, 182 and 195 were inoculated subcutaneously with the suspension of the following non-diazotized ether of cellulose:



with antibiotics (12,500 units of penicillin and 15,000 units of streptomycin). Each animal was injected with 125 mg of cellulose ether. This procedure was carried out 7 months after the administration of protein–cellulose immunosorbent. Tests for antibodies were performed 1, 2 and 3 weeks after the injection of the suspension of cellulose ether. The results are given in Table 1, which shows that antibodies specific to the insoluble portion of sorbent were not detected in the sera of experimental animals.

The data obtained demonstrate that the animals produced similar amounts of antibodies in response to the first administration of protein antigen which was firmly conjugated with insoluble cellulose, as they did in response to the administration of soluble antigen.

Over the 3 months of observation, immunosorbent produced a more protracted output of antibodies than did soluble antigen. Apparently some pronounced immunological reorganization occurs under these conditions. This is shown by the fact that animals first immunized with immunosorbent produce much more antibody in response to the new immunization than if they are first immunized with soluble antigen only.

The possibility of obtaining antisera with high levels of antibodies (up to 10–15 mg/ml.) recommends this method of immunization for the preparation of hyperimmune sera.

The data presented here show the significance of prolonged antigen action. They also show the necessity of

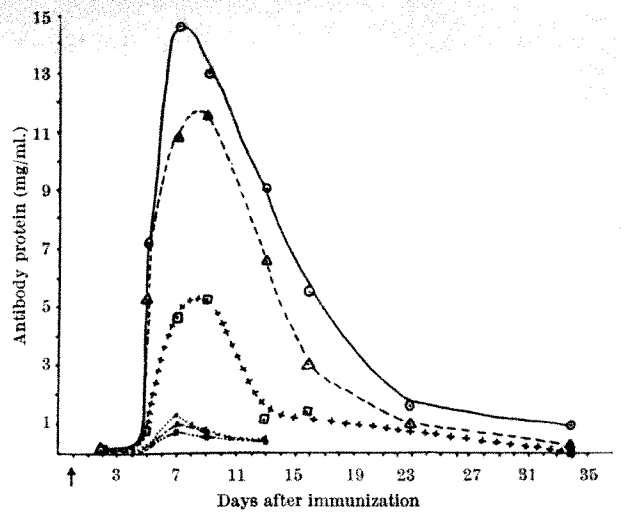


Fig. 1. Plot of antibody protein (mg/ml.) versus number of days after immunization for six rabbits. ○——○, 195; △ - - - △, 182; ⊡ + + ⊡, 176; ● ··· ●, 094; ▲ — · — ▲, 096; ··· – ■ – ···, 097

Table 1

No. of rabbits	Weight of rabbits at second immunization (kg)	First immunization			Second immunization		Maximal amount of anti-cellulose ether (mg/ml.) antibody protein
		Amount of soluble HGG (mg)	Amount of HGG fixed on the cellulose ether (mg)	Maximal amount of anti-HGG antibody protein (mg/ml.)	Amount of soluble HGG (mg)	Maximal amount of anti-HGG antibody protein (mg/ml.)	
094	3·2	100	—	0·55	150	1·34	—
096	2·9	100	—	0·33	150	1·00	—
097	3·0	100	—	0·43	150	0·79	—
176	2·8	—	25	0·67	150	5·36	0
182	3·4	—	25	1·10	150	11·52	0
195	3·7	—	25	0·77	150	14·56	0

carrying out further experiments to find the most effective dose and periods for immunization with this product and to investigate the mechanism of its action. It is very likely that, in the future, it will be possible to prepare sera with still higher levels of antibodies by means of such immunosorbents.

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BIOLOGY

Test-plants for Investigations of the Physiology of Fruiting in *Vitis vinifera* L.

Experimental work on the physiology of fruiting in woody plants is often made difficult by the large size of most mature fruit trees and vines. Many potentially useful techniques are denied the research worker, for example, growth of plants under closely controlled environmental conditions. The annual occurrence of the crop raises another difficulty because treatments must be applied and data recorded within the space of a few days or weeks, and the information obtained must suffice until the following year, as repetition of experiments in the same year is impossible.

A way of overcoming some of the difficulties inherent in research with woody plants would be to use small test-plants, that is, fruiting plants with essentially the same characteristics as field vines or fruit trees, but which are more amenable to experimentation. In this way, understanding of the factors controlling fruit set and development may be facilitated, and information from test-plants may ultimately provide the basis for less empirical field experiments.

In the orange and the grape vine (*Vitis vinifera* L.), progress has been made in producing fruiting test-plants. Using softwood cuttings, Gates, Bouma and Groenewegen[1] have propagated small orange trees which bear mature fruits within 15 months of striking the cutting. In the sultana vine (syn. Thompson's 'Seedless'), two methods of producing small fruiting plants have been developed. The first is by aerial layering of the present year's shoots[2], and the second by taking softwood cuttings from the present year's shoots after flower initiation in the primary buds[3]. These techniques overcome the problem of size of the experimental unit, but involve much labour and the maintenance of very large numbers of container-grown plants. In this laboratory, techniques have been developed which greatly simplify the production of small fruiting vines. Test-plants are raised in large numbers throughout the year, and used in physiological studies of fruiting. These plants are produced from single node one-year-old hardwood cuttings.

Hitherto, dormant one-year-old vine cuttings were unsuitable as a source of test-plants because inflorescence growth often ceases soon after bud burst. The rachis fails to elongate, and further differentiation of individual flowers is attenuated. Inflorescences remain attached to the plant for a few weeks, but later shrivel and drop off. Hence, in cuttings of most commercial varieties of *Vitis vinifera*, the potential of fertile buds to produce a bunch of grapes is rarely realized.

Experiments were carried out to detect the cause of failure of inflorescence growth in vine cuttings. Growth analysis showed little increase in the total dry weight of cuttings (that is, dry weight of old stem, new stem, leaves and roots) during the first month after bud burst. Initially, it appears that growth and respiration are supported to a large extent by reserves of the parent stem. Hence, leaves contribute little to growth, and may be considered as importers rather than as net suppliers of photosynthate. In cuttings, therefore, failure of the inflorescence to grow may be related to its inability to compete with leaves and other organs for available food supplies. This theory is supported by autoradiographic studies on field vines by Hale and Weaver[4], who showed that the inflorescence has limited ability to attract photosynthate until after the stage of fruit set. These observations suggest that surgical removal of competing organs might allow inflorescence growth to proceed unhindered.

Leaf removal proved the most successful treatment, for when leaves basal to the lowermost inflorescence, together with the leaf adjacent, were removed as soon as they appeared, growth and differentiation of that inflorescence took place. Plants were pruned to a single inflorescence for best results. After removal of the lower leaves, leaves produced distal to the inflorescence were allowed to grow out normally. Using the leaf dissection technique it was found possible to produce bunches, containing up to 100 grapes, from single node cuttings within 12 weeks from planting (Fig. 1).

Details of the technique are as follows. Dormant one-year-old vine shoots are stored under refrigeration (4° C) in sealed plastic containers. In collecting parent shoots care is taken to select for uniformity of diameter and internode length. Furthermore, shoots comprise six nodes, that is, buds number 4–10 inclusive from the point of insertion of the shoot on the vine. In this way, cuttings can be produced from buds of known position on the shoot should this be so desired. Shoots of the required variety are removed from cold-storage, and cut into single nodes. Each single node cutting is weighed and planted in a numbered 3 in. × 1 in. plastic propagating tube. At an arbitrary stage of bud burst, cuttings of similar original fresh weight, and at a similar stage of growth, are selected into groups. Dissection of leaves is carried out as soon as the first basal leaves appear beyond the brown bud scales, and continued as leaves appear until the required number have been removed. Within each group of treated plants further selection is made on the basis of inflorescence size (number of flowers), leaf number distal to the inflorescence, and shoot length.

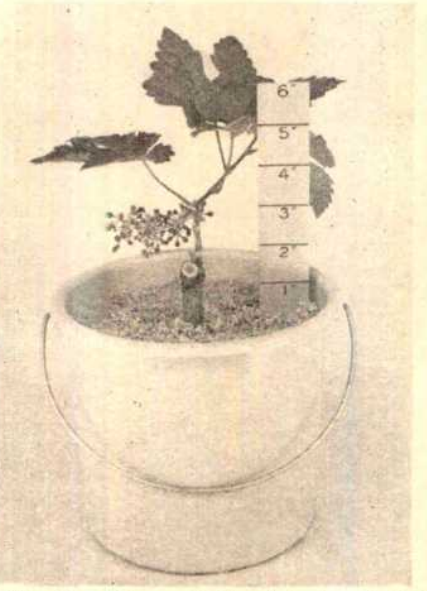


Fig. 1. Fruiting vine cutting var. 'Carbernet Sauvignon'. Photographed 12 weeks from bud burst. Leaves basal and adjacent to the inflorescence were removed as they appeared. Leaves distal to the inflorescence were allowed to grow out normally

After this final selection, plants destined for an experiment are transferred to 6-in. pots containing vermiculite, perlite or other suitable medium. Nutrients are provided by frequent application of half-strength standard Hoagland solution. In practice, more than 60 per cent of treated plants are discarded as a result of selection for uniformity. In this laboratory, 600 cuttings are propagated each month to provide continuity of supply of experimental material.

It might be argued that the test-plants described here are so unlike grape vines that their behaviour gives little indication of circumstances in the field. Experience so far does not support this criticism. Weaver *et al.*[5,6] have shown that the kinin '*SD* 8339', 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H purine, has marked effects in increasing fruit set and size in field vines, including the 'Black Corinth' (syn. 'Xante Currant'). Using test-plants of this variety, parallel effects of '*SD* 8339' on fruiting have been observed. These results suggest that test-plants may be of particular value in the initial evaluation of effects of new growth substances on fruiting in vines.

I thank Dr. J. VanOverbeek for supplies of '*SD* 8339'.

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Field Resistance in *Lolium* sp. to Leaf Rust (*Puccinia coronata*)

A high degree of 'field' resistance to infection by leaf rust (*Puccinia coronata* var. *lolii*) has been developed in perennial ryegrass (*Lolium perenne*), an open pollinated plant, by selecting individual plants for resistance (as one of several agronomic characters) from the progenies of open pollinated strains. The strains, selected by Corkill *et al.*, were tested for combining ability and other agronomic characters. Several were allowed to inter-pollinate to form the synthetic variety released as New Zealand perennial ryegrass[1].

A high degree of heterozygosity has been maintained in this variety. Although leaf rust occurs on a few individual plants it can rarely be transferred to neighbouring plants even though these may be infected; this indicates that physiological races of the pathogen are present. Neither the characteristics of the physiological races nor the genetic nature of the resistance against the pathogen are at present known. Resistance would appear to be due to an accumulation of genes within the variety but, because of the considerable and intentionally high degree of heterozygosity of individual plants, it seems probable that neighbouring plants are of different genotypes, and therefore owe their resistance to leaf rust to various combinations of a multiplicity of different genes.

This resistance to many physiological races of a pathogen, termed 'horizontal resistance' by van der Plank[2], though treated as a minor character by the plant breeders, was developed by breeding for 20 years a crop that previously had no noticeable resistance to the disease. A significant factor would appear to be that the breeding nursery was established in a locality which experiences severe natural infection of *P. coronata* var. *lolii* every summer.

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Effect of Plant Regulators on the Incorporation of Phosphorus-32 into the Alcohol-soluble Fractions of Pea Seedlings

This communication reports experiments which we have carried out to examine and to compare some of the relations of the quantitative changes that ensue in the alcohol-soluble phosphorus fractions of pea seedlings, which have been induced by the effect of uncoupling and growth-regulating substances. In the course of the experiments, the changes in pea seedlings, which were treated with uncoupling and growth-regulating compounds *in vivo*, were recorded by labelling the phosphorylated products with ^{32}P-phosphate and paper chromatography.

If the uncoupling effect ensues, one may expect that the inorganic or the ester phosphate fractions accumulate and that the quantity of the nucleotides containing energy-rich phosphate decreases. Therefore, the changes in the following phosphorus fractions were analysed by measuring their phosphorus-32 activity: (1) inorganic phosphate; (2) ester phosphate; (3) phosphate coupled to an indole skeleton; (4) nucleotid phosphate. The ester fraction contains hexose and pentose phosphates, glyceric acid phosphate, adenosine monophosphate and a few unknown compounds with low phosphorus-32 activity. The indole phosphate fraction is an unknown compound which reacts in a similar manner to indole, sugar and phosphate, and if labelled with phosphorus-32 it becomes radioactive. The nucleotide phosphate fraction contains compounds which react in a similar manner to purine, sugar and phosphate, and their R_F-values are identical with those of the nucleotide polyphosphates.

Before the experiments were carried out, the plants were placed for 2 h in Knop's solution, diluted ten-fold and then (during the experiment) transferred into a solution containing ^{32}P-labelled potassium dihydrogen phosphate and the regulator. The controls were initially placed in a similar Knop's solution. The experimental plants were kept in the dark at 22° C for 24 h, and then the various phosphorus fractions were analysed by means of paper chromatography. Simultaneously, measurements were taken to determine the growth of the roots and shoots, and the c.p.m. and distribution of radioactivity established as a percentage of the total activity of the single phosphorus fractions.

The data in Table 1 reveal that the total activity is highest in the control plants, thus all three compounds applied decrease the amount of the soluble phosphorus compounds in the following order: DNP > SA > IAA. Similarly, all the substances examined decrease the incorporation of the phosphorus-32 into the nucleotide fraction. The proportion of the total impulses is as follows: the quantity of inorganic phosphorus-32 decreases in the sequence of C > IAA > SA > DNP; in the ester fraction the sequence is approximately the opposite—SA > DNP > IAA > C (C, control; SA, salicylic acid; DNP, 2,4-dinitrophenol; IAA, indolyl-3-acetic acid). The participation of the nucleotide fraction in the total activity is parallel to that of the inorganic fraction. It is present in the highest percentage in the control plant and decreases in the order IAA > SA > DNP. The activity of the fraction of indole-phosphate-32 is the highest from the point of view of both its total and percentile activity in the plants treated with IAA: the percentile ratio is IAA > C > SA > DNP; however, the difference between the c.p.m. and the percentile values is not significant.

Among the results, the most characteristic is the decrease which ensues in the phosphorus-32 activity of the nucleotide fraction in plants treated with regulators. This effect is greatest if typical uncoupling substances are applied, but it may also be observed in the case of IAA. The percentile increase of the ester fraction activity and the simultaneous decrease of the activity of the nucleotide phosphate fraction are striking.

Table 1. DISTRIBUTION OF ALCOHOL-SOLUBLE ^{32}P-ACTIVITY FOR THE SINGLE FRACTION INCORPORATED IN PEA SEEDLINGS

Fraction	Control		Salicylic acid 10^{-4}			Indolyl-3-acetic acid 10^{-4} M			2,4-Dinitrophenol 10^{-4} M		
	C.p m./ fraction	% Total impulses	C.p.m./ fraction	% Total impulses	% Control	C.p.m./ fraction	% Total impulses	% Control	C p.m / fraction	% Total impulses	% Control
Inorg.-P	30,582	57·82	12,442	42·84	40·6	15,615	45·84	51·0	10,357	47·23	33·8
Ester-P	18,660	35·27	16,092	55·41	86·2	17,072	50·12	90·49	11,176	50·97	55·1
Indole-P	991	1·87	340	1·17	34·3	1,153	3·37	116·34	273	1·25	27·5
Nucleotide-P	2,664	5·04	167	0 58	6 27	228	0·67	8·56	121	0 55	4·5
Total	52,897	100 00	29,041	100·00		34,068	100 00		21,927	100 00	

Conflicting views appear in the literature concerning the uncoupling effect of the growth regulators from the point of view of their function in growth. Various authors have established that there is a close connexion between growth and phosphorus metabolism. Some authors interpret only the inhibitory effect; others[1–3], on the basis of the uncoupling of the oxidative phosphorylation, also the stimulating effect. According to the various opinions, the regulators exert their effect on the phosphorus metabolism directly[3–5], or indirectly[6,7]. Our experiments support the view that the IAA and the SA act directly on phosphorylation. This view is confirmed by the following: (1) the decrease of the nucleotide phosphate present and the simultaneous increase of the accumulation of the ester phosphate; (2) the similarity of the effect of DNP, SA and IAA; (3) the fact that it was possible to identify phosphorylated glycosides of indole compounds and phenolic acids in different plant organs.

The correlation between the uncoupling and growth-regulating effect is, however, very complex, because growth promotion or inhibition may both be associated with the uncoupling effect. This means that the growth regulation activity influences numerous other physiological processes. Further difficulties arise through the interpretation of the specific character of the regulating substances on the basis of the uncoupling effect. Thus, it seems that we cannot consider the uncoupling of the oxidative phosphorylation as a fundamental and general reaction of growth regulation.

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Availability of Biologically Fixed Atmospheric Nitrogen-15 to Higher Plants

THE direct utilization by higher plants of nitrogen fixed by blue-green algae could play a substantial part in the Earth's nitrogen cycle. That certain species of blue-green algae can fix nitrogen when grown in solution culture is well established[1]. The maintenance of soil nitrogen supplies in rice paddy has been attributed to the growth and fixation of nitrogen by blue-green algae. Recently it was postulated that the blue-green algae may be important in the nitrogen economy of grazing lands[2–4].

The blue-green algae are known actively to excrete nitrogenous materials in solution culture[5]. However, the fate of nitrogenous products fixed by the blue-green algae and/or their associated organisms *in situ* has not been completely delineated. In rice culture it was suggested that algal-N became available to the rice plant following decomposition of algal tissue[6].

Surface soil encrustations of blue-green algae and associated organisms are found on semi-arid grassland soils[1]. Grass seedlings may be found rooted in the algal crusts where moisture and fertility conditions may be more favourable than on non-encrusted soils. Several grass seeds (*Artemesia* sp.) unexpectedly germinated in crusts under experimentation to assess the magnitude of nitrogen fixation. The use of atmospheric nitrogen-15 provided a means of tracing the products of nitrogen fixation and the possible ecological contribution of nitrogen to higher plants.

Native algal crusts were removed intact from the surface of desert-grassland soils. Excessive adhering soil was removed from the underside and 1 cm diam. cores were cut from the crust. Composite samples consisting of 60 random cores were placed in a desiccator containing 74·8 per cent nitrogen; enriched with 0·53 per cent atm. ^{15}N; 4·9 per cent carbon dioxide, 20 per cent oxygen and 0·3 per cent argon. The samples were moistened with de-ionized water by means of a cotton wick, and incubated in a plant growth chamber with 12 h days of 35° C with light intensity of 2,000 ft.-candles and 12 h nights at 18° C.

After incubation the seedlings were cut 1 cm above the algal crust and successively rinsed with 1 N HCl and de-ionized water. Nitrogen was determined by the Kjeldahl method using 1 : 3 : 1 sample : acid : salt ratio and a salt composition of K_2SO_4 : $CuSO_4$: Se in a 100 : 10 : 1 ratio on a weight basis. Digestion was continued for 6 h after clearing as recommended by Bremner[7]. Ammonia was recovered by alkaline distillation and prepared for isotopic nitrogen analysis by the hypobromite method[8]. We thank Dr. Arthur Edwards, Agronomy Department, Iowa State University, for the determination of ^{29}N/^{28}N ratios on the Consolidated Electrodynamics model 21–620 mass spectrometer.

Grass seeds (*Artemesia* sp.) germinated and grew for some time on an algal crust sample incubated for 160 days. At harvest the grass tops contained 1·22 mg nitrogen which contained 0·3733 per cent ^{15}N while the host crust nitrogen was enriched with 0·3813 per cent ^{15}N. Both enrichments were significantly greater than either the control crust sample (0·3648 per cent ^{15}N) or a commercial ammonium sulphate standard (0·3663 per cent ^{15}N).

The algal crust materials were lyophilized and ground with mortar and pestle to pass an 80-mesh sieve. Approximately 3·4 per cent of the total algal crust nitrogen was found to be water soluble, which included 1 per cent as water soluble ammonium-nitrogen. Both forms of soluble nitrogen contained significant isotopic enrichments of ^{15}N and may have been the form of labelled nitrogen taken up by the higher plant.

Thus, it appears that nitrogen taken up by the grass originated as atmospheric nitrogen, which was fixed by organisms in the algal crust and subsequently released in a form suitable for utilization by higher plants. Whether release of fixed nitrogen occurred via active excretion of nitrogenous products or followed decomposition of the biological nitrogen fixing organisms or both was not resolved. However, active nitrogen fixation was occurring in the algal crust at the 160 day harvest at the same rate as found on previous harvest dates (3, 6, 12, 24 and 52 days). The accumulation of fixed nitrogen was found[9] to fit a linear regression line with a correlation of $R = 0{\cdot}98$.

Soil nitrogen in grassland areas is depleted by removal by grazing animals and may be lost by soil erosion and denitrification processes. These losses gradually lead

to an impoverishment of soil nitrogen except where it may be replenished by biological fixation of atmospheric nitrogen or by artificial means. The evidence provided here indicated that the algal crust organisms fix atmospheric nitrogen and release it in forms available for uptake by higher plants growing in association with the crusts. The crust organisms appear to be an important source of nitrogen on semi-arid grassland.

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Suppression of Schistosomiasis in Snails by Chloramphenicol

Chloramphenicol is known to inhibit protein synthesis in bacteria and in proliferating mammalian cells[1,2]. The experiments described here were undertaken to determine the effect of chloramphenicol on the protein synthesis which must occur during certain stages of the schistosome cycle when there is rapid growth or proliferation of the parasite. Following the penetration of a snail by a miracidium of *S. mansoni*, marked proliferation of the parasite occurs, leading to the development of tens of thousands of cercariae[3]. Snails exposed to miracidia were maintained in water containing chloramphenicol in an effort to inhibit protein synthesis and thereby to suppress formation of cercariae.

Fifty-two laboratory-reared snails approximately 12 mm in diameter, descended from a Puerto Rican strain of *Australorbis glabratus*, were used in these experiments. The snails were exposed overnight in 3 ml. of dechlorinated water to 15–20 miracidia of a Puerto Rican strain of *Schistosoma mansoni*. The miracidia came from eggs obtained from the livers of mice infected for 7–8 weeks.

On the day following exposure, the snails were divided into one group of twenty-six snails and two groups of thirteen snails each. Each group was put into 4 l. of dechlorinated water in covered stainless steel pans. The room temperature was maintained at 23° C; the water was aerated and the snails were fed lettuce *ad libitum*. The larger group (twenty-six snails) was untreated and served as the control group. Thirteen snails were kept in water containing chloramphenicol in a concentration of 625 parts per million (p.p.m.); the other thirteen snails were kept in water containing chloramphenicol in a concentration of 312 p.p.m. The water was changed each week and fresh chloramphenicol was added to water containing the two experimental groups. The treatment groups did not receive the drug between the third and fourth weeks as the water alone was causing some signs of toxicity in the snails. Five weeks after exposure to the miracidia and four weeks after exposure to drug, the snails were all placed in fresh water.

At six weeks the snails in each of the three groups were each placed in a medicine glass containing 3 ml. of water to see if they were shedding cercariae. At this point two of the control snails were dead but six of the surviving twenty-four snails were shedding. There were no deaths among the treated snails and none was shedding cercariae.

The shedding of cercariae by the control and experimental groups is summarized in Fig. 1. At 7 weeks, twenty-two of the twenty-four control snails were shedding cercariae, but at 7, 8 and 9 weeks none of the snails exposed to chloramphenicol was putting out cercariae. At 10 weeks, twenty of twenty-two control snails were shedding cercariae, two more having died. There was still no shedding of cercariae in either of the treated groups at 10 and 11 weeks and one death occurred in the snails that had been maintained at 625 p.p.m. At 12 weeks, however, four of the twelve remaining snails previously maintained at 625 p.p.m. started shedding cercariae, as did eight of the thirteen snails kept at 312 p.p.m. At 13 and 14 weeks the number of snails shedding from the greater concentration of chloramphenicol rose to 8/10 and to 8/8 as two more snails died, and those in the lesser concentration rose to 10/13 and 12/13. At 14 weeks the ten remaining control snails were shedding.

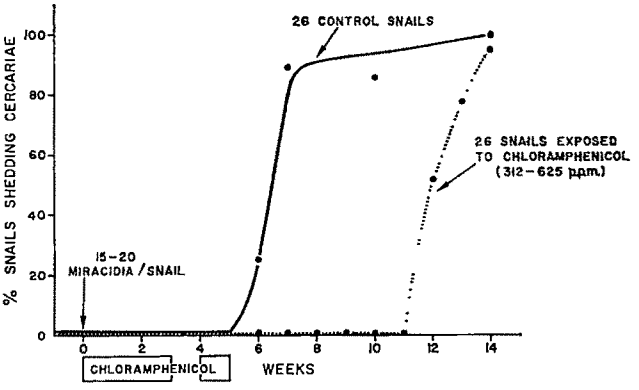


Fig. 1 The effect of chloramphenicol on the shedding of cercariae after exposure of *A. glabratus* to miracidia of *S. mansoni*

One major method of controlling schistosomiasis has been through the use of molluscicides[4]. To our knowledge no one has attempted to break the cycle through the prophylaxis or treatment of the disease in snails. The possibility of inhibiting protein synthesis during the rapid proliferation of the parasite from the miracidium to cercariae provided the rationale for an attempt to treat the infection in snails. The results indicate that, under the conditions used, chloramphenicol can suppress the development of the disease in snails, and that this suppression can be obtained with low concentrations which are not toxic to the snails. No deaths occurred until there was prolonged exposure to more than 1,000 p.p.m. of chloramphenicol. We have confirmed our initial results with an experiment now in progress using 280 snails in groups of 40 each. The drug at a concentration of 40 p.p.m. was completely effective in delaying the appearance of cercariae. However, the delay in development of cercariae was proportional to the time during which the infected snails were exposed to chloramphenicol. Thus, snails exposed to chloramphenicol over a 5-week period exhibited a 5-week delay in the shedding of cercariae as compared with the control groups. Similarly, in our present experiments, snails exposed to chloramphenicol for a shorter period of time exhibited a proportionally shorter delay in shedding of cercariae as compared with control groups.

The smallest concentration of chloramphenicol which is effective in suppressing the development of cercariae in snails is at present being determined. The possibility that *S. mansoni* may be more susceptible to chloramphenicol during other phases of the life-cycle of the parasite is also being examined. Conceivably other inhibitors

of protein synthesis may be more effective than chloramphenicol in interrupting the life-cycle of *S. mansoni*.

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Dependence of the Olive Fly, *Dacus oleae*, Larvae on Symbiosis with *Pseudomonas savastanoi* for the Utilization of Olive

THE extracellular bacterium *Pseudomonas savastanoi* (Smith) Stevens has been suspected as a mutualist of *Dacus oleae* Gmelin for more than fifty years. Petri[1] described in detail how the bacterium was preserved through the pupal and adult stages in an oesophageal diverticulum in the head of the olive fly, and also discovered how the bacteria were transmitted from generation to generation. During oviposition the egg is smeared with the bacteria which are housed in invaginations near the distal end of the ovipositor. The bacteria enter the egg through the micropyle and ultimately become established in caeca arising from the anterior end of the larval mid gut. The functions of this symbiote, however, were not determined.

Other Tephritidae contain extracellular bacteria in special structures[2,3], but the nature of the symbiotic relationships has not been elucidated. Hellmuth[3] cultured isolated bacteria from various tephritids and concluded from the metabolic products in the culture substrate of *P. savastanoi* and *P. mutabilis* Hellmuth that these species produce aldehyde-like substances from proteins and that they can grow on media containing urea and uric acid as nitrogen sources. *Agrobacterium luteum*, also found in *D. oleae* at times[1], could only convert uric acid and not urea to protein-like compounds.

With the recent development of artificial media for larvae and techniques for culturing of *D. oleae* in the laboratory without olives[4], the symbiotic relationships were modified through the inclusion of antibiotics in the adult diet and acidification of the larval medium.

Forty-five pairs of adult *D. oleae* reared from olives were divided into three groups (Table 1). They were exposed to a standard adult diet consisting of 8 g fructose, 2 g enzymatic protein hydrolysate of brewer's yeast, and 10 mg choline chloride, in 10 ml. distilled water. The tests were conducted at 25° ± 1° C, 60–80 per cent relative humidity and under normal diurnal light.

There were three diets: a standard, the standard plus 50 mg of streptomycin sulphate, and the standard plus 50 mg streptomycin sulphate and 50 mg of uricase. Uricase was added to the third test diet when the possible uric acid metabolism by *P. savastanoi* was precluded by streptomycin.

The fertility of the flies was determined in the following way: on three different dates after an initial feeding of 20 days, green olives were exposed to the different groups of flies for a day and then held for at least 15 days at 25° C to allow development and pupation of *D. oleae* larvae. After 15 days all olives were dissected and the eggs or larvae were counted.

Table 1. EFFECT OF STREPTOMYCIN IN THE ADULT FOOD OF *Dacus oleae* ON THE DEVELOPMENT OF PROGENY IN OLIVES

Adult diet*	Oviposition date	No. of each larval instar dead in olives L1	L2	L3	No. flies produced
Control	2/17	0	0	0	12
	3/2	1	0	0	28
	3/15	0	0	0	13
Streptomycin	2/17	14	0	0	0
	3/2	25	0	0	0
	3/15	16	0	0	0
Streptomycin + uricase	2/17	18	1	0	0
	3/2	30	0	0	0
	3/15	11	0	0	0

* Control diet: 8 g fructose, 2 g enzymatic protein hydrolysate of yeast, 10 mg choline chloride and 10 ml. distilled water. Streptomycin sulphate, uricase and enzymatic protein hydrolysate of yeast from Nutritional Biochemicals Corp., Cleveland, Ohio.

Table 2. INFLUENCE OF DIETS CONTAINING ENZYMATIC PROTEIN HYDROLYSATES ON THE DEVELOPMENT OF APOSYMBIOTIC *Dacus oleae* LARVAE

Larval diet*	Amount (g)	No. eggs	Days to pupation	Wt. puparia $\bar{x}$ range (mg)	Per cent recovery $\bar{x}$ range
Soy meal	3	320	—	—	None
Soy hydrolysate†	3	331	11	6·1 (5·7–7·0)	34 (20–45)
Casein†	4	286	15	5·6 (5·3–6·0)	2 (0–11)
Casein hydrolysate†	4	398	11	7·0 (6·5–8·0)	33 (22–50)

* Basal larval medium: 25 g dehydrated carrot powder, 15 g brewer's yeast, 2 g agar, 0·05 g choline chloride, 15 ml. olive oil, 5 ml. 'Tween 80', 0·3 g sodium benzoate, 0·05 g *n*-butyl parahydroxybenzoate, 125 ml. distilled water and *p*H adjusted to 4·1 with 7 ml. 2 N hydrochloric acid.
† Nutritional Biochemicals Corp., Cleveland, Ohio.

The data (Table 1) show that flies fed the standard adult diet produced a normal progeny, while those adults that fed on diets with streptomycin produced larvae which failed to attain the third instar. With this latter group all eggs hatched in the olives, but all save one larva had died in the first-instar stage after tunnelling considerable distances. Thus, it appears that the bacterial symbiotes are indeed necessary to *D. oleae* larvae for the utilization of the olive flesh.

In order to answer the question as to whether streptomycin actually inhibited the bacteria or directly hindered the growth of the *D. oleae* larvae, the following tests were conducted. Using the techniques described by Hagen *et al.*[4], eggs were obtained from flies exposed continually to streptomycin; these eggs were placed on various prepared media. The types of media tested and the data obtained are shown in Table 2.

From these data it can be seen that enzymatic protein hydrolysates of either soy protein or casein permitted faster larval development, production of larger puparia and greater larval survival than when the unhydrolysed proteins were used. This suggests that *P. savastanoi* hydrolyses the protein in the olive flesh. This method of protein hydrolysis is perhaps reflected by the nature of the feeding habits of *D. oleae* larvae. The solitary larva of the olive fly feeds on fresh, usually solid, and relatively uncontaminated tissue as it bores through its host instead of feeding in a decaying substrate such as that normally ingested and re-ingested by a group of larvae in a single fruit characteristic of most other species of *Dacus*.

Prepared larval media adjusted to *p*H 4·1 also evidently destroyed *P. savastanoi* in the larvae of *D. oleae* even though the symbiotes made their entry into the embryo during oviposition by flies not fed streptomycin. The larvae developed normally in the acid medium if it contained hydrolysed protein, and adults from such aposymbiotic larvae are normal except where the larval morphological sites of the usual symbiotes were invaded by acidophilus bacteria, which at times were numerous enough to block mechanically the ovipositor in adults about 30 days old[4]. The first-instar larvae that hatch from eggs deposited in olives by flies containing these acidophilus bacteria do not develop to the second-instar, but eggs obtained from these infected adults and placed on media containing hydrolysed protein develop to adult flies. This verifies the significance of *P. savastanoi* acting as a mutualist, and indicates the inability of acidophilus bacteria to play the part of the normal mutualistic bacteria.

Besides performing protein hydrolysis, *P. savastanoi* may also be involved in the synthesis of methionine and threonine. Olive flesh analysed by one- and two-dimensional paper chromatography did not reveal the presence of these two essential amino-acids[5]. However, Diptera in which amino-acid requirements have been determined invariably require these two amino-acids for completion of larval development[6]. Since cystine also was not detected

in olive flesh[5], the possible sparing action of cystine for methionine is not likely either. The fact that aposymbiotic larvae of *D. oleae* cannot complete their development in olive flesh, with the assumption that these larvae require methionine and threonine, suggests that these amino-acids are synthesized by *P. savastanoi*.

It is interesting that *P. savastanoi* occurs as a pathogen of olive trees causing olive knot, and survives without the presence of the olive fly. In California, olive knot is common, and there are no *D. oleae* established in the new world. Therefore, *D. oleae* is not obligatory for the existence of the bacterium, but the olive and the bacterium are apparently essential in the ecosystem of the olive fly to-day.

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Some Apparent Effects of Bithionol ('Actamer') on *Fasciola hepatica*

For some years my research has been concerned with the invasive stages of *Fasciola hepatica* in mammalian hosts. Young flukes have been tracked through the intestinal wall, the abdominal cavity and the hepatic parenchyma, and adults have been studied in the bile duct. Much evidence has shown that the flukes can break down many soft tissues which they encounter and has indicated that the fluke is, at all stages, a tissue feeding animal and not a blood feeder, as is commonly supposed. When I infected mice experimentally, dead flukes were not seen, although my investigations of X-irradiated flukes[1] have shown that flukes weakened in this way may be killed by phagocytosis. Band-type neutrophil leucocytes (polymorphs) penetrate the cuticle, spread throughout the cuticular core, and raise the epicuticle in blister-like formations which break down, leaving a stripped cuticle in a dying fluke. However, the leucocytes do not penetrate the base of the cuticle (lamina vitrea) and the disintegration of dead flukes is by secondary phagocytosis, so that this process differs from that reported by Standen[2] for drug-treated schistosomes. Standen has suggested that phagocytosis and drug action are related processes in schistosomes, and the possibility that this is so seemed to be worthy of investigation in liver flukes. However, my aim in a recent investigation has been to study any histological effects on the organs of *F. hepatica* when the rat hosts are treated with a drug.

My choice of bithionol as a suitable drug to use was made because this liver fluke and the lung fluke, *Paragonimus westermani*, display similar feeding activities and burrowing powers, and also because bithionol has proved to be potent in the treatment of paragonimiasis. Yokogawa[3] supported this contention of mine (see Dawes and Hughes, ref. 4) by noting the resemblance between the contents of the intestinal caeca of *P. westermani* during feeding operations and those of *F. hepatica* as described by me[5]. Regarding the potency of bithionol, it is known that dilutions less than 10^{-6} will kill excysted forms of *Paragonimus*, and Yokogawa (*loc. cit.*) considers this to be the most effective drug ever used in paragonimiasis, adding (p. 152) that this disease in man "will gradually die out in areas where mass treatment with bithionol has been thoroughly carried out". The antiseptic properties of bithionol (trade mark 'Actamer') are utilized in certain detergents and cosmetics, and the characteristics of the drug are described by the Hilton-Davis Chemical Co., Cincinnati, Ohio, in an undated publication.

In my experiments, ten young rats (weight about 300 g) each received five metacercarial cysts of *F. hepatica* by stomach tube and the infections were allowed to continue normally for 18½–34½ weeks. Eggs recovered from the faeces of the rats provided miracidia which infected laboratory-reared *Lymnaea truncatula*, proving that all infections were successful. Each of seven rats (*A–G*) began to receive drug treatment on day 131 and autopsy was carried out one day after administration of the final dose. The drug is almost insoluble in water and it was administered in suspension after 10 min shaking, using a stomach tube, and 10 mg constituted a single dose. Dosage was daily, so far as possible, and the rats received 2, 3, 4, 6 or 10 doses. Rat *H* did not receive any drug, and this control infection ran for 240 days, then yielding one living fluke measuring 18 × 10 mm and at least two flukes in serial sections of the bile duct. Two rats (*I* and *J*) carried their infections for 34½ weeks; each of them received three double doses of drug (20 mg daily on days 243–245) and was killed for autopsy on day 246.

Great effort was expended in the preparation of serial sections of the enlarged bile ducts of all these rats, together with any flukes which they contained, and sometimes recovered flukes, which were otherwise stained as whole mounts. From rats *A–G*, ten flukes 11–20 mm in length and 6·5–9 mm in breadth were taken alive, but these and also sectioned flukes all showed structural abnormalities which mainly affected the testes and the process of egg production, sometimes the cuticle and, to some extent, the vitellaria. Abnormalities of the testes are difficult to pinpoint at this stage for such a ramified organ, but numerous residual cytoplasmic bodies containing periodic acid–Schiff-positive materials are conspicuous in the tubules, within which there are numerous abnormal, curled spermatids, but relatively few typical sperms. The spermatids may have been liberated prematurely from their cytophores with consequent failure to complete their development.

This apparently general effect on the testes seems to have been brought about as a result of damage to the cuticle of the flukes, which is never phagocytosed but is often vacuolated extensively and sometimes loosened from underlying tissues and even stripped from some parts of the body both dorsally and ventrally, while there is no apparent damage to the intestinal caeca. Derangement of the egg-producing mechanism is probably due to faulty liberation of shell-forming materials from vitelline cells in the absence of sperms or else the presence of abnormally small numbers of sperms. It is evident in flukes from rats *B*, *C* and *D*, which received only three doses of drug, although the uterus of each of these flukes contains numerous eggs. Flukes from rats *E*, *F* and *G*, which received 4, 6 and 10 doses of drug respectively, show progressively diminishing numbers of eggs *in utero* until, in those from rat *G*, only very few malformed eggs appear. The ovary is apparently unaffected by drug treatment and isolated ova are plentiful in the oviduct and the nearby parts of the uterus. However, vitelline cells are not aggregated around the ova in the characteristic manner and typical sperms are evidently in short supply. Isolated vitelline cells, or small groups of such cells, sometimes have abnormal shells around them, and these cells also appear to be present only in sub-normal numbers, along with droplets of liberated secretion.

Neither of the rats *I* and *J* had any flukes in the hypertrophied bile ducts, which were filled with coarse, darkly coloured materials which are presumed to be the remains of flukes. Rat *J* was given a second infection of twenty cysts on day 211 and at autopsy (day 246) one living fluke of this infection was recovered entire from the liver, while sections of the liver revealed flukes in a dead or dying condition. There is thus some evidence, albeit scanty, that bithionol interferes with the reproductive function of *F. hepatica* and may kill flukes which are mature in the bile ducts or are maturing in the liver.

My collection of stained mounts of *F. hepatica* from various mammals includes two remarkable adults which are devoid of eggs. These specimens from the rabbit are notable because they also show derangement of the egg-forming mechanism. The oviduct is blocked with fragments of shell-forming secretion, the vitelloducts are closely packed with vitelline cells unable to continue their journey to the site of egg formation, and the vitellaria are completely degenerate. Sexual sterility has resulted in this instance from a simple physical effect on what might be called the assembly line of egg-production. The normal process by which the egg of a trematode receives its shell is subtle, and not fully understood. From the oviduct, vitelloducts and receptaculum seminis, respectively, an ovum, a cluster of vitelline cells and a rivulet of spermatozoa pass by a synchronized process into a special part of the oviduct (the ootype) and they become organized as a characteristic group, after which the shell appears by almost instantaneous liberation of secretion from the vitelline cells. From the time of witnessing the apparent disruption of this precision mechanism in the sterile flukes my opinion has strengthened to a belief that in seeking a drug that will eradicate flatworms it would be appropriate to try to interfere with this basic activity of producing shelled eggs rather than to try to kill or paralyse these worms. It would appear that bithionol shows some promise of providing just such an effect.

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Oligomycin Inhibition of Ion Transport in Plant Roots

INVESTIGATIONS of ion accumulation by isolated animal[1-4] and plant[5] mitochondria indicate that a high-energy intermediate of oxidative phosphorylation provides the necessary energy for the ion transport process in these organelles. The intermediate can be formed by either substrate oxidation or directly by ATP. The antibiotic, oligomycin, which had earlier been shown to block one of the terminal steps of the phosphorylation sequence[6,7], does not inhibit the substrate-driven ion transport system but does inhibit the ATP-driven system[1-5].

It is not known whether ion transport across other plant cell membranes, such as the plasmalemma or tonoplast, is similar to ion transport across mitochondrial membranes. However, it seems possible that a similar ATP-driven system with the formation of a high-energy intermediate or other phosphorylated substance could also be operative in these membranes. Such a system might also be sensitive to oligomycin. Indeed, in the case of several animal tissues, that is, liver slices[9], red blood cells[10,11], and kidney cortex slices[11], oligomycin has been shown to inhibit K^+ uptake. Whittam *et al.*[11] have also shown that the $Na^+ + K^+$-stimulated ATPase activity of membranes from renal cortex tissue is inhibited by oligomycin in a fashion similar to the inhibition caused by ouabain. They further showed that Na^+ stimulated the incorporation of phosphorus-32, from terminally labelled ATP, into an acid insoluble fraction and that K^+ stimulated its discharge. Similar results were obtained by Charnock and Post[12]. Oligomycin and ouabain inhibited the K^+ stimulated discharge of phosphorus-32 from the protein. Thus, in the case of various animal membrane systems, it appears that a phosphorylated protein serves as an intermediate in ion transport and that oligomycin directly inhibits the transport process by blocking the functioning of the phosphorylated intermediate.

Although attempts have been made to demonstrate a comparable salt-stimulated ATPase system in higher plant tissues, the efforts have met with only limited success[13]. Therefore, in view of the effects of oligomycin already mentioned here, it seemed reasonable that an investigation of the effect of oligomycin on ion transport by whole roots might shed light on the nature of the ion transport system across plant membranes.

Roots of *Avena sativa* (oats) were used in most experiments. The seeds were planted on paper towels saturated with 0·1 mM calcium chloride and grown in a dark germinator (30° C) for 5 days. Entire root systems were excised, washed several times in 0·1 mM calcium chloride and then transferred into the experimental solutions. For examining the ion uptake the experimental solution contained 1 mM potassium chloride and 1 mM calcium chloride. Rubidium-86 (approximately 10,000 c.p.m./ml.) was added as a tracer of potassium. Absorption periods were generally for 1 h at room temperature (24°–25° C). The stock oligomycin was suspended in 95 per cent ethanol and all treatments were adjusted to contain equivalent amounts of ethanol. Experiments were terminated by draining off the experimental solution, rapidly rinsing the roots three times with cold de-ionized water and then placing the roots in cold de-ionized water for 15 min. The water wash was followed with a 15-min wash in an ice-cold 1 mM potassium chloride + 1 mM calcium chloride solution to remove exchangeable K(^{86}Rb). Aeration was provided during both the experimental periods and the washes. The roots were then blotted, weighed and extracted three times (10 min each) with 0·5 N nitric acid in a boiling-water bath. Aliquots of the extracts were plated and counted in a gas flow counter. Results are presented as μmoles K^+ accumulated/g fresh weight on the basis of the accumulated isotope.

Respiration studies were performed with the conventional Warburg apparatus. The experimental solution for the root respiration studies was identical to the ion absorption experiments.

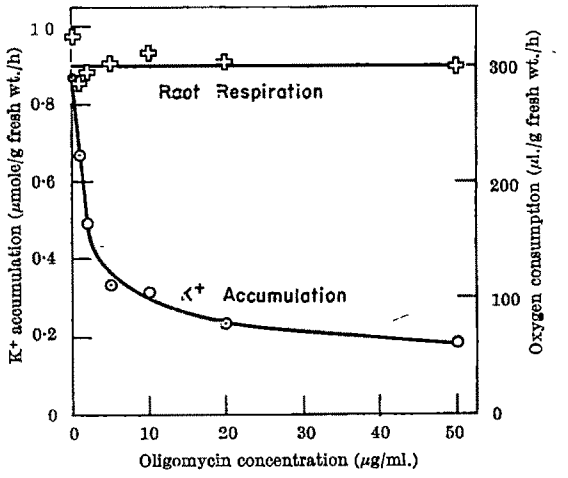


Fig. 1. Effect of oligomycin on K^+ accumulation and root respiration. See text for experimental procedure

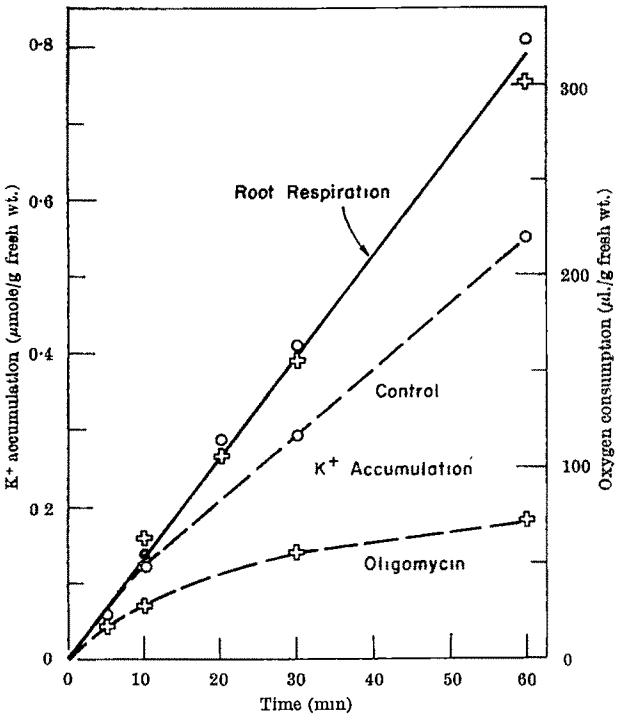


Fig. 2. Time course of K^+ accumulation and root respiration in the presence and absence of 20 μg/ml. oligomycin. – – –, K^+ accumulation; —, root respiration

The inhibitory effect of oligomycin on K^+ accumulation by excised oat roots is shown in Fig. 1. As little as 1 μg/ml. oligomycin caused a significant inhibition with half maximal inhibition occurring at a concentration of 2–3 μg/ml. These same concentrations of oligomycin had no significant effect on respiration of similar roots (Fig. 1). Fig. 2 shows that the inhibition of K^+ accumulation by 20 μg/ml. oligomycin is apparent within 5–10 min, while the root respiration is unaffected over a 1-h period. Additional experiments have shown that root respiration rates are unaffected by as much as 50 μg/ml. oligomycin over at least a 2-h period. It is well known that both oxygen uptake and phosphorylation by isolated mitochondria are inhibited by oligomycin[6–8]. This has been confirmed for mitochondria isolated from both the roots and shoots of oats. Thus, the lack of inhibition of whole-root respiration would imply that the antibiotic did not penetrate appreciably into the cell, and therefore, mitochondrial activity and the associated production of ATP should not have been limiting. This would suggest then that oligomycin was functioning to inhibit K^+ uptake by blocking ATP utilization in a process occurring primarily at the cell surface.

The inhibitory effect of oligomycin on K^+ accumulation was also found in other plant species although to varying degrees (Table 1). Roots which were most efficient in accumulation of K^+ showed the greatest inhibition to oligomycin.

Oligomycin inhibited accumulation of Cl^- and $PO_4^{\equiv}$ to about the same degree as K^+ (Table 2). However, accumulation of Ca^{++} was not significantly affected. There is some question, however, as to how much of the calcium uptake measured in experiments such as these actually represents active ion transport[14].

Table 1. Oligomycin Inhibition of K^+ Accumulation in Various Plants

Species	K^+ accumulated (μmoles/g fresh wt./h) – Oligomycin	+ Oligomycin	Inhibition (%)
Avena sativa (oats)	1·02	0·26	74·5
Lolium perenne (ryegrass)	0 38	0·14	63·2
Pisum sativum (peas)	0·30	0 17	43·4
Phaseolus vulgaris (bush bean)	0 17	0 16	5·8

The ryegrass was grown in the greenhouse for six weeks in sand culture receiving daily watering with complete nutrient solutions. The peas and beans were grown in the dark in vermiculite and distilled water for six days. Roots were washed free of adhering sand and vermiculite, excised and placed into the experimental solutions. Oligomycin concentration was 20 μg/ml

Table 2. Effect of Oligomycin on Ion Accumulation by Oat Roots

Ion	Accumulation (μmoles/gm fresh wt./h) – Oligomycin	+ Oligomycin	% inhibition
K^+	0·725	0 287	60 4
Ca^{++}	0·373	0·358	4·0
Cl^-	0·376	0·173	54·0
$PO_4^{\equiv}$	0·292	0·113	61·3

^{86}Rb, ^{45}Ca, ^{36}Cl and ^{32}P were used as the tracers for potassium, calcium, chloride and phosphate, respectively. Solutions contained approximately 10,000 c.p.m./ml. Oligomycin concentration was 20 μg/ml.

Inasmuch as oligomycin inhibits respiration of isolated mitochondria but does not affect the respiration of roots, it can be deduced that the antibiotic is not penetrating into the cells in any appreciable quantity. Furthermore, the inhibition of salt uptake is noticeable within 5–10 min. These observations are taken to mean that oligomycin is acting at the cell surface. The plasmalemma must contain an oligomycin-sensitive transport system, similar to that found in various animal membranes[9–11]. There is one distinction to be made: ouabain apparently acts like oligomycin on animal membranes[11], but I have been unable to detect any ouabain inhibition of accumulation of K^+ in plant roots. In addition, oxidative phosphorylation and the ATP-driven accumulation of calcium and inorganic phosphorus in isolated plant mitochondria[5] are unaffected by ouabain.

A search is now being made to determine whether the plasmalemma contains a salt-stimulated ATPase, and to determine its response to oligomycin.

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Differences in the Lactic Dehydrogenases of Primate Brains

In mammals, lactic dehydrogenase (LDH) has five molecular forms which show species and tissue specific patterns by starch-gel electrophoresis[1]. These molecular forms appear to be tetramers of two different polypeptide sub-units in their five possible combinations[2,3]. The two types of sub-units have been termed '*A*' and '*B*' (ref. 2), or '*M*' and '*H*' referring to their predominance in skeletal muscle and heart respectively[3]. The *M*(uscle) type of lactic dehydrogenase is best suited for anaerobic metabolism and the *H*(eart) type is best suited for oxidative metabolism[3,4]. Since glycolysis or anaerobic metabolism is phylogenetically more ancient that aerobic metabolism[5], a shift in certain tissues from *M* LDH to *H* LDH would be expected during the phylogenetic development of higher animals. From a comparative study of lactic dehydrogenases in a small series of vertebrate brains, Bonavita and Guarneri[6] provided evidence that this trend occurred during the emergence of warm-blooded

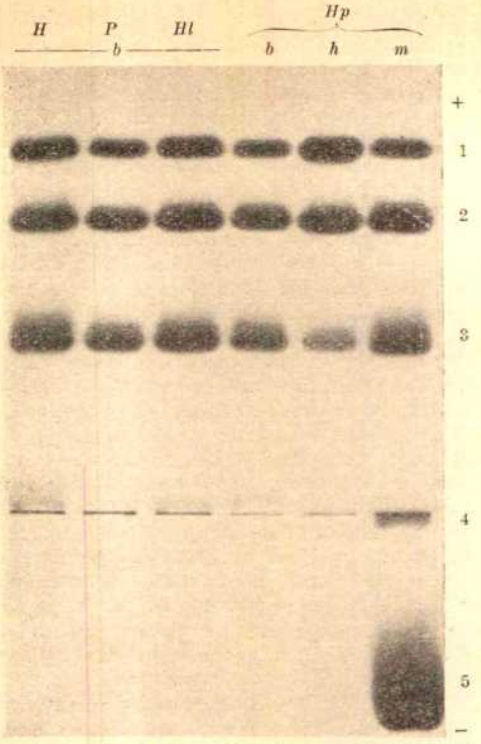


Fig. 1. LDH patterns of brains (*b*) from *Homo sapiens* (*H*), *Pan troglodytes* (*P*), and *Hylobates lar* (*Hl*), and of brain (*b*), heart (*h*), and skeletal muscle (*m*) of *Hylobates pileatus* (*Hp*)

vertebrates. The present communication provides evidence that such a shift was still occurring during the evolution of the order Primates.

The primate forms (Table 1) used in this study consisted of tree-shrews, lorisoids, tarsiers, ceboids, cercopithecoids, and hominoids, which show in the sequence mentioned an increasing phylogenetic relationship to man[7]. Brain, heart, and skeletal muscle, recovered as quickly as possible from these primate forms, were used immediately or stored for a few days at −18° C before being used. The tissues were homogenized in cold 0·05 M *tris* buffer *p*H 7·4, using three parts buffer to one part tissue. The supernatants obtained by centrifugation at 20,000*g* for 20 min were subjected to vertical starch-gel electrophoresis at 4° C for 16 h by the technique of Smithies[8], using the gel conditions described by Boyer *et al.*[9]. The gel was then bisected and the halves placed in a solution of 0·05 M sodium lactate, 0·0004 M NAD, 0·3 mg/ml. nitro blue tetrazolium, 20 μg/ml. phenazine methosulphate, and 0·05 M *tris* buffer, *p*H 7·4. After incubation for 1 h at 37° C the zones or bands of LDH activity developed in the gel. The pure *H* LDH gave the band of fastest anodic electrophoretic mobility (band 1); the pure *M* LDH gave the band of slowest mobility (band 5); and the hybrids of *H* and *M* gave the three intermediate bands. The formulae for these five bands are: (1) H_4; (2) H_3M_1; (3) H_2M_2; (4) H_1M_3; and (5) M_4.

Table 1. The Primate Forms and Number (in Parentheses) of Animals Examined in this Investigation

Hominoidea
- *Homo sapiens*, man (6).
- *Pan troglodytes*, chimpanzee (3)
- *Hylobates lar*, gibbon (2)
- *Hylobates pileatus**, gibbon (1)

Cercopithecoidea
- *Colobus polykomas*, colobus (3)
- *Macaca nemestrina*, pig-tail macaque (1)
- *Macaca mulatta*, rhesus monkey (1)
- *Macaca irus*, cynomolgus monkey (2)
- *Cercopithecus aethiops*, vervet (2)

Ceboidea
- *Ateles*†, spider monkey (1)
- *Alouatta*†, howler monkey (2)
- *Cebus*†, capuchin monkey (3)
- *Saimiri sciureus*, squirrel monkey (3)
- *Callicebus*†, titi monkey (2)
- *Aotes trivirgatus*, night monkey (2)
- *Oedipomidasoedipus*, cotton-top marmoset (2)

Tarsioidea
- *Tarsius syrichta*, Philippines tarsier (2)

Lorisoidea
- *Galago crassicaudatus*, greater bush-baby (5)
- *Galago senegalensis*, lesser bush-baby (1)
- *Nycticebus coucang*, slow loris (4)
- *Perodicticus potto* (1)

Tupaioidea
- *Tupaia glis*, tree-shrew (7)

The chimpanzee, gibbon, colobus, rhesus and squirrel monkey tissue were shipped to us in dry ice from the Delta Regional Primate Research Center of Tulane University. Tarsiers were sent from the Philippines and died in flight just before arriving in Detroit. Human brain was from autopsy made 6 h after death. All other animals were killed in the laboratory.

* The species name used for this specimen by the Delta Regional Primate Research Center is considered a synonym for *Hylobates lar* by Fiedler (ref. 7).

† The exact species classification of these specimens has not been determined.

Fig. 1 shows the LDH patterns in the brains of different members of the Hominoidea (man, chimpanzee, and the gibbons). In these patterns there are large amounts of bands 1, 2 and 3, and only trace amounts of bands 4 and 5. As can be noted in the figure, the brain patterns are much more similar to the heart pattern of gibbon than to its skeletal muscle pattern. No variations were observed among these hominoids in the mobilities of their LDH bands. This uniformity of brain LDH pattern extended to all the catarrhine primates listed in Table 1: the various members of the Cercopithecoidea (colobus, pig-tail macaque, rhesus, cynomolgus and vervet monkeys) as well as to the members of the Hominoidea.

Fig. 2 compares the brain LDH patterns of six ceboids (howler, capuchin, squirrel, night and titi monkey and cotton-top marmoset) to that of the vervet (a catarrhine monkey). The *H* LDH of these ceboids had the same mobility as *H* LDH of the catarrhines. However, the ceboid *M* sub-units had a faster anodic mobility than the catarrhine *M* sub-units since bands 2, 3 and 4 had more anodic positions in the ceboids than in the catarrhines. A spider monkey had a brain LDH pattern similar to that of the ceboids shown in Fig. 2. However, two capuchin monkeys had an *H* LDH of slower mobility. Furthermore, one of the two titi monkeys may well have been heterozygous at the genetic locus for *M* LDH, since the LDH of this monkey showed five separate electrophoretic components in band 5, four in band 4, three in band 3, and two in band 2. The most important difference between the ceboid group and the catarrhine group was the larger quantity of *M* LDH in the ceboid brains than in the catarrhine brains. The intensity of staining in

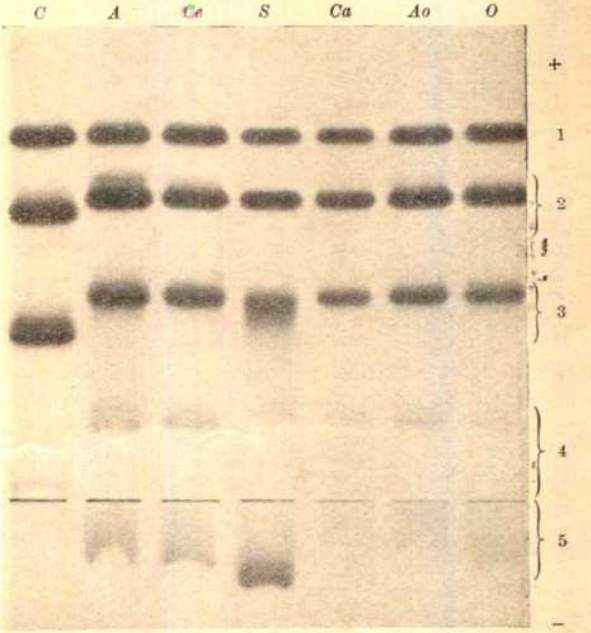


Fig. 2. Brain LDH patterns of *Cercopithecus aethiops* (*C*), *Alouatta* (*A*), *Cebus* (*Ce*), *Saimiri sciureus* (*S*), *Callicebus* (*Ca*), *Aotes trivirgatus* (*Ao*), and *Oedipomidas oedipus* (*O*)

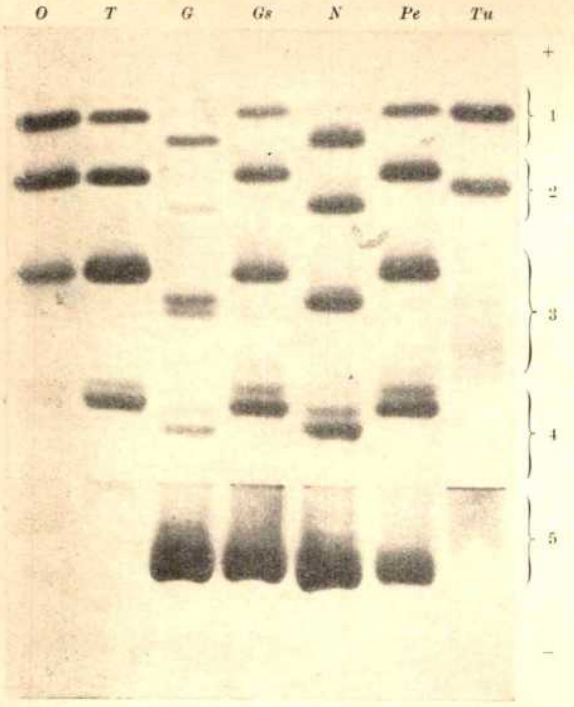


Fig. 3. Brain LDH patterns of *Oedipomidas oedipus* (*O*), *Tarsius syrichta* (*T*), *Galago crassicaudatus* (*G*), *Galago senegalensis* (*Gs*), *Nycticebus coucang* (*N*), *Perodicticus potto* (*Pe*), and *Tupaia glis* (*Tu*)

bands 4 and 5 was distinctly higher in the ceboids. Nevertheless, *H* LDH still predominated over *M* LDH in the ceboid brains.

Fig. 3 extends the comparisons of the brain LDH patterns from the Anthropoidea or sub-order of higher primates (represented by the cotton-top marmoset) to the more distant primate relatives of man (represented by the tarsier and different lorisoids, and also the tree shrew). The tarsier pattern was similar to the ceboid pattern in the mobilities of the LDH bands as well as in the greater proportion *H* enzyme to *M* enzyme in the brain. However, in the tarsier pattern, band 4 stained as intensely as band 1, whereas in the ceboid pattern band 4 did not stain so heavily as band 1. Thus, the proportion of *H* to *M* appeared to be somewhat less in the tarsier brains than in the ceboid brains. Among the Lorisoidea, the lesser bush-baby and *potto* had LDH bands with the same mobilities as those of the tarsier and most of the ceboids, while the greater bush-baby and slow loris had an *H* LDH of slower mobility. All members of the Lorisoidea had much larger amounts of band 5 than band 1 in their brains. Thus the lorisoids, in contrast to the other primates, had much more *M* enzyme than *H* enzyme in their brains. *Tupaia glis* of the Tupaiodea, a group long separated from other placental mammals and not regarded by all authorities as members of the order Primates[10], showed a preponderance of the *H* enzyme in their brains. Thus the LDH pattern of the tree-shrew was like that of the higher primates rather than the lorisoids. On the other hand, the African hedgehog, *Atelerix*, and the masked shrew, *Sorex cinereus* (representing the oldest order of placental mammals, the Insectivora), were also examined, and their brains were rich in *M* LDH.

Just as the overlap of the midbrain by an enlarged cerebrum occurred independently and to different degrees in different lineages of placental mammals during the Tertiary epoch[11], the biochemical evolution of the brain must also have progressed independently in these lineages. Thus the tree-shrew results need not deter us from deducing from the lorisoid, tarsier, ceboid, and catarrhine results that a shift in brain metabolism from synthesis of a preponderance of muscle-type to heart-type lactic dehydrogenase occurred in the evolutionary development of man. The lorisoid results suggest that man's primate ancestors at the beginning of the Tertiary epoch still had in their brains the form of lactic dehydrogenase which is best suited for anaerobic energy metabolism.

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MICROBIOLOGY

Stability of β-Galactosidase in Starved *Escherichia coli*

The endogenous metabolism of micro-organisms in aqueous suspension in the absence of added nutrients may involve the utilization of endogenous protein and RNA[1], as well as amino-acid pool constituents and reserve substances such as glycogen and polyhydroxybutyric acid (see reviews by Dawes and Ribbons[2]). Investigations

Table 1. Changes in Dry Weight, Viability, Protein and Induced β-Galactosidase Activity in *Escherichia coli* starved in Aerated Saline Phosphate Buffer (*p*H 6·5) at 37°

Hours		0	1	4	6	22·5	25	30	48
Viable count	(10⁸/ml.)	5·9	6·1	6·3	5·8	5·6	5·9	5·6	5·0
	(per cent)	100	103	107	98	95	100	95	85
Viability by slide culture	(per cent)	99	99	97·5	96·5	96	97	95	90
Dry weight	(mg/ml.)	2·42	2·32	2·18	2·15	2·03	—	1·85	1·86
	(per cent)	100	96	90	89	84	—	76·5	77
Total protein	(mg/ml.)	1·52	1·47	1·42	1·38	1·29	1·27	1·20	1·17
	(per cent)	100	96·6	93·5	90·8	84·8	83·5	79	77
β-Galactosidase in suspension	(units)	94·5	93·6	82·8	80·4	76·5	73·2	66·9	62·9
	(per cent)	100	99	87·6	85	81	77·4	70·8	66·5
β-Galactosidase in bacteria	(units)	93·8	90·1	82·5	77·4	74·4	71·6	66·3	61·3
	(per cent)	100	96	88	82·5	79·4	76·4	70·7	65·3
NH_3-N in supernatant fluid	(μg/ml.)	2·2	4·7	11·8	14·8	23·6	24·4	26·0	33·4

Bacteria were grown and starved as described in the text. β-Galactosidase activity is expressed as μmoles *O*-nitrophenylgalactoside hydrolysed/h/mg initial dry weight bacteria at 37° in sodium phosphate buffer (0·2 N Na^+, *p*H 7·2).

with *Aerobacter aerogenes*[1], *Escherichia coli* and *Pseudomonas fluorescens* have shown that starvation in aerated saline buffer at 37° results in a considerable loss of protein during a period when viability of the populations remains at 95–100 per cent. Since analyses of ultracentrifugal fractions separated from *A. aerogenes* after various periods of starvation showed decreases in the protein content of the insoluble, ribosomal and soluble fractions[3], the loss apparently represents a depletion of various cell proteins. It is interesting, therefore, that induced β-galactosidase in *Escherichia coli* was found to be relatively stable while other proteins were being rapidly degraded during turnover[4]. The relative stability of induced β-galactosidase in a wild-type strain of *E. coli* during starvation has been investigated and this communication records the relevant data.

Escherichia coli (M.R.E. 162: from human faeces) was grown at 37° for 16 h in shaken flasks (2 l.) containing a defined ammonium-salts medium[1] (100 ml.) with lactose (1 per cent w/v) as the sole source of carbon. Stationary phase organisms were separated from the culture and washed twice with saline phosphate buffer (0·055 M NaCl and KH_2PO_4 + K_2HPO_4 (0·01 M PO_4), *p*H 6·5) by centrifugation. The β-galactosidase concentration in these bacteria was about 90 units whereas bacteria grown in a medium with mannitol as the sole source of carbon contained less than 0·2 units of enzyme. The bacteria were re-suspended (1·5–2·5 mg dry weight/ml.) in saline buffer and the suspension was aerated with washed air in a Dreschel bottle at 37°; effluent air escaped through a water reflux condenser fitted with an air filter. At intervals samples were removed for determinations of total protein by a Biuret method[5] (colour obeyed Beer's law in the range used; coefficient of variation, 1·1 per cent for fifteen replicates), β-galactosidase with *O*-nitrophenylgalactoside[6] (*O*-nitrophenol colour obeyed Beer's law; coefficient of variation, 2·6 per cent for twenty-two replicates), viability by viable plate count (coefficient of variation, 4 per cent for twelve replicates with mean count of 835 colonies) and viability by slide culture[7] (coefficient of variation, 1 per cent for twelve replicates with mean of 95·7 per cent). The enriched agar medium for viable counts and slide culture was as described by Postgate, Crumpton and Hunter[7] except that glucose (0·5 per cent w/v) replaced glycerol.

The results (Table 1) show that during 30 h at 37°, when loss of viability was 5 per cent, losses of total protein and β-galactosidase activity were 21 and 29 per cent of the initial concentrations, respectively; the total protein-loss accounted for 56 per cent of the dry-weight loss and one of the products was ammonia. When starvation was continued for 48 h, further small losses of total protein and galactosidase activity occurred accompanied by a further small loss of viability. At each time-interval, β-galactosidase was determined in the whole suspension and in the bacteria separated by centrifugation, and re-suspended in saline buffer; under the latter conditions results were slightly lower in some cases due, perhaps, to incomplete recovery of bacteria or failure to re-suspend to exactly the same volume. When bacteria were starved in saline buffer containing thiomethylgalactoside[8] (TMG; 1 mM), β-galactosidase activity remained at the initial level for at least 24 h, while loss of protein was similar to that in the absence of inducer.

Thus, while under some conditions, β-galactosidase and indeed other induced enzymes in non-growing bacte may be relatively stable (see ref. 4), the present res indicate that in the absence of nitrogen and c sources at growth temperature, induced β- activity in *E. coli* is lost at a higher rate th rate of degradation of all cellular prote according to two methods of assay, was level that excludes bacterial lysis as t of protein or β-galactosidase activ

of *A. aerogenes* after various periods of starvation, by the present method, and by a copper-iodometric α-amino nitrogen method (after hydrolysis of the bacteria with 6 N hydrochloric acid for 20 h at 105°), gave similar results for protein losses. The stabilizing effect of TMG on β-galactosidase in starved *E. coli* may be due to the formation of a stable inducer-enzyme complex or to resynthesis of enzyme stimulated by the inducer.

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VIROLOGY

A Cytopathogenic Virus from the Brain of Fowl

A virus has been isolated from the brains of chickens which have died or been killed while suffering from a disease indistinguishable by clinical signs or histopathological lesions from infectious avian encephalomyelitis ('epidemic tremor').

The virus has multiplied in monkey kidney cell culture and has been repeatedly passaged in that system, in which it produces a cytopathic effect similar to that produced by an enterovirus. The agent appears to be completely resistant to the effect of ether. An antiserum produced against the Van Roekel strain of infectious avian encephalomyelitis virus has been shown to have a neutralizing effect against this virus in monkey kidney cell culture.

Further investigations of the virus are in progress, the results of which will be published in the near future.

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GENETICS

Evolution of Over-dominance during the Initial Spread of New Alleles

Bodmer and Parsons[1–3] have discussed a model for the initial increase in the frequency of newly arising, favourable mutations in a large undivided population and under different mating systems. They argued that over-dominance (higher fitness of heterozygotes over the [illegible]) has evolved in outbreeding species during [illegible] follows: (1) a new allele, say A_1, at locus [illegible]ally in the heterozygotes when it is [illegible]y in the population; (2) selection [illegible]te of increase in [illegible]at primarily [illegible] linked, [illegible]d

solutions for the conditions and relative rates of gene frequency changes under one- and two-locus models. The crucial feature (3 above) of their theory has not yet been established quantitatively. In fact, some calculations by Haldane[6] on the postulated role of dominance modifiers suggest that allelic substitution occurs largely through selective forces operating on the primary locus rather than through modifiers. These ideas are rather easily checked by simulation on a digital computer. This communication briefly re-examines the arguments involved in the theory of evolution of over-dominance with the help of exact numerical solutions obtained by simulation.

Following Bodmer and Parsons[1], consider first a model in which a new allele A_1 is introduced at a low frequency in an A_2A_2 population. Given the initial frequencies p^2 (A_1A_1), $2pq$ (A_1A_2), q^2 (A_2A_2), and their relative selective values, a, h, b, respectively, the frequency of A_1 in the following generation is:

$$p' = \frac{ap^2 + hpq}{ap^2 + 2hpq + bq^2};$$

the condition for increase in p is simply that $p' > p$. Ignoring terms involving p^2, this requires $h > b$, and thus, the authors concluded that "the heterozygotes must be superior in fitness to the original wild homozygote . . . since the frequency of the homozygotes A_2A_2 is of the second order in p, its viability does not affect the final progress". However, it can be easily shown that over-dominance is not a necessary condition and that the selective value of A_2A_2 is important in terms of the relative rates of change in p. Consider the condition for increase in p under the following situations: (1) no dominance:

$$(a = 1 + x,\ h = 1,\ b = 1 - x),\ p'/p = 1 + \frac{xq}{1 + x(p - q)}$$

(2) allele A_1 recessive:

$$(a = 1 + x,\ h = b = 1),\ p'/p = 1 + \frac{xpq}{1 + xp^2}$$

(3) over-dominance:

$$(a = b = 1 - x,\ h = 1),\ p'/p = 1 + \frac{x(p^2 + q^2 - p)}{1 - x(p^2 + q^2)}$$

(4) under-dominance:

$$(a = 1 + x + y,\ h = 1,\ b = 1 + x),$$

$$p'/p = 1 + \frac{p(x + y) - x(p^2 + q^2) - yp^2}{1 + x(p^2 + q^2) + yp^2},$$

so that for $p' > p$,

$$y > \frac{x(p^2 + q^2 - p)}{pq}.$$

In cases (1–3), clearly $p'/p > 1$, or $p' > p$ (since $x > 0$), and also in case of (4), this condition is satisfied with an appropriate value of y.

Table 1 gives a few numerical examples of the relative rates of initial progress in the frequency of A_1 under random mating versus varying proportions of mixed selfing and random mating. Clearly, all three selective values (a, h, b) influence the changes in gene frequency and note that under random mating, even under-dominance would allow an increase in p, given appropriate initial p and a large enough value of $y(= a - b)$. Bodmer and Parsons[1] imply that this could occur only under inbreeding, in contrast to the random mating situation in which "heterozygote advantage is a pre-requisite for a new gene to increase in frequency". On the other hand, they ruled out any role of over-dominant loci under inbreeding where, in fact, the difference in the selective values of two homozygotes (a–b) is very restrictive in terms of the long-term maintenance of polymorphic loci unless there is involved substantial heterozygote advantage[7,8].

The relative rates of initial increase in the frequency of allele A_1 are also relevant to the calculations of the cost of such a substitutional process, since, as shown by Haldane[9], the total cumulative load is influenced by the degree of dominance and the breeding system. Haldane's method was criticized by Bodmer[3], who considered the proportion of $AA : Aa$ more relevant than the relative proportions of all three genotypes. However, the numerical results given in Table 2 show that Haldane's calculations are essentially correct; inbreeding reduces the cost of allelic substitution by a favourable gene proportionately more with under-dominance or no dominance than with partial or complete dominance. It should be emphasized, however, that instead of the total cost, the cost incurred per generation is more important as a measure of loss in the reproductive potential, especially in view of the different rates of change in gene frequencies. The cost per generation involved in this selective process is indeed nearly the same for various proportions of selfing(s) although it varies in relation to the allelic frequency of A_1 in different ranges. It should also be noted that introducing recurrent mutation rates in the model only slightly changes these cost figures.

Using a two-locus model, Bodmer and Parsons[5] showed that "a modifier B the only effect of which is to enhance the heterozygote advantage of Aa will only be selected if it is sufficiently closely linked to locus A–a". The example they used to illustrate the effect of linkage on such a mechanism for the evolution of over-dominance assumed

Table 1. Frequency of the Newly Arising Allele (p) after 200 Generations

Selective values (a, h, b)	Initial $p^*_{(0)}$	Proportion of selfing (s)							
		0	0·10	0 30	0 50	0 70	0 90	0 95	1·0
1·0, 0·98, 0·96	0·00005	0 003	0·004	0 006	0 012	0 026	0 075	0·102	0·142
(No dominance)	0·005	0 232	0·272	0 382	0·538	0 726	0·890	0·919	0·943
1·0, 0·98, 0·95	0·00005	0·024	0·029	0·047	0·085	0 174	0 300	0 475	0·571
(Partial dominance)	0·005	0·622	0·668	0·764	0·859	0·936	0·982	0·988	0·993
1·0, 0 90, 0·90	0·00005	0·0001	0 00	005	0·087	0·978	0·999	1·0	1·0
(A_1 recessive)	0·005	0·0056			0·999	1 0	1·0	1·0	1·0
0·95, 1·0, 0·90	0·00005	0·060			0·764	0·823	0·838	0 805	0 713
(Over-dominance)	0·005	0·665			0·801	0 905	0·984	0·992	0·996
1 0, 0·795, 0 80	0·00005	0			1·0	1·0	1·0	1·0	1·0
(Under-dominance)	0·05				1·0	1 0	1·0	1·0	1·0

* The new allel… frequency; alternatively, the use of a constant rate of recurrent mutation μ ($A_…$

…uring the Increase of $p_{(0)} = 0\ 00005$ to $p \sim 0{\cdot}1$* and $p \sim 0{\cdot}5$*

	…0 98, 0 95)		Under-dominance (1·0, 0·795, 0 80)			
	Cost	$N_{p\sim 0\cdot 1}$	Cost	$N_{p\sim 0\cdot 5}$	Cost
	5·0	—	—	—	—
	4·4	910	175 8	115	22·2
	2	95	18 9	53	9 9
		42	8·3	48	8·4
		34	6·8		

Table 3. ALLELIC FREQUENCIES p_A AND p_M AFTER 100 AND 1,000 GENERATIONS (n)

Effect of modifier α	K	λ	Initial $p_A = p_M = 0{\cdot}0005$				Initial $p_A = 0{\cdot}0005$, $p_M = 0\ 50$			
			$n = 100$		$n = 1{,}000$		$n = 100$		$n = 1{,}000$	
			p_A	p_M	p_A	p_M	p_A	p_M	p_A	p_M
0·50	0 02	0·25	0 0023	0 0005	0·9594	0·0017	0 0028	0·5001	0·9452	0 6280
		0·50	0 0014	0·0005	0·9259	0·0118	0 0020	0·5002	0·9576	0·7451
	0·20	0·25	0 9735	0 0018	1·000	0·0019	0·9570	0 6447	1·000	0·6481
		0 50	0 9765	0·0155	1·000	0·0160	0·9738	0·7889	1·000	0·7940
0·25	0 02	0 25	0 0023	0 0005	0 9593	0 0032				
		0 50	0·0014	0·0005	0 9233	0 0650				
	0·20	0 25	0·9734	0 0035	1 000	0 0036	0 9431	0 7014	1 000	0·7094
		0 50	0·9740	0 0777	1 000	0·0309	0·9555	0·8530	1·000	0·8651
0	0 02	0·25	0 0023	0·0005	0 9590	0·0061				
		0·50	0·0014	0 0005	0 9058	0·2720				
	0 20	0 25	0 9732	0 0067	0 9999	0 0070	0 9252	0 7481	0·9956	0 7677
		0·50	0·9586	0·2952	0·9992	0 3198	0 9224	0 8878	0 9953	0 9189
−0·25	0 02	0 25	0 0023	0·0005	0 9583	0 0120				
		0·50	0·0014	0 0005	0 8634	0 5831				
	0·20	0·25	0 9727	0 0125	0·9486	0·0855	0 9039	0 7858	0 9451	0·8363
		0·50	0 9049	0 6203	0 9006	0 8343	0·8771	0 9026	0·9002	0 9731

ab/ab to be the prevailing genotype and gametes *AB* and *Ab* to be in very low initial frequencies. The relative viabilities were: *ab/ab*, 1·0; *Ab/ab*, 1·05; and *AB/ab*, 1·15 (other viabilities were not specified). Such a model is clearly inadequate to illustrate the evolution of over-dominance through selection for modifiers since over-dominance at locus *A–a* is assumed to begin with. Let us consider a more pertinent model similar to the one discussed by Haldane[6] in which a dominant allele *M* modifies the fitness of *Aa* genotype such that the relative selective values are:

$$\begin{array}{c} \\ AA \\ Aa \\ aa \end{array} \begin{array}{c} \begin{array}{ccc} MM & Mm & mm \end{array} \\ \begin{pmatrix} 1 & 1 & 1 \\ 1-\alpha\lambda K & 1-\alpha\lambda K & 1-\lambda K \\ 1-K & 1-K & 1-K \end{pmatrix} \end{array}$$

Here, λ measures the degree of dominance (namely, $\lambda = 0$, allele *A* dominant; $\lambda = 1$, allele *a* dominant; $\lambda = 0{\cdot}5$, no dominance, etc.), and K determines the selection intensity at locus *A–a*. Let the loci *A* and *M* be linked with recombination value, *c*. A large number of computer runs were made to determine the relative effects of λ, α and K with different initial frequencies of alleles *A* and *M*. Table 3 gives a sample of the results obtained for the case of random mating and recombination value $c = 0{\cdot}001$, in combination with other parametric values. Points to note are: (1) the frequency of allele *A* increased rather rapidly with large values of K and the relative rates of this increase varied with λ and the initial p_A, irrespective of the modifier effect, α; (2) the frequency of modifier *M* increased in frequency with $\alpha = 0$ or $-0{\cdot}25$; this follows from the fact that $\alpha \leqslant 0$ gives allele *M* an advantage over *m* in combination with *Aa* and due to linkage disequilibrium, genotypes *AaMM*, *AaMm* occurred at a higher frequency than *Aamm* individuals; (3) thus, modifier frequency did not seem to influence the rate of initial spread of allele *A* (Compare the values of p_M for $\lambda = 0{\cdot}25$ and 0·50, Table 3) which is largely determined by the selection parameters K and λ, as pointed out earlier by Haldane[6]. In fact, with $c = 0{\cdot}50$ (no linkage), it was found that the frequency of modifier *M* did not increase significantly in any case and yet the values of p increased slightly at higher rates than those observed with linkage.

It therefore seems unlikely that over-dominance has evolved through the accumulation of linked modifiers during the initial spread of a newly arising favourable allele as postulated by Bodmer and Parsons. The role of over-dominance in the maintenance of stable polymorphism at linked, interacting loci is, however, a different matter, as shown by Kimura[10]. Investigations by simulation of wide variety of selection models showed that in models without intrinsic over-dominance (for example, optimum model) linkage disequilibria result in some marginal over-dominance which is a necessary condition for the stability of gene frequency equilibria[11]. The initial increase in frequency of any rare allele would be inevitable in a large population if there is a stable non-trivial peak in the neighbourhood. The effects of selection, mating system, linkage and the nature and stability of resulting gene frequency equlilibria must be analysed jointly, if we are to understand the evolutionary properties of over-dominant linked gene complexes.

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VETERINARY SCIENCE

Adaptation of Bovine Sweat Glands to a Hot Environment

THE increase in human sweating following acclimatization to heat is well known[1]. Bianca[2] found that, with cattle, repeated exposure to heat (37° C) in a psychrometric laboratory resulted in a progressive reduction in body temperature, respiratory rate and heart rate. However, there is little evidence of any causal adjustment of sweat gland activity under these conditions. Experiments by McLean[3] and Banerjee *et al.*[4] suggest that the sweating rates of cattle under artificial heating are directly related to the prevailing temperatures animals have been previously exposed to.

To determine any possible acclimatization of sweat gland production to heat, two 17-month-old bulls were transferred from a warm (Armidale, 30° 30′ S., 151° 35′ E., 3,333 ft. altitude) to a hot (Moree, 29° 20′ S., 150° 0′ E., 700 ft. altitude) environment. One of the animals was a pure-bred Hereford, the other was a ¾ Santa Gertrudis–¼ Shorthorn. The animals were transferred from Armidale to Moree on February 26, 1963, and remained at Moree for 4 weeks. Mean monthly maximum and minimum temperatures for Armidale in February were 82° and 58° F respectively, while the corresponding figures for Moree in March were 91° and 67° F.

Sweating rate from the back thoracic[3] region was measured using the ventilated capsule technique[6], rectal temperature by a clinical thermometer, respiratory rate by counting flank movements and skin temperature with a copper-constantan thermocouple. Two days of observations are reported. Days I and II refer to data collected after the animals had been at Moree for one day and four weeks respectively. On each of the two days, observations were made between 10 a.m. and 3 p.m. while the animals were exposed to direct solar radiation. Mean dry bulb temperatures on days I and II were 90° and 89° F respectively.

Table 1. Mean Physiological Responses of the Hereford and the ¾ Santa Gertrudis before and after the Four-week Acclimatization Period at Moree

Observation	Hereford		¾ Santa Gertrudis	
	Day I	Day II	Day I	Day II
Rectal temp. (° F)	102·5 ± 0·7	102·3 ± 0·2	101·8 ± 0·3	101·8 ± 0·2
Respiratory rate (No./min)	94 ± 19	69 ± 11	65 ± 8	52 ± 7
Sweating rate (mg/31·8 cm²/5 min)	129 ± 32	141 ± 37	165 ± 18	150 ± 24
Skin temp. (° C)	39 5 ± 2·1	35 8 ± 1·6	38·9 ± 1·4	35·9 ± 1·1

Of the two animals, the ¾ Santa Gertrudis was least affected by the higher temperatures encountered at Moree on day I, this being demonstrated by its lower rectal temperature, respiratory rate and skin temperature (see Table 1). After the 4-week acclimatization period (day II) both animals recorded marked reductions in respiratory rate and skin temperature, while rectal temperature showed little change.

As illustrated in Fig. 1*a*, the sweating rates of the animals on day I followed a similar but rather irregular pattern of response. Compared with maximum sweating rates obtained previously at Armidale[6] (¾ Santa Gertrudis 158, and Hereford, 157 mg/31·8 cm²/5 min), only the ¾ Santa Gertrudis showed any increase in sweat gland activity. On day II (Fig. 1*b*) the sweating rates of both animals followed a definite trend with maximum rates occurring about midday. This was similar to the pattern observed earlier at Armidale except that the range of sweating rates was greater at Moree (76–184, compare 122–158 mg/31·8 cm²/5 min).

This experiment shows that although the diurnal pattern of sweat production in cattle of both the types *Bos indicus* and *Bos taurus* can be altered by a sudden transfer to a hotter environment, only the sweat glands of the former are capable of increased secretion. Furthermore, the pattern of sweat production after the animals became acclimated to the Moree environment provides further[7] indirect evidence of the importance of direct solar radiation in the control of sweat gland secretion.

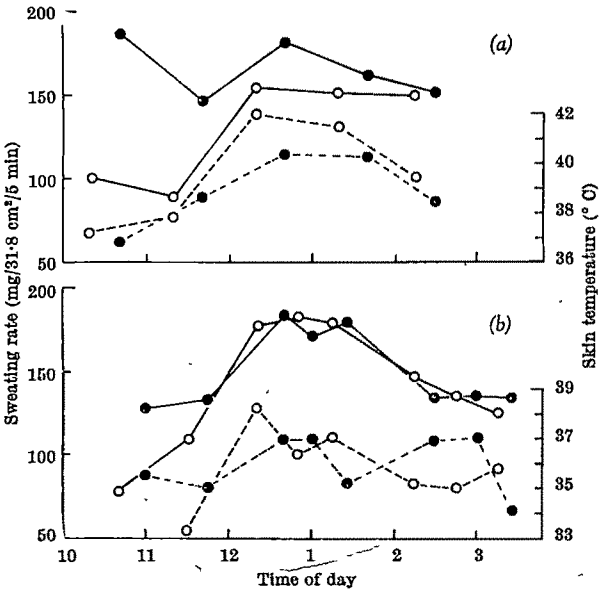


Fig. 1. Sweating rates and skin temperatures of the Hereford and ¾ Santa Gertrudis on days I (*a*) and II (*b*) at Moree. Hereford: sweat (O—O); skin temperature (O - - - O). ¾ Santa Gertrudis: sweat (●—●); skin temperature (● - - - ●)

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PSYCHOLOGY

Effect of Previous Exploratory Activity on the Exploration of a Simple Maze

There has been a good deal of discussion about the nature of the motivation involved in exploratory behaviour, and various theories have been put forward[1–3]. Most such theories suppose that this motivation is aroused directly by novel stimuli, and therefore that the strength of the motivation is relatively immune to deprivation effects. On the whole the experimental evidence bears out this view, though what may possibly be a deprivation effect has been found with visual exploration in monkeys[4]. Another type of test has not, however, been used. If we wish to examine a motivation such as hunger we can vary the period of deprivation and see how this affects the subsequent behaviour; alternatively we can use a fixed period of deprivation, give the animals varying amounts to eat, and see whether this appears to alter drive level. The first procedure has been attempted in the exploratory case with negative results (in rats at least)[5]; the second procedure has not. If the motivation involved 'builds up' in the rat when it is not exploring and is reduced by exploration, then exploration of one environment should reduce the motivation and so reduce the amount of exploration, immediately afterwards, of another quite different environment. If, on the other hand, the motivation is aroused primarily by the stimuli in the environment, then exploration of one environment should not reduce subsequent exploration of another dissimilar environment.

The subjects in this experiment were twenty-four satiated female hooded rats about 100 days old. They were given three days of pretraining in which they were allowed to move around a table-top for a few minutes and were picked up and handled a number of times. The animals were then randomly assigned to three groups of eight animals each. On the fourth day the animals in Group I were given exploration for 3 min in an elevated Y-maze, with arms 18 in. long, 4 in. wide and 18 in. high; they were removed from the Y-maze, kept in a small delay cage for 0·75 min and then allowed to explore an enclosed T-maze for 2 min. The plan of this maze is given in Fig. 1; the walls were 6 in. high and of unpainted metal. The animals in Group II had 3 min exploration of an open field 3 ft. × 3 ft., with 2 ft. high dark green and white striped walls; they were then kept in the delay cage for 0·75 min and allowed to explore the enclosed T-maze for 2 min. The animals in Group III were put directly into the delay cage for 0·75 min and then allowed to explore the T-maze for 2 min. The activity of the rats was recorded in terms of 'section entries' during each 30 sec of exploration. In the elevated Y-maze each arm was divided into two imaginary 9-in. 'sections'; in the open field the 'sections' were nine, 1 ft. × 1 ft., squares marked on the floor; the 'sections' in the T-maze are shown by the dotted lines in Fig. 1. A rat was considered to have

entered a section if its head and three of its paws were over the dividing line.

The results in terms of section entries are shown in Fig. 2. The decline in activity between the first and last minutes of the first trial for Groups I and II is characteristic of this type of situation and is in each case highly significant ($P < 0.008$, signs test). It is clear from the graph that there is no appreciable difference between the groups on the second trial. The decline between the first and second minutes of the second trial is significant at below the 0·008 level for Group I and Group II and at the 0·07 level for Group III (two tailed signs tests). There are no significant differences in activity between the three groups individually either overall or in any 30-sec period, but Groups I and II combined are slightly more active than Group III in the first 30 sec of the second trial ($P = 0.05$ signs test).

It appears, therefore, that after a rat has explored one type of environment, and has to some degree reduced the capacity of that environment to evoke exploration, it is just as exploratory in a new environment as a rat with no immediately preceding exploratory experience. The slight difference between the groups in the first 30 sec of the second trial is, of course, in the wrong direction for any view which predicts that previous exploratory activity has a depressing effect on subsequent exploration. It may perhaps be due to some sort of 'warm up' effect, but is too slight to merit much discussion. In this type of situation at least, the motivation for exploratory behaviour seems, therefore, to be aroused by the stimuli in the environment, rather than to be present in the animal, perhaps as a result of 'deprivation', and reduced by exploratory experience. In this respect, at least, this motivation is therefore more analogous to such externally aroused drives as fear than to hunger or thirst.

I thank Mr. A. J. Watson for his advice.

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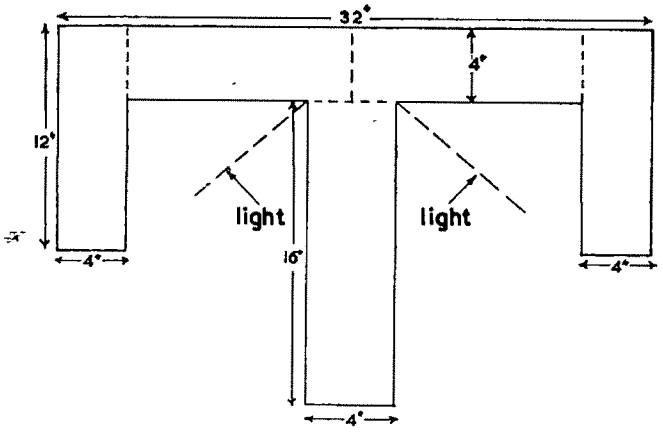


Fig. 1. Plan of T-maze. The dotted lines represent maze sections

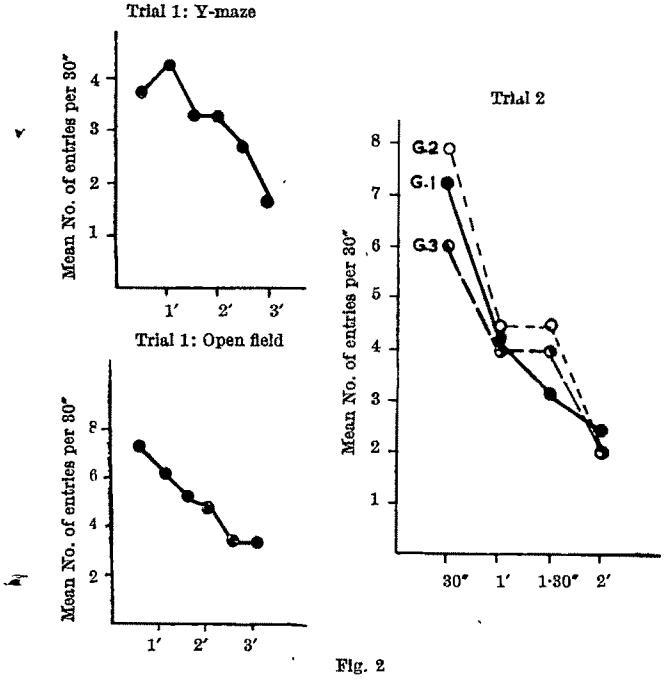


Fig. 2

Time Contraction and Psychomotor Performance produced by 'Psilocybin'

It is particularly rewarding to investigate the experience of chronosystole, or time contraction during sympathetic excitation, as this state, which is connected with an increase in metabolic rate, can be elicited at will through psychotomimetic, pyretogenic drugs such as mescaline, marihuana, D-lysergic acid diethylamide (LSD), 'Psilocybin'[1,2]. We may define time contraction in terms of an alteration in the signal per noise ratio of the sensor, or in an adaptation of Stroud's[3] terminology, an increase in data content. In short, if more events are happening within a chronological time unit, the phenomenon of time contraction is experienced. Hofmann's self-observation, in what may be called the first description of time contraction after a dose of 0·25 mg of LSD (ref. 4), relates "the impression of being unable to move from the spot" on his way home from the laboratory on a bicycle. Delay's[5] systematic description of the psychic effects elicited by 'Psilocybin' also refers to experiences which may be considered phenomena of time contraction: "J'ai l'impression que le temps passe plus lentement, qu'il a plus de densité". "Tout est d'une longuer indéfinissable."

Within the framework of space–time equivalence, time contraction can be illustrated through the concomitant expansion of space; specifically, by the increased size of handwriting samples at the peak of the psychotomimetic drug experience[2]. When finger-tapping rates are used as a measure of intensification of time, the magnitude of the drug reaction may be assessed in relation to the control value, the baseline of a subject. (See in this connexion Wilder's[6] law of initial values.) Therefore, patients in various states of excitation and depression cannot be expected to experience changes in time perception analogous to those of an average healthy subject, that is, the patient's experience of time contraction may be different in degree or may not occur at all. (This explains the divergent results of Kenna and Sedman[7].)

Among the various attempts to define 'Psilocybin' reactivity, the most notable is that of Isbell[8], who found a close correspondence between the maximum effect of various drug doses on pupillary size and that on mental response. No effort has been made, however, to quantitatively differentiate the degrees of reactivity in subjects who have ingested the same dose of 'Psilocybin'. Long experience has shown us that a dose of 115 μg/kg 'Psilocybin' is likely to produce one of the following reaction types: 'non-reactors', 'mild reactors', 'reactors' and 'strong reactors'. Hence the problem presents itself of characterizing them with relatively simple, quantifiable parameters.

We have refined the use of two already existing variables for the measurement of time contraction—handwriting samples and finger-tapping rates. As shown by the following representative examples, the former proved useful for the characterization of 'strong reactors' and 'non-reactors', the latter for that of 'reactors' and 'mild reactors'.

Handwriting size can be quantified to measure the extent of psychomotor drug reactivity. A planimetric comparison of pen-and-ink handwriting samples on flat paper[9] reveals that whereas only 60·00 cm² is needed to cover the first five written lines for our 'strong reactor' under control conditions, 81·30 cm² is required at drug peak—an increase of 31 per cent (compare the upper and lower halves of Fig. 1). On the other hand, the handwriting space of a 'non-reactor' shows no increase at drug peak as compared with the control performance (see upper and lower halves of Fig. 2).

Table 1. Quantification of Time Contraction

Mean tapping rates and variances (at an 8-min period)

	At peak of 'Psilocybin' experience		Next day (control) at the same time	
	Mean	Variance	Mean	Variance
Subject *A* ('mild reactor')	2·21/sec	0·018	1·16/sec	,0 003
Subject *B* ('reactor')	1·31/sec	0 001	0·42/sec	0·001

Mean tapping rates and variances at drug peak, that is, 100 min after oral ingestion of the drug, were obtained using silent Morse-key tappings at a freely chosen speed during an 8-min period. These were also measured during a control period the following day. Afterwards, power density spectra were derived according to the procedure of Ralston and Wilf[11], using a 'Bendix' *G*-20 digital high-speed computer. (The results of spectral analysis are to be published[10].) The tabulated mean values of the fluctuations, and the variance in tapping rate for the 8-min periods for two subjects—a 'mild reactor' and a 'reactor'

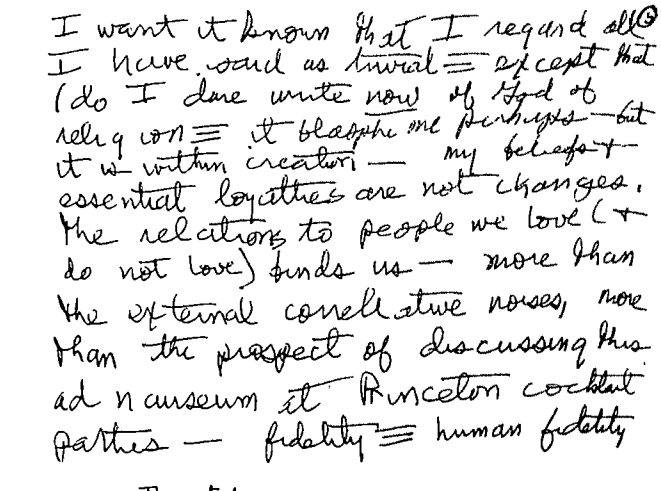


Fig. 1. Handwriting samples of a 'strong reactor' 2 h after the ingestion of 115 μg/kg 'Psilocybin' (upper portion of figure) as contrasted with control sample obtained on the following day (lower portion); half the original size

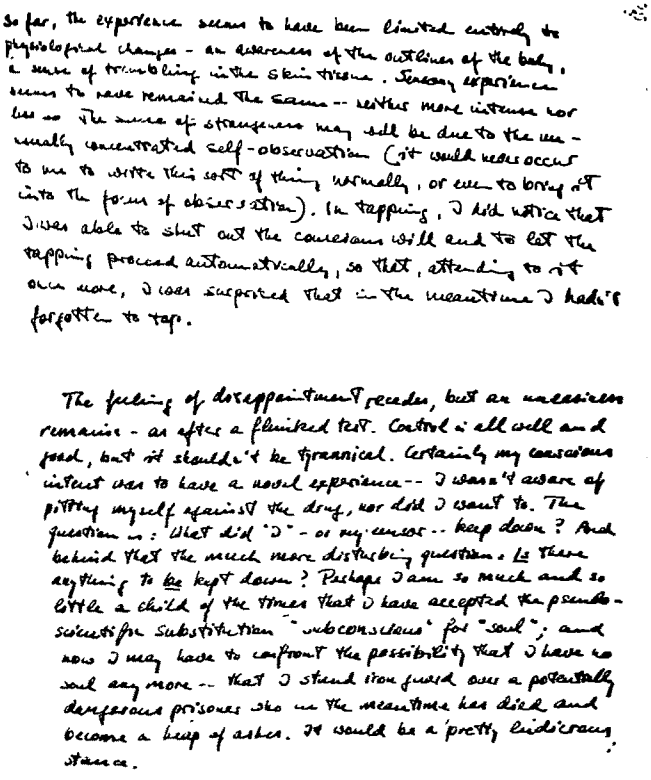


Fig. 2. Handwriting samples of a 'non-reactor' 2 h after the ingestion of 115 μg/kg 'Psilocybin' (upper portion of figure) as contrasted with control sample obtained on the following day (lower portion); half of the original size

—show that there is a significant increase in tapping rate at the peak of the 'Psilocybin' experience relative to the respective control value (see Table 1).

This increase also reflects the degree of the excitation syndrome which 'Psilocybin' can produce in an individual. It can be seen from Table 1 that subject *B* with a low mean control tapping rate (0·42/sec) and a low variance (0·001) increased his performance more than threefold at drug peak, whereas subject *A* with a high baseline (mean control tapping rate 1·16/sec) increased his performance not quite twofold at drug peak with a larger variance. This again indicates that the baseline (control value) of a subject is important in the assessment of his drug reactivity.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 24

INSTITUTE OF ACTUARIES (at Staple Inn, High Holborn, London, W.C.1), at 5 p.m.—Sir Paul Chambers, K.B.E., C.B., C.I.E.. "Problems in Forecasting in the Chemical Industry" (Twelfth Alfred Watson Memorial Lecture).

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. W. N. Aldridge. "Hydrolytic Enzymes and Their Inhibitors" (further lecture on January 31).*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. H. Ahmed, Mr. C. E. Maloney, Mr. J. D. Sankey and Mr. A. H. Beck: "Recent Studies of Dispenser Cathodes".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Gaseous Insulation".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. M. W. Thring: "Towards a Creative Society: The Philosophy of Technology". (Second of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

Tuesday, January 25

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Short Paper Meeting.

CAROLINE HASLETT MEMORIAL TRUST (at the Royal Commonwealth Society, Craven Street, London, W.C.2), at 5 p.m.—Mrs. Elizabeth Walker: "The Electrical Way of Life in Canada".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Use of Programmed Learning for Remedial Work in Higher Education" opened by Mr. D. Unwin and Mr. A. K. Spencer.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. W. Pearson: "Method for the Prediction of the Fading Performance of a Multisection Microwave Link".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. T. S. West: "Inorganic Trace Analysis" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. Cotchin: "Tumours of Domestic Animals". (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Prof. P. L. Shinnie: "Problems of West African Archaeology".*

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. R. G. Croissant: "V.T.O.L. Aircraft".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. R. Moore and Dr. B. M. Tidswell: "Instrumentation of Molecular Weight Measurements".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Eccleston Square, London, S.W.1), at 7 p.m.—Mr. D. Daniels: "Moulding Machinery Utilization and Unit Cost per Article".

Wednesday, January 26

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. H. L. Burstyn: "Foucault's Pendulum and the Progress of Oceanography".

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (at Imperial College of Science and Technology, London, S.W.7.), at 5 p.m.—Mr. P. M. Stocker: "The Integration of Man and Computer".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. COMPUTER GROUPS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "The Linking of Computers" opened by Mr. T. Spinks and Mr. D. Rowland.

INSTITUTION OF ELECTRONICS AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "The Problems of PAL in Relation to Receivers".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. L. J. Clark: "The Carriage of Liquefied Petroleum Gases" (Thomas Lowe Gray Lecture).

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, Exhibition Road, London, S.W.7), at 6 p.m.—Mr. T. H. Carr: "Plastics Foams for Packaging".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. W. Taylor: "Problems in the Manufacture of Dehydrated Potato Flake"; Dr. T. P. Williams: "Chemistry of the Potato Cell Wall in Relation to Texture of Mashed Potatoes".

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE WITH IMPERIAL COLLEGE CAVING CLUB (in the Department of Physics, Imperial College of Science and Technology, London, S.W.7), at 7.30 p.m.—Mr. Andrew Watson: "Bats in Caves".

Thursday, January 27

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. P. H. Kemp: "Sea and Shore".*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 1.30 p.m.—Mr. Patric Dickinson: "Computer versus Creator" (last of three lectures).*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. G. Deeks: "*D*-Region Electron Distributions in Middle Latitudes Deduced from the Reflexion of Long Radio Waves"; Mr. N. O. Weiss: "The Expulsion of Magnetic Flux by Eddies"; Mr. R. L. Parker: "Reconnexion of Lines of Force in Rotating Spheres and Cylinders".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Electrical Analogue Techniques in Ground Water Studies" introduced by Mr. J. A. Cole.

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. H. Noé and Mr. T. P. Flanagan: "Ionization Techniques Applied to Gas-Flow Measurement".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. Kao: "Optical Surface Waves".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. G. S. Dawes. "New Views on O_2, Transfer Across the Placenta". (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in Lecture Theatre No. 1, Physics Building, Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Dr. Walter Marshall: "Investigations of Magnetic Materials Using Neutron Scattering. III. Neutron Investigation of Many Body Problems: Critical Phenomena in Magnetic Materials".*

SOCIETY FOR ANALYTICAL CHEMISTRY, AUTOMATIC METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Dr. A. W. Foster: "The Economics of Automatic Analysis".

Thursday, January 27—Friday, January 28

CHALLENGER SOCIETY, and REPRESENTATIVES FROM THE MARINE LABORATORIES (Natural Environment Research Council Scheme) (at the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10 a.m. daily—Scientific Papers.

Friday, January 28

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 2 p.m.—Colloquium on "Microwave Noise-Measuring Techniques".

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Determination of the Geoid from Satellite Data". Speakers: Mr. D. G. King-Hele, Dr. R. R. Allan, Major-General R. C. A. Edge, Sir Harold Jeffreys, F.R.S., and Prof. S. K. Runcorn, F.R.S.

TELEVISION SOCIETY (in the Conference Suite I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. W. J. Morcom: "U.H.F. Translators".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Admiral of the Fleet, The Earl Mountbatten of Burma, K.G., P.C., O.M., D.Sc.: "Controlling the Information Explosion".

Saturday, January 29

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mrs. S. C. Coryndon: "African Faunas Past and Present".*

Monday, January 31

CIBA FOUNDATION FOR THE PROMOTION OF INTERNATIONAL CO-OPERATION IN MEDICAL AND CLINICAL RESEARCH (at The Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5 p.m.—Prof. C. P. Leblond, F.R.S. (McGill University): "Role of the Golgi Complex in Secretion" (18th (Annual) Ciba Foundation Lecture).

UNIVERSITY OF LONDON (at the London School of Economics, Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. R. J. Harrison-Church: "Some Geographical Aspects of West African Development" (Inaugural Lecture).*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "The Problems of Radiation Pressure and Associated Mechanical Forces".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. F. Hoskins: "The Definition and Properties of the Delta Function".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. M. W. Thring: "The Status and Training of Engineers and Technologists". (Last of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. J. Kohn: "The Use of Balsa Wood in Association with Reinforced Plastics".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

EXPERIMENTAL OFFICER (preferably with a degree or equivalent qualification and experience in the field of electron optics) to assist in the operation of a high resolution electron microscope—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester, 1 (January 29)

ASSISTANT LECTURER (preferably with an interest in enzymology) IN BIOCHEMISTRY; a LECTURER and an ASSISTANT LECTURER (with an interest in electrochemistry) IN CHEMISTRY; and a LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS or APPLIED MATHEMATICS or MATHEMATICAL

STATISTICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (January 31).

LECTURER IN COMPUTER SCIENCE IN THE DEPARTMENT OF MATHEMATICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (January 31).

LECTURER or ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Registrar, University College of Wales, Aberystwyth (January 31).

LECTURER (preferably candidate interested in metabolism) IN BIOCHEMISTRY IN THE RHEUMATISM RESEARCH CENTRE AND THE DEPARTMENT OF MEDICAL BIOCHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 235/65 (February 2).

LECTURER/ASSISTANT LECTURER IN THE DEPARTMENT OF MORAL PHILOSOPHY—The Secretary, The University, Aberdeen (February 5).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. CV/356 (February 5).

ASSISTANT LECTURER or LECTURER IN PSYCHOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (February 7).

LECTURER (with a good honours degree and subsequent research or industrial experience in the general field of electrical machines) IN THE DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Registrar, The University, Leeds, 2 (February 7).

ASSISTANT PROFESSOR (with medical qualifications together with at least three years teaching experience and independent research interests, and preferably a knowledge of simple workshop practice) IN PHYSIOLOGY/PHARMACOLOGY IN THE INSTITUTE OF MEDICAL SCIENCE, Haile Selassie I University, Ethiopia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 10).

RESEARCH STUDENT (with a first- or second-class honours degree or an equivalent qualification, for example, G.R.I.C.) IN THE DEPARTMENT OF CHEMISTRY, to undertake research in high temperature inorganic chemistry, studying crystallization of oxide glasses, with a view to the degree of M.Sc. or the degree of Ph.D.—The Secretary, University of Aberdeen, Marischal College, Aberdeen, Scotland (February 11)

LECTURER (with a good degree in physiology, biochemistry, pharmacology, chemistry, medicine, or zoology, and postgraduate experience of Ph.D. or equivalent standard) IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Deputy Secretary, The University, Southampton (February 12).

ASSISTANT LECTURER (man or woman graduate) IN PHYSICS—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (February 13).

LECTURER or ASSISTANT LECTURER (preferably with practical and/or research experience in structural analysis and in the design of both steel and reinforced concrete) IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Leeds, 2 (February 14).

LECTURER IN ENGINEERING MATHEMATICS (COMPUTATION)—The Secretary, The Queen's University, Belfast, Northern Ireland (February 15).

LECTURER or ASSISTANT LECTURER (with a degree in physics, chemistry, metallurgy or engineering, and postgraduate research experience in the field of structure and properties of materials) IN MATERIALS SCIENCE AND ENGINEERING—The Deputy Secretary, The University, Southampton (February 15).

ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Hull (February 16).

LECTURERS (2) IN PSYCHOLOGY (an interest in social and/or educational psychology would be relevant to one of the posts) IN PSYCHOLOGY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (February 26).

CHAIR OF PHYSIOLOGY AT MONASH UNIVERSITY, Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University, Clayton, Victoria, Australia (Australia and London, February 28).

DIRECTOR OF THE COMPUTER LABORATORY, University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 28)

LECTURER IN MEDICAL BIOLOGY at the University College of Rhodesia and Nyasaland, for duties which will include responsibility for the teaching of first-year medical students—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 28).

LECTURERS or ASSISTANT LECTURERS IN THE DEPARTMENT OF PHYSICS (for one post preference will be given to candidates with qualifications in theoretical physics) IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Leeds, 2 (February 28).

RESEARCH FELLOWS FOR INDEPENDENT RESEARCH—The Assistant Registrar (Room 28, O.R.B.), The University, Reading (February 28).

SENIOR RESEARCH FELLOW or RESEARCH FELLOW IN THE DEPARTMENT OF DEMOGRAPHY, Research School of Social Studies, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 28).

ASSISTANT LECTURER or LECTURER (preferably with interests in structural geology and geophysics) IN THE DEPARTMENT OF GEOLOGY—The Deputy Secretary, The University, Southampton (March 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (normally below the age of 30, with the degree of Ph.D. or equivalent research experience) IN CHEMISTRY, PHYSICS, METALLURGY, ENGINEERING, GEOLOGY, AGRICULTURAL SCIENCE OR RELATED SUBJECTS—The Registrar of the University of Wales, University Registry, Cathays Park, Cardiff (March 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph.D. or equivalent research experience, and normally below the age of 30) IN CHEMISTRY or PHYSICS—The Registrar, University of Keele, Keele, Staffs (March 1).

VETERINARY SURGEON to work on virus infections of domestic animals with special reference to the respiratory tract of the pig—The Secretary, Institute for Research on Animal Diseases, Agricultural Research Council, Compton, Berkshire (March 1).

ASSISTANT LECTURER IN GEOGRAPHY—The Registrar, The University, Hull.

BASIC GRADE SCIENTIFIC OFFICER (Bacteriology) (with an appropriate science degree) mainly for work in a routine laboratory—The House Governor, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, 339 Goldhawk Road, London, W.6.

ORGANIC CHEMISTS and PHYSICAL CHEMISTS (with a Ph.D., a good honours degree or H.N.C. qualification, and preferably under 30 years of age) for work which is in many cases of a fundamental nature and every encouragement is given to publish the results and study for a higher degree—The Director, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian.

PRE-DOCTORAL RESEARCH ASSISTANT to work on flat slab construction—Dr. J. C. Chapman, Civil Engineering Department, Imperial College of Science and Technology, London, S.W.7.

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (preferably with an honours degree in agriculture or in an appropriate biological subject) FOR THE DAIRY HUSBANDRY DEPARTMENT—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 66/T/1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. Animal Disease Surveys Report No. 2, Part 2: Fluorosis in Cattle—Development and Alleviation: Experimental Studies. By Ruth Allcroft, K. N. Burns and C. Nancy Hebert. Pp. vi+59+12 plates. (London: H.M. Stationery Office, 1965.) 3s. 6d. net. [2611

Agricultural Research Council. Experimental Farm Buildings—Report No. 4: The Use of Concrete Staved Silos for Storing High-Moisture Barley. By H. J. M. Messer and J. M. Hill, Dr. R. Whittenbury and Dr. J. Lacey. Pp. 24. (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 3s. 6d. net. [2611

University of Strathclyde Association of University Teachers. The Modern University: A Symposium, Friday, Saturday, 12th and 13th November 1965. Pp. 125. (Glasgow: University of Strathclyde Association of University Teachers, 1965.) [2611

Greater London Council: Scientific Branch. Annual Report of the Scientific Adviser 1964. Pp. 109+4 plates. (Publication 23.) (London: Greater London Council, 1965.) 1s. 3d. [2611

Philosophical Transactions of the Royal Society of London. Series A. Mathematical and Physical Sciences. No. 1089, Vol. 258 (11 November 1965): The Adsorption of Krypton and Xenon on Evaporated Metal Films By D. Brennan and M. J. Graham. The Adsorption of Carbon Monoxide on Evaporated Metal Films. By D. Brennan and F. H. Hayes. The Adsorption of Carbon Dioxide on Evaporated Metal Films. By D. Brennan and D. O. Hayward. Pp. 325–386. 23s.; 3.45 dollars. No. 1090, Vol. 258 (11 November 1965): Multiple Scattering and the Scattering of Light by Large or Small Molecules in Solution. By R. K. Bullough. Pp. 387–420. 12s.; 1.80 dollars. No. 1091, Vol. 258 (25 November 1965): The Application of Phased Arrays to the Analysis of Seismic Body Waves. By J. W. Birtill and F. E. Whiteway. Pp. 421–493. 44s.; 6 60 dollars. (London: The Royal Society, 1965.) [2611

Royal Observatory Bulletins. No. 102: Time and Latitude Service 1964, October–December. Pp. B389–B403. (London: H.M. Stationery Office, 1965.) 3s. net. [2611

Planning, Vol. 31, No. 493 (November 1965): The National Plan—Its Contribution to Growth. Pp. 331–362. (London: Political and Economic Planning, 1965.) 6s. [2611

Proceedings of the British Ceramic Society, No. 3 (September 1965): Fabrication Science. Pp. 273. (Stoke-on-Trent: British Ceramic Society, 1965.) 70s. [2611

Other Countries

Tea in Kenya: The Story of a Great Industry. Pp. 48. (Nairobi: The Tea Board of Kenya, 1965.) [2911

Publications of the National Research Council of Canada 1965, Supplement No. 1. Compiled by the Staff of the National Science Library. Pp. iii+37. (Ottawa: National Research Council of Canada, 1965.) [2911

Authors of Plant Genera. By Sydney W. Gould and Dorothy C. Noyce. (Vol. 2 of the International Plant Index.) Pp. 336. (New York: The New York Botanical Garden; New Haven, Conn.: The Connecticut Agricultural Experiment Station, 1965.) [2911

World Meteorological Organization. Technical Notes, No. 58: Tidal Phenomena in the Upper Atmosphere. By B. Hauriwitz. Pp. x+27. 3 Sw. francs. Technical Notes, No. 63: Sites for Wind-power Installations. (Report of a Working Group of the Commission for Aerology.) Pp. x+37. 5 Sw. francs. Technical Notes No. 67: The Present Situation with regard to the Application of Numerical Methods for Routine Weather Prediction and Prospects for the Future. Pp. x+64. 6 Sw. francs. Technical Notes, No. 69: Meteorology and the Desert Locust. (Proceedings of the WMO/FAO Seminar on Meteorology and the Desert Locust, Tehran, 25 November–11 December 1963.) Pp. xx+310. 30 Sw. francs. (Geneva: World Meteorological Organization, 1965.) [2911

Twentieth Annual Report of the Council of the Queensland Institute of Medical Research to the Honourable the Minister for Health for the year ended June 30, 1965. Pp. 14. (Brisbane: The Queensland Institute for Medical Research, 1965.) [2911

Nederlandse Vereniging voor Weer- en Sterrenkunde Observations of Variable Stars, January–June 1965. Pp. 4. (Groningen: Kapteyn Astronomical Laboratory, 1965.) [2911

Commonwealth of Australia. Tertiary Education in Australia. Report of the Committee on the Future of Tertiary Education in Australia to the Australian Universities Commission, Vol. 3. Pp. viii+172. (Canberra: Australian Universities Commission, 1965.) [2911

Instituto Antartico Argentino Contribucions No. 77: Notas sobre el Cruce Aero del Pasaje Drake Por E. H. Acuña Pp. 7. No. 93: La Campaña Antartica 1963–64. Por J. E. Zimmermann. Pp. 11. No. 81: Movimiento de la Barrera de Filchner, Antarctica. Por C. A. Lisignoli. Pp. 17 (Buenos Aires: Instituto Antartico Argentino, 1965.) [2911

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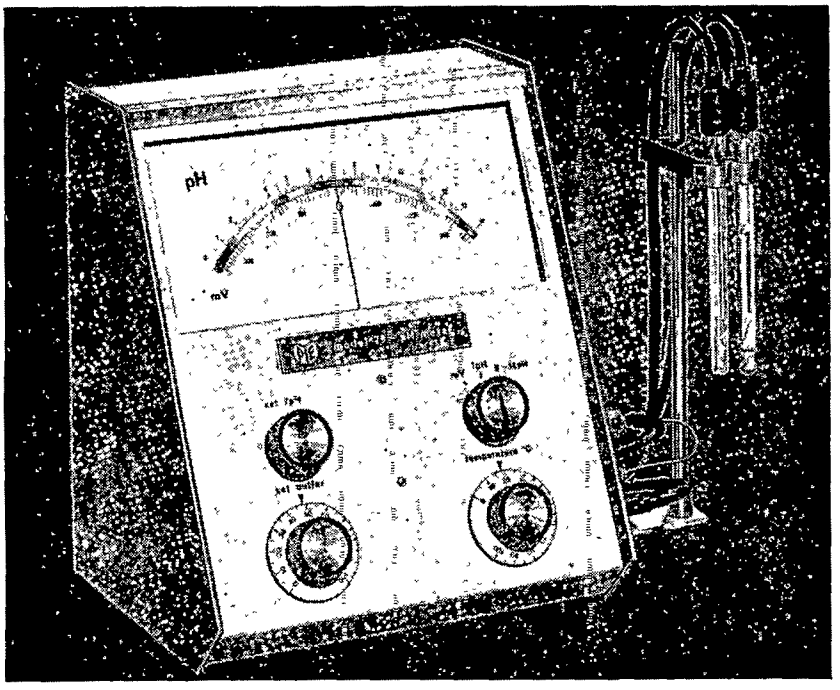
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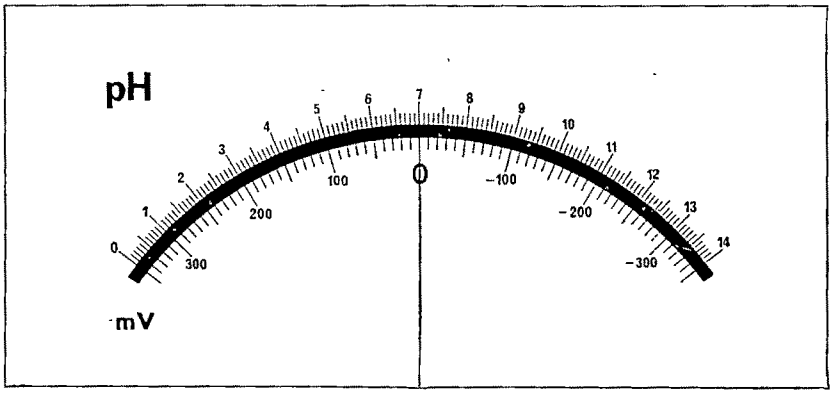
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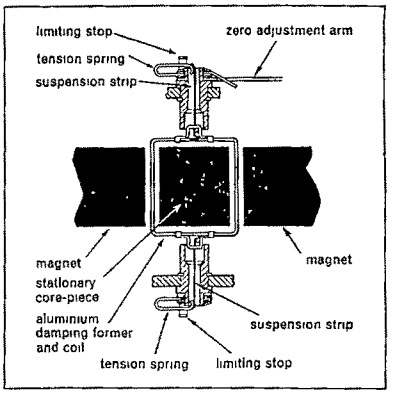
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OVERSEAS AID IN ITS PROPER PERSPECTIVE

IN *The Decade of Development**—an address delivered in London on May 3, 1965, under the auspices of the Overseas Development Institute, and now published by the Institute—Miss Barbara Ward looks back over the first 5 years of the Decade, and emphasizes the numerous lessons learnt about the processes of development which could form a springboard for better action in the rest of the Decade. The whole idea of the United Nations' 'Decade of Development' lay in the belief that the development of the continents in the southern hemisphere could be so accelerated that 10 years would be a meaningful period for fundamental change.

In basic terms, it was visualized that the 3·5 per cent annual rate of growth in gross national product achieved by the southern developing countries in the 1950's could be increased to 5 per cent and, if possible, towards 6 per cent by the end of the Decade. It was also hoped that the share of these developing countries in world trade would increase from 26 per cent to 28 per cent, and that the terms of trade (which, after the end of the post-Korean War boom, had moved rather sharply against the primary producing countries) might move back, if not by the whole 12 points lost, then at least by 8. Finally, it was hoped that the developed countries, defined by some such criterion of national income as 700 dollars *per capita* per annum, would aim by the middle of the 'Decade of Development' to be giving 1 per cent of their national income in economic assistance. There was equal emphasis on aid and trade.

At the end of the 5 years, however, the gap between the richer and the poorer nations is greater still—not because the poor have necessarily grown poorer, but because the rich have become disproportionately richer. In most of the developed countries around the North Atlantic, the annual growth rate has risen from about 5 per cent to 6 per cent. Moreover, the relative shares in world trade have not improved, not because the trade of the developing world has not increased, but because the share of the developed countries, and of their trade with each other (which constitutes more than 70 per cent of world trade), has increased even more. In proportion to their national income, moreover, the assistance given by the wealthier countries has begun to fall; with the notable exception of France, these countries give considerably less than 1 per cent of their national income. This percentage is at the moment tending to fall even further, and with the exception of those countries which have been growing at phenomenal rates of more than 7 per cent *per capita* per annum (such as Greece, Taiwan or Israel) the world in the mid-'sixties has made no progress in lessening the gap between north and south.

Against this, Miss Ward suggests that a much more judicious estimate of the difficulties in the way of development is replacing the old optimism of 'development in a decade': we now have a sense of 'development in half a century'. The relative lack of progress over the past 5 years is not necessarily a cause for discouragement, but Miss Ward insists that there is need for much more analysis, for more and better designed strategies and for a great deal more determination. She points out that colonial and semi-colonial dependence has itself hindered more rapid development. Almost to the very end of the colonial period, local leaders were in no position to make decisions; yet in any process of development there must be a power to make decisions to deal with blocks and barriers which are not overcome by the sheer momentum of the actual economic process.

Beyond this, there are the differing consequences of different sequences of cause and effect. Here Miss Ward emphasizes that, while one of the real achievements of the past 20 years in the developing world has been an increase in life-expectancy from 25 to 45 years, this has resulted in a population growth far in advance of any possible modernization of the economy. With the possible exception of Liberia, censuses held under United Nations auspices in developing countries since 1960 have shown that populations were higher and rates of expansion more rapid than expected. Growth of population, moreover, occurring after a breakthrough into greater mechanization and productivity, acts as a spur to further growth; if population expands ahead of productivity without such a breakthrough, the outlook is very different. To introduce the full technology of modernization in farming and industry requires greater saving, but an ever-increasing population living longer pushes up consumption at the expense of saving; it is important to remember that because industry was developed first, and health later, the Atlantic nations were spared the profound dilemma that confronts the developing world to-day.

Next, Miss Ward makes a point which has been repeatedly emphasized by Prof. P. M. S. Blackett and others, namely, that technology, despite its predominant role in the world of to-day, is not always appropriate to the needs of the developing countries. The whole weight of economic research and of investment in further research is virtually confined to the developed countries, and for more than 50 years has taken the form of a search for labour-saving methods of production, with the consequent substitution of machines for man-power. Large-scale labour-saving technologies at this stage of development, however, constitute the least suitable methods of production for continents such as Asia, where the one abundant resource is labour, or for countries, like many in Africa, in which the one potentially competitive resource is labour, which is still relatively cheap.

Policies which seek to promote the introduction of advanced industrial technology into countries where there is neither the market nor the skills, nor the managerial capacity or other pre-conditions for such a technology to work successfully, constitute a block in the way of development, and do little to improve economic relations between the northern and the southern hemispheres. Moreover, the disproportion in technology spills over into the field

* The Overseas Development Institute, Ltd , 1965. *The Decade of Development—a Study in Frustration?* (A lecture delivered in London under the auspices of the Overseas Development Institute on May 3, 1965.) By Barbara Ward. Pp. 23 (London. The Overseas Development Institute, Ltd., 1965.) 3*s* 6*d*.

of trade. In the developed world, the use of substitutes is constantly increasing because contemporary research concentrates largely on the products of the industrial countries. In the present world pattern of research, those adjustments which are necessary have continuously to be made by those countries which can least afford to do so, which have least economic choice and flexibility, and which are least able to adapt to rapid change.

Miss Ward thus underlines not only the comments of Prof. Blackett, but also the reasoned argument which Dr. S. Dedijer and others have advanced, for considered policy in regard to the use of science in the developing countries. Like Dr. Dedijer, moreover, she recognizes that there is a psychological difficulty, and that here again the colonial experience has not been helpful, if only because it has induced nationalist leaders to think that the economic basis of colonialism was sheer exploitation. There exists, in fact, a profound psychological disproportion between what Governments want, and what they are able quickly to achieve.

Beyond this, however, Miss Ward points out that progress has been made in selecting some of the strategic points for the most effective attack on these difficulties. She believes, for example, that we may be on the verge of a breakthrough in the field of population control. India and Pakistan are preparing to put a completely new emphasis on population policy in their next Plans; at the same time, the biological sciences are making striking advances in the understanding of the control of fertility. Taking these factors together, she suggests that within 20 years the lessening of the growth of population will not be the over-riding priority it is rightly felt to be to-day. We may, in the future, be giving far greater attention to the social aspects of population policy, namely, strengthening and enriching family life, enhancing the status and education of, and opportunities for, women and seeking to base relations between the sexes, not on licence but on friendship and respect.

She goes on to point out that far more attention is nowadays being directed to the essential inputs of mind, skill and experience required in policies of development. Education has surged forward, especially in Africa, to become the essential priority of modernization. Technical assistance to schools at every level has become the largest category of aid in manpower, while as a result of the Ashby Report, new strategies are being evolved in Nigeria to relate educational strategy to the proposed pattern of development. An effort is also being made to increase the number of people who are participating in this field of development planning: this is part of a wider realization that investment in brain-power may perhaps be even more important than investment in tools and should precede any massive increase in physical development.

One aspect of this deficiency of trained manpower is the shortage of people who can prepare projects for financing. More and more, assistance and skills must be developed to the preparation of projects, and although, in one sense, this is simply another aspect of the right administrative approach, it requires special skills. To this category of effective preparation and survey, creative foreign enterprise must be added, and here, unfortunately, the legacy of colonialism still disrupts a potentially creative field. Miss Ward questions whether the developed world sufficiently appreciates the difficulties experienced by local Governments when more than a certain proportion of their private industrial sector is foreign-owned. She suggests that in this essential field of investment far greater investigation of alternative methods of local participation is required. The idea of foreign enterprise as the spearhead, opening up new fields for ultimate local control, could be politically very attractive to many of the developing countries, while entailing no less to the investing companies; as a technique, it applies equally well to industrial as to agricultural enterprises.

There are also new perspectives in the field of trade, and she points out that the Trade and Development Conference of the United Nations sought to direct the attention of the developed world to the degree to which, by a whole sum of largely unconscious discriminations, it had so fixed world trade that most of its advantages tended to accrue to the rich rather than to the poor. A new range of proposals are now before us for dealing with these disproportions, all of which belong to a new realization of the degree to which patterns of world trade must be altered by specific political decisions. A new focus is also needed, and already a modest scheme is being prepared by the International Monetary Fund which gives primary producers virtually automatic access to the first part of their quotas from the International Monetary Fund. To encourage the provision of working capital for world trade, Miss Ward strongly supports the further proposals that the Fund should be empowered to offer certificates equal to 5 per cent increase in world trade. These certificates could be lodged with the World Bank as part of its near-liquid resources, but the International Development Authority would issue an equivalent amount of credit for long-term development loans to the poorer countries. This scheme for compensatory finance and world liquidity seems to her to cover more of the difficulties than any other strategy at present available.

Still more important, if more general, is her emphasis on the importance of an imaginative approach, rooted in neighbourliness and goodwill, to this whole question of overseas aid and development. Ultimately, in the pattern of world development, in the disproportion between the wealthy economies and the poverty of the rest of the world, policy will be decided, neither by the techniques nor the difficulties which have been described, but by an act of acceptance and an act of will. Aid as a policy will be safe when it is seen as the normal attribute of a developed society in this age of affluence.

Finally, Miss Ward suggests that, in spite of present economic difficulties, a special responsibility rests with Britain, for Britain is one of the leading industrial members in one of the few world-wide associations which includes rich and poor nations, Coloured and White, ex-imperial and ex-colonial, north and south. Membership of the Commonwealth offers a training ground, a field of experience as well as of need and contact, and of problems which present an unrivalled opportunity. Miss Ward suggests that the greatest aspiration for the British Commonwealth in the future might be to become a forcing-house, an experimental field, a partnership in the work of development which could set the pace in remaking the face of the Earth. That there is realism as well as optimism here can be seen even in the shadows of the Rhodesian situation in the attitude which Britain has so far adopted in continuing aid to those countries besides Rhodesia which have severed diplomatic relations. Miss Ward's address places the whole question of overseas aid in its proper perspective in overseas policy. In so doing it challenges further constructive thinking in those vital fields of technology and education where the scientist has so much to offer.

WILLIAM HARVEY

William Harvey
The Man, The Physician, and The Scientist. By Kenneth D. Keele. (British Men of Science.) Pp. xi+244+18 plates. (London: Thomas Nelson and Sons, Ltd., 1965.) 42*s*. net.

WILLIAM HARVEY: The Man, The Physician, and The Scientist is one of the "British Men of Science" series and is in no sense a biography, although it contains some biographical details. It is divided into three parts: Harvey the man, Harvey the physician, and Harvey the scientist. The first part contains such biographical details as are needed in this study. Harvey's career as a physician is presented fully and in an interesting way.

The main bulk of the book is devoted to Harvey the scientist and easily transcends in both interest and excellence the other two parts. *De Motu Cordis* is discussed and presented in many aspects, including the previous ideas of the functions of the heart and blood. Harvey's great book is presented and analysed in a way that makes the relevant chapters almost a companion to the work. Especially valuable is the chapter on Harvey's scientific methods, a theme which is continued throughout several chapters and becomes of great interest. Another chapter deals with the reception of Harvey's theory of the circulation of the blood; a discussion of particular interest when we can study the campaign with hindsight.

After *De Motu Cordis* the author deals thoroughly with *De Generatione Animalium* and again hindsight makes survey of the problems much easier. Especially interesting is Harvey's conclusion that fertilization of the ovum is due to some process he calls a "contagion, much the same way as medical men observe contagious diseases such as leprosy, lues venerea, plague, phthisis, creep through the ranks of men, and by mere extrinsic contact excite diseases similar to themselves".

This theme, that inevitably leads to and is explained by the spermatozoon and by bacteria, had to await the revelations of the microscope.

The author concludes inevitably that *De Generatione* compares unfavourably with *De Motu Cordis*. The essence of the contrast lies in the experimental basis of the one as opposed to the philosophical basis of the other. This is developed in a fascinating chapter on Harvey's scientific lineage, on his place in the history of discovery.

The neglect of the clinical application of Harvey's discovery is well presented and its gradual recovery through the work of Stephen Hales and the progress from Priestley's work to Lavoisier's recognition and description of oxygen and his finding of carbon dioxide in the expired air. The elucidation of the function of the pulmonary circulation thus opens the way for the clinical understanding of the whole circulation. The introduction of the Fick principle carries us further forward until Forssman's and Cournand's work on cardiac catheterization brings us near to the time when "we are at last reaping the fruits of this discovery in terms of the diagnosis of cardiac disease and treatment of the patient".

Dr. Keele concludes his invaluable book with a significant paragraph: "The true essence of greatness lies in the conquest of time. And as we now leave Harvey we can see this little sallow-faced man with the bright eyes standing like a colossus astride the great space of 2,300 years, with one foot in the ancient Greek library of Aristotle, and the other in the operating theatre of the modern cardiac surgeon".

This is an excellent book which I strongly recommend. It should be read by all those interested in the circulation, either physiological or clinical; it is much more than an essay in the history of medicine. BROCK

CONSERVATION: WHAT IS IT?

Readings in Resource Management and Conservation
Edited with Introductions by Ian Burton and Robert W. Kates, with the assistance of Lydia Burton. Pp. xi+609. (Chicago and London: University of Chicago Press, 1965.) 8.50 dollars; 63*s*.

WHEN will someone write a text-book on conservation and resource management that draws its examples and conclusions from British situations and experience and is written in simple and straightforward English? The question is prompted by two unconnected statements in the admirably succinct preface to *Readings in Resource Management and Conservation*, in which the editors explain their aim. It is "to acquaint students in courses in conservation and resource management with some of the wide variety of material to be found outside the standard texts" available in the United States and Canada. In Britain, where broadly based courses in conservation and resource management are a novelty to be counted on the fingers of one hand, there is not a single wide-ranging text-book available that has been written with the British student in mind. That such a book is required is undoubted; if one may quote from Burton and Kates (out of context) it should seek to provide "an adequate conceptual framework for resource management and conservation"—a field that is "wide, varied and amorphous". Existing (American) texts have not been notably successful in this particular endeavour, either in content or expression.

Readings in Resource Management and Conservation is an anthology of writings on conservation and the "social and behavioural science" aspects of resource management. It incorporates a wide range of selections of historical interest—including polemical statements of the type so difficult to present in traditional text-books—and papers on topics at present of concern in the United States, as well as some not readily accessible or fugitive material. All this has been arranged, and to some extent selected, in such a way as to resemble a monograph more than a text, short bridge-passages providing a linking introduction to each new chapter. This treatment is extremely effective and the editors have undoubtedly, as they express it, "taken a step towards a conceptualization of a field which lacks structure and form as it also lacks theory". (Incidentally, it was this sentence that inspired the second half of the question with which this review opened. Far too much jargon and unnecessary word-coining are blotting the literature of ecology and conservation; it is time for a cleansing and a return to simple and readily understandable English.)

A short, four-page introduction to the anthology traces the pattern of its contents: in the papers selected, "man is seen variously as a biological population, an exploiter, a conserver and a manager of resources; as an economic optimizer, a nature lover and in many other guises". Nature also is variously regarded: "now as an impersonal force that moulds the destinies of man, now as a complex web of life arranged in a delicate balance that we disturb at our peril, now as a malleable set of resources that may be used at will". The selections present a diversity of views ranging from the determinist extreme—"Malthusian in its concern with the adequacy of resources and conservationist in its prescription for policy"—to the attitude towards Nature that is "optimistic in its view of technological advance and the sufficiency of resources" and concerns itself mainly with the technical and managerial problems of development.

Part 1 of the book, "The Malthusian Equation", considers the problem of population growth and the finite earth; Part 2 is concerned with "The Conservation of Limited Resources"; Part 3 with "The Management of an Expanding Resource Base". Particularly revealing of much loose thinking are the selections in Part 2 which deal with the growth of the conservation movement and

the conservation ethic. After reading them, every reader will feel constrained to seek to define for himself (since no one else has yet done so satisfactorily) just what conservation is, what it means, and whether it is a way of life, a state of mind or a field of knowledge, or some or all of these things, or none.

Readings in management occupy more than half the book and cover a wide range of subject, type of resource, regional setting, objective and research technique. They are grouped into chapters dealing with the managerial experience, resources and economic development, scientific and technological change, and the choice of alternatives. Among the selections offered (and to quote only about a third of the titles) are accounts of the Columbia Basin and Tennessee Valley Authority projects; the management of the Harvard Forest; the conflict between fish and power resources in the Pacific Northwest; soil erosion control; conservation and stock reduction on a tribal range; wilderness perception and use; natural resources and economic development; the diffusion of agricultural innovations; and a safe minimum standard as an objective of conservation policy. Taken together, the papers "suggest a picture of managerial activity marked by less success than is normally assumed", and a "failure to anticipate change or to accommodate differences in value", both of which are intractable managerial problems.

This is a happily conceived, efficiently contrived and unusually stimulating book; every chapter of it arouses thought. It is well produced, tastefully laid out, a pleasure to handle and good value for money. It should be compulsory reading for resource managers and conservationists, however defined. W. J. Eggeling

JURASSIC STRATIGRAPHY

Jura

By H. Holder. (Band IV of the Handbuch der stratigraphischen Geologie.) Pp. xii + 603. (Ferdinand Enke Verlag, Stuttgart, 1964.) DM 129.

THE appearance of a new book on world Jurassic stratigraphy is bound to provoke comparisons with the well-known synthesis of W. J. Arkell published ten years ago, almost exactly a century after the publication of *Der Jura*, by F. A. Quenstedt, the first major treatise on the Jurassic. *Jura*, dedicated appropriately to the memory of these two eminent scholars, differs in important respects from its immediate predecessor, to which, indeed, it forms an admirable complement.

While a three-page 'retrospect' at the end of Prof. Hölder's book is no substitute for the five general chapters in *Jurassic Geology of the World*, which make stimulating reading for all types of geologist, there are a number of compensating advantages. In the first place there is a much fuller emphasis on the classical areas of Europe, especially central Europe. An abundance of illuminating text-figures replaces the admittedly often beautiful but less useful photographs in Arkell's work. Taking into account also the flood of new literature in the past decade, which receives due attention, these features suggest that Holder's book may actually prove more valuable as a reference work for the practising Jurassic stratigrapher.

The book is divided into two sections, with emphasis respectively on chronology and regional geology. Some compromise is involved here. Thus the type sections of the English Middle and Upper Jurassic are considered better dealt with in the chronological section, whereas the English Lias and south German Malm, where type sections are not considered necessary, are treated in the regional section. Such an approach is rather inconvenient for the reader, who is obliged to consult both sections to learn all he can about a given part of the Jurassic in a classical European area.

After a critical review of the evolution of the zonal concept, the development of successive Jurassic stages in the type areas and neighbouring regions is fully considered; the section ends with a palaeontological appendix devoted to a discussion of several interesting fossil groups.

Difficulties concerning the definition of the top of the Jurassic are not hedged, and Casey's correlation of the Jurassic–Cretaceous boundary in the boreal realm with the Tithonian–Berriasian boundary in the Mediterranean is viewed with scepticism. The regional section includes a concise but fair review of recent work on the British Jurassic, though the author adheres to the outdated concept of posthumous axes of uplift.

In contrast to the ample treatment of the European Jurassic, the rest of the world is dealt with somewhat cursorily and the information is not always up to date. Thus the Franciscan Group of California is now known to range in age from Tithonian to Cenomanian, and is considered to be a eugeosynclinal equivalent of the Knoxville. The Nevadan orogeny is likewise known to straddle the Jurassic–Cretaceous boundary, and no major foundered Pacific landmass need be invoked to account for the origin of the Franciscan sediments.

The cost of Prof. Hölder's fine and well-produced book is unfortunately high, but it deserves a place on the shelves of geological libraries throughout the country. One is provoked to the reflexion that, with its traditional emphasis on stratigraphic correlation by means of ammonites, it may prove to be the last comprehensive Jurassic treatise of its kind. While important correlation problems remain, notably in the Upper Jurassic, interest has begun to shift towards a much fuller understanding of the rocks and of the fossils as representatives of past life. It is indeed astonishing how few detailed sedimentological and palaeoecological investigations have been undertaken in a geological system that has received intensive scrutiny for more than a century. A. Hallam

ATMOSPHERIC AND SPACE ELECTRICITY

Problems of Atmospheric and Space Electricity

(Proceedings of the Third International Conference on Atmospheric and Space Electricity, Montreux, Switzerland, May 5–10, 1963.) Edited by Samuel C. Coroniti. Pp. xiv + 616. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 200*s*.

PROBLEMS of Atmospheric and Space Electricity is a valuable document, well edited and handsomely produced. The proceedings and extensive discussions will certainly be regarded as historic; within a very few years space-probes and directed satellites will have produced information on the atmospheres of other planets to compare and contrast with that of our own.

Though the workers represented at Montreux and their predecessors have had great successes, including the discovery and elucidation of the cosmic rays, knowledge of the electrical state of our own atmosphere is still far from complete. We have, as yet, a very limited amount of information about its outer edges, the ionosphere and the exosphere. It was, therefore, with some sense of urgency that this gathering of 160 workers in terrestrial atmospheric electricity met to survey their subject before it becomes translated to other worlds. Cosmic rays have already left their purview, the maintenance of the general ionization of the lower atmosphere has been largely reduced to order, though bedevilled by the fouling from atomic-bomb fallout. Two outstanding problems occupied most of their attention: the mechanism of thunderstorms and the electrical state of the ionosphere.

Thunderstorms offered the opportunity for a full-scale debate, almost a battle, between rival experimenters and theorists. On the one side were Latham and Mason with their well-authenticated evidence that thunderstorm charges arise from the impact of supercooled droplets of water on larger particles of soft hail, effectively at a

lower temperature, the former giving rise to minute, positively charged splinters to be blown upwards relative to the larger rime-covered and negatively-charged hail.

On the other were Workman and his colleagues from New Mexico, equally convinced that the charging arises from multitudinous contacts between hailstones and already frozen drops of water. Argument was quickly switched, therefore, to such evidence as there is about the scale of the two different activities and about the milieu in which they occur. The key question which soon emerged was to what extent the thundercloud can accumulate charge of one polarity in one region and of the opposite polarity in another. As Dr. Ross Gunn was to emphasize, full understanding can come only when we have reliable determinations of the nature and the concentrations of the various charge carriers as well as their velocities. Existing evidence is little more than speculation and the difficult task of distinguishing between the two schools (not to mention a third claimant, Dr. Vonnegut, who has propounded a purely convective theory of the thunderstorm) now lies with the meteorologists. At the present time, in the words of *Exodus* IX, verse 24, we are left with "hail, and fire mingled with the hail, very grievous".

On the second major question an important paper was presented by E. C. Whipple, jun., on the electrification between the troposphere and the bottom of the *D* layer of the ionosphere, a region inaccessible to balloons and difficult to explore from rockets, which disturb the local environment. This is, however, considered the outstanding task for space-vehicles. The day is not far distant when the problems of the dusty atmosphere of Venus and the gigantic atmosphere of Jupiter with its turbulent red spot will present new targets for our ingenuity. In the meantime we have, as an urgent task, to study intensively the language of the radio-emissions from both of them with earth-bound equipment.

A peaceful battle was followed by a feast of informative papers. Many will be stimulated by them. All are worth reading.

BASIL SCHONLAND

ABERRATIONS OR REVELATIONS?

Drugs of Hallucination

The Uses and Misuses of Lysergic Acid Diethylamide. By Sidney Cohen. Pp. xviii + 268. (London: Secker and Warburg, 1965.) 30*s*. net.

DR. COHEN, who is a psychiatrist, has written a good, popular account of the hallucinogens, especially lysergic acid diethylamide (LSD), at a cost of less than a penny a page. *Drugs of Hallucination* is open-minded but not detailed, fair but not neutral. The dangers of therapy with LSD, to therapist as well as patient, are emphasized no less than the enlightenment of "instant Zen". The book describes admirably the terrors as well as the joys of LSD-taking, with the aid of lengthy extracts from subjective accounts. These were, of course, made after the experience had ended: at the time, the subject is relatively (sometimes absolutely) mute, either catatonic or entranced with his jewel-studded umbilicus. It is worth pointing out once again that even those who have enjoyed the experience are invariably dissatisfied with their subsequent attempts to put it on paper. Whatever Mr. Wilson and Mr. Trocchi say, drugs do not help the user to express himself. They cannot help those who have never written to write at all, and those who are at best indifferent writers continue to write indifferently.

The volume contains an elementary account of hallucinogen chemistry in one appendix, and the sketchiest of phenomenological comparisons of sensory deprivation, psychotic experiences and experiences of LSD in another. There is also an index. What there is not—and this is really unforgivable in a book on a serious subject, even if it is intended for a lay audience—is adequate documentation of statements. There are fourteen titles in the bibliography, which are perhaps enough, for the literature on this subject is not of such outstanding quality that it needs to be quoted extensively. Even so, they are not altogether the best titles, nor relevant at all in some cases: and, what is most serious, they contain no mention of his own experimental work; and Dr. Cohen draws on this continually in the body of his book. Since he nowhere in the book describes what he did, to how many, when and with how much (all important matters to the psychopharmacologist), the omission is, to say the least, a nuisance.

Dr. Cohen is a respected and careful scientist, however, whose professional papers can easily be found by reference to the proper sources. This cannot be said for many so-called 'workers' in the field of the hallucinogens, reports of whose activities are all too frequently found in sensational rather than scientific literature. It is an ethical condition of work that the scientist should make his results available in a form that allows any other scientist to attempt to reproduce them, as a preliminary step to building on them. This condition is too seldom met in a field of research that may be as important to the future of mankind as the wheel or nuclear fission. Any tool that may possibly help unravel the torments of our existence is worth close, critical and documented examination.

Most of us are inclined to regard the intellect as the most interesting characteristic of man: but his future development may well depend on deeper understanding and use of his emotions, and research of the best kind into the hallucinogens may be invaluable here. It may be that the effects of LSD are so variable, even in the same individual, because its substrate is the minute-to-minute emotional state of the subject. Such drugs as alcohol or amphetamine are also variable in their effects but these are relatively predictable, perhaps because they depend on mood. If LSD itself works below the level of the intellect, it is all the more important that those who investigate it should not do so as well. As Dr. Cohen points out in his simple but good book, a disturbingly high proportion of those who administer LSD to others go mad themselves!

C. R. B. JOYCE

USE AND MISUSE OF LSD 25

Utopiates

The Use and Users of LSD 25. By Dr. Richard Blum and associates. Pp. xv + 303. (London: Tavistock Publications, Ltd., 1965.) 58*s*. net.

LYSERGIC acid diethylamide (LSD) has been widely used as a research tool in neuropsychopharmacology, and as an adjunct to psychotherapy. It is also, unfortunately, being misused, particularly in the United States, as a drug purveying what has succinctly been called 'instant Zen'. *Utopiates: The Use and Users of LSD 25* is a sociological survey of this phenomenon to try to determine who uses the drug in this manner and why. Naturally, the material was not easy to obtain, but Dr. Blum and his co-workers managed to contact ninety-two persons who had taken LSD. These they divided into four groups: (1) an informal professional group, mainly psychiatrists, who had taken the drug outside any recognized research project; (2) a therapy patient sample who had been given LSD by a psychiatrist as part of treatment; (3) an informal black-market sample; (4) the religious-medical centre group associated with an organization (IFIF*)–using the LSD experience for a defined religious purpose. These were analysed with respect to a very large number of factors, including type of person involved, their motives, types of reaction obtained, repeated use, proselytizing activity, community attitudes, group interactions, etc. This provides a mine of interesting data on the present-day American scene. The over-all theme appears in the form of earnest attempts by followers of Aldous Huxley to use

* International Federation for Internal Freedom.

LSD for social, personal and religious purposes, and the no less earnest opposition by others who believe this to be dangerous, misguided or wicked. Dr. Blum's survey is factual and liberal in attitude. It turns up some facts of general psychiatric interest. For example, he reports that six out of a known population of 300 LSD users had psychotic reactions of such severity to necessitate hospital admission "for short or long periods". It was felt to be impossible to predict who would get an LSD psychosis on the basis of previous personality aberration. The second fact is that one-third of subjects who had taken LSD as volunteers in a recognized research project went on to take it again on their own, in some cases regularly.

A brief but admirable chapter by Keith Killam follows on the psychopharmacological aspects of LSD, particularly its dangers. This is followed by reports on the "Mexican Psychedelic Training Centre", established by Leary and Alpert. The first is by a psychiatrist outside the group—J. J. Downing. This is again tolerant and yet does not gloss over the difficulties involved, which finally led to the expulsion of the group from Mexico. The second is by Leary and Alpert themselves and gives their own point of view.

Joel Fort, a specialist in the treatment of alcoholism, puts LSD in its proper perspective against the grisly facts of the misuse of alcohol, barbiturates, amphetamine, etc., in our culture.

A particularly fascinating chapter is a survey by Blum of police attitudes towards drug uses. A sample of 27 police officers was asked to rate people of different categories as to their degree of menace to the community. Members of the United States Communist Party came out on top (score 27), followed by heroin addicts (26); LSD users (20) came middle in the list below the Mafia and Black Muslims (23), but above members of the John Birch Society (16).

The essays conclude with a forthright statement by Edward Comber, director of the Bureau of Criminal Information of the San Francisco Police Department, that LSD use, outside medical and research projects, is both dangerous for the individual and society and morally wrong.

J. R. Smythies

MASTERS OF MICROBIOLOGY

Three Centuries of Microbiology

By H. A. Lechevalier and M. Solotorovsky. Pp. 536. (New York, London and Sydney: McGraw-Hill Book Company, 1965.) 12.50 dollars; 100*s*.

Three Centuries of Microbiology opens with a preface which contains the following explanatory statement: "Since we feel that nobody can explain a discovery as well as the person who made it, our text contains numerous, often extensive, quotations from classical papers that outweigh the volume of our own writing. Since many of the papers that we are quoting were not written in English, our work has often been that of translators." This adds considerably to the value of the book.

The book is essentially a collection of biographies of outstanding microbiologists and of other scientists whose work has influenced them. It is divided into 12 chapters. Each chapter has its own useful list of references, usually to biographies of the scientists concerned, and the book closes with a good working index.

The first chapter is a historical survey, starting with Girolamo Fracastoro (1483–1553), whom the authors describe as "the father of the germ theory of disease". It briefly describes the general scientific background to the development of the microscope and the gradual development of microbiology up to the time of Pasteur, a period of more than 300 years, illustrating this with some twenty 'microbiographies'. One chapter each, of about fifty pages, is devoted to the lives and work of Pasteur and Koch. Most of the information is readily available in other published biographies, but it is interesting to note that the origin of the key word 'microbe' is given by Pasteur in a joint paper with Joubert and Chamberland (*C.R. Acad. Sci., Paris*, 86, 1037; 1878) as a "new and fortunate expression proposed by M. Sédillot" (*ibid.*, 86, 634; 1878).

A lengthy chapter entitled "Bacteria as Agents of Disease" deals with work in both Europe and America on tuberculosis, diphtheria, bubonic plague, tetanus, dysentery and bacterial plant diseases. The workers mentioned include Villemin, whose "Studies on Tuberculosis" (1868) proved an inspiration to Koch and who furnished conclusive proof that tuberculosis was contagious, Loffler, Gram, whose method of differential staining of bacteria made an important contribution to bacteriology, Roux, Yersin, Kitasato, the pioneer Japanese bacteriologist, Shiga, T. J. Burrill, who was the first to indicate the role of bacteria as disease-producers in plants, and Erwin F. Smith, whom the authors describe as "the Koch of phytopathology".

The next two chapters discuss immunology, cellular and humoral. The first of these chapters is concerned chiefly with the lives of Metchnikoff and Almroth Wright and their work on phagocytes. The second traces the development of serology, work on anti-toxins for diphtheria and tetanus, BCG vaccine for tuberculosis and antigens, referring extensively to the work of Nuttall, Behring, Calmette, Guérin, Bordet, Richet and others. Under the heading "From Soil Microbiology to Comparative Biochemistry" the authors describe the work of Winogradsky and Beijerinck on nitrogen fixation in the soil by microorganisms, and the work of Kluyver on microbial biochemistry. The chapter headed "Viruses and Rickettsiae" traces the elucidation of the nature and properties of these micro-organisms and deals in detail with the work on the diseases caused by them and with the introduction of the electron microscope which proved such an important tool in the study of viruses and in the whole field of microbiology. A chapter on mycology gives an account of the origin of interest in fungi and describes the development of fungicides and of medical mycology. A chapter on protozoology describes the early work on the nature and classification of protozoa by Hooke, Leeuwenhoek and others which led to the realization of the responsibility of pathogenic protozoa for diseases such as malaria, and ends with good accounts of such workers as Theobald Smith, Bruce, Laveran, Manson and Ross. Under the title "Chemotherapy" the authors trace the three major paths in its development: the use of natural products such as quinine, the search for synthetic agents resulting in salvarsan and prontosil, and the investigation of the metabolic products of micro-organisms which led to the discovery and development of the antibiotics penicillin, streptomycin, chloramphenicol, the tetracyclines and the semi-synthetic penicillins. In connexion with penicillin, I wish to correct a statement, which appears on page 468 of this book and in almost all the numerous books on this important mould metabolite, to the effect that the work carried out in my laboratory at the London School of Hygiene and Tropical Medicine was brought to a premature close after the publication of the paper by Clutterbuck, Lovell and Raistrick (*Biochem. J.*, 26, 1907; 1932) because of the death of Clutterbuck in 1932 and Lovell's departure to the Royal Veterinary College in 1933. In fact, Dr. P. W. Clutterbuck died at the end of 1937, having, in the interval since 1932, published a number of valuable papers.

The final chapter on genetics starts with Mendel, described as "the founder of genetics", born in 1822, and de Vries, who, working with plants, introduced the concept of mutation, and goes on to describe the work of microbial geneticists including Beadle, Tatum, Avery, Lederberg and Pontecorvo.

The authors conclude the book with an epilogue in which they say: "More than a hundred years have passed

since Pasteur started to investigate the secrets of the simplest forms of life, which have turned out to be so complex". They are to be warmly congratulated on having produced such a readable history of this fascinating subject. I recommend the book to all microbiologists and other scientists of catholic tastes.

HAROLD RAISTRICK

ALL THE MAMMALS

The Mammals

A Guide to the Living Species. By Desmond Morris. Pp. 448 (300 photographs). (London: Hodder and Stoughton, Ltd., 1965. Published in association with The Zoological Society of London and Granada TV Network.) 63*s*. net.

THE Mammals: A Guide to the Living Species deals with the orders of mammals in turn. We are given a classified list of the living species of each order, followed by photographs and brief descriptions of a selection of the species. The lists will presumably be used mainly by zoologists and it is rather surprising to find them in a book which is otherwise designed for a much wider public.

The lists have been compiled mainly from a dozen fauna lists which between them cover the world. They give the systematic position, scientific name and English name (if any) of each species, with an indication of distribution and a reference to a taxonomic work where fuller details may be found. The authors of species, and synonyms, are omitted. The lists of bats and rodents have been kept reasonably short by giving the number of species, and not their names, whenever a genus includes several species. Twice as much space would have been required to list the mammals if this had not been done. The lists of the other orders include all the specific names. Dr. Morris estimates that a complete list of the species of living mammals, with synonyms and authorities, would occupy at least 2,000 pages. We may regret that he has not been able to give us such a list, but he has provided a very useful list in the 80 or so pages that he (or his publisher) has felt able to spare for the purpose. There does not appear to be any other modern list of the mammals of the world.

There are, it appears, 4,237 known species of mammals. 300 of them have been selected for illustration and brief description. These include a much higher proportion of the primates, carnivores and ungulates than of the insectivores, bats and rodents. This is inevitable, because the latter group of orders is so large and because the former one is so well represented in zoos. Dr. Morris is curator of mammals to the Zoological Society of London, and most of his readers must depend largely on zoos for their experience of non-domestic mammals. Nevertheless, I would gladly have given up a few of the duller monkeys in return for some more zalambdodont insectivores.

The descriptions deal with the more remarkable features of the habits and anatomy of each species. The selection of material for them has been excellent, though it was, perhaps, a mistake to give every species the same amount of space. In some cases, when other very similar species have been described, the requisite 250 or so words seem to have been hard to find. In other cases, they are not nearly enough. In the account of the European rabbit, for example, burrows and refection are discussed, but there is too little space to cover the equally interesting topics of myxomatosis and the reabsorption of embryos. A few opportunities have been missed; there is, for example, no mention of the difference in echo-location mechanism between rhinolophid and vespertilionid bats, which could be illustrated very well by photographs. No authorities are cited for the information given in the descriptions.

Most of the photographs are of captive animals. A red deer does not look its best against a background of bars, wire netting and a perambulator. A few of the photographs are poor, but most illustrate their subjects adequately.

The introduction includes a very worthwhile attempt to explain the success of the mammals in popular terms. Fuller explanations of some points would have been welcome, and it seems unfair to write about the mammals "eliminating the inefficient mixing of venous and arterial blood" since recent experiments indicate that very little mixing occurs in reptile hearts[1,2].

This is a useful book, and an attractive one.

R. MCN. ALEXANDER

[1] White, F. N., *Anat. Rec.*, **135**, 129 (1960).
[2] Khalil, F., and Zaki, K., *Z. vergl. Physiol.*, **48**, 663 (1964).

PLANT ANATOMY

Plant Anatomy

By Prof. Katherine Esau. Second edition. Pp. xx + 767 (96 plates). (New York and London: John Wiley and Sons, Inc., 1965.) 105*s*.

ALTHOUGH plant anatomy can no longer claim so great a share of a well-balanced botany course as in former years, it is equally certain that no botanist should be without a sound knowledge of plant form and structure. The corresponding need for a good modern text-book of plant anatomy has now been met by this second edition of Prof. Esau's *Plant Anatomy*.

The previous edition of this book, published in 1953, received much praise from many users, and all the good things said about the first edition apply equally to the present one. The clearly written text is illustrated by many text-figures and by a large number of photographs, which have been increased to 96 plates in this edition, as compared with 85 in the first edition. There have been few changes in the general plan of the book, and with one exception, Chapter 13, with the new title "Secretory Structures", as opposed to the previously more restricted topic of "Laticifers", the chapter heads remain the same. A great deal of new information has been incorporated in the text, but appropriate condensations and omissions have enabled this integration to be made without an undue increase in length, now 767 pages instead of 735.

The principal changes have naturally been in the sections where recent research has been active, notably in the chapters dealing with "The Protoplast" and "The Cell Wall", which now include condensed, but informative, accounts of modern ultra-structural research. The chapters on "Meristems and Differentiation" and "Apical Meristems" have been extended to cover new developments, such as the culture of isolated cells and experimental studies by various workers on plant morphogenesis.

But this new edition, like the previous one, is primarily concerned with the broad treatment of fundamental aspects of plant anatomy. As a consequence of this approach it is inevitable that individual readers may be disappointed with the curtailed accounts of certain special topics. Thus, there is little consideration of the interesting differences in the detailed structure of secondary xylem which permit the identification of commercially valuable timbers. There is also only brief mention of the varied anatomical modifications characteristic of plants with different ecological requirements. On the credit side, the omission of details of this kind has permitted the inclusion of chapters dealing with "The Flower", "The Fruit" and "The Seed".

In sum, this book is of value both as a reference work for any student of botany, and even for general reading by those interested in acquiring a good grounding in plant anatomy. It is above all a scholarly treatment in which the author has carefully selected the more significant items from the great mass of detailed information available. The book is well produced and the two drawings of transverse sections of collenchyma make an attractive and appropriate dust-cover.

A. ALLSOPP

NEW BIOCHEMISTRY BUILDING, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

THE new biochemistry building, which is under the direction of Prof. E. B. Chain, was formally opened on November 4, 1965, by H.M. Queen Elizabeth the Queen Mother, chancellor of the University of London, at a ceremony presided over by Lord Sherfield, chairman of the Governors of the Imperial College of Science and Technology, in the presence of Sir Patrick Linstead, rector of the College, Sir Thomas Creed, vice-chancellor of the University, and its principal, Sir Douglas Logan.

The construction of such a large and well-equipped university biochemistry department is an exceptional event and marks the importance which the Imperial College of Science and Technology attaches to this rapidly expanding discipline.

While this represents a new phase of the College's interest in biochemistry, it is now more than 50 years since the subject first received official recognition. Thus, in 1913 a small Sub-department of Biochemistry was set up in the Department of Botany under the direction of S. B. Schryver, who was appointed full professor in 1920. On his death in 1929 he was succeeded by a former pupil, A. C. Chibnall, who was subsequently made full professor in 1936. Prof. Chibnall resigned from the College in 1943 to take up the Sir William Dunn chair of biochemistry at Cambridge, after which the chair at Imperial College remained vacant, although some research was continued under the direction of Dr. H. W. Buston.

With the rapid post-war progress in biochemistry, it was felt, in the mid-1950's, that the College should modernize and considerably expand the existing facilities for biochemical research to a level commensurate with that of the other well-established scientific and engineering departments. As a first step it was, therefore, necessary to re-establish the chair of biochemistry. In the course of a visit to Rome, Prof. P. M. S. Blackett saw Prof. E. B. Chain, who was responsible for the creation of the internationally recognized Biochemistry Department of the Istituto Superiore di Sanità. His Department was comprehensively equipped for metabolic studies and included an extensive fermentation pilot plant, and the possibility of setting up a similarly equipped department at Imperial College was discussed. Following a visit to Rome by the rector, Sir Patrick Linstead, a tentative project was prepared. Unfortunately, analysis of the estimates for constructing a department of the desired dimensions showed the cost to exceed the funds then available and the scheme was temporarily shelved.

In 1959, Prof. Chain discussed the financial difficulties of the project with the late Lord Nathan, who was the first chairman of the recently founded Wolfson Foundation. Lord Nathan suggested that the College should apply for support to the Trustees of the Foundation who, following numerous prolonged discussions, agreed, subject to the appointment of Prof. Chain to the chair, to contribute £350,000 towards establishing the new department. This would include the building and equipping of modern laboratories for biochemistry and microbiology to be known as the Wolfson Laboratories.

The original plan was to build the Wolfson Laboratories, including the fermentation pilot plant, at the rear of the Royal College of Science block and to meet the remainder of the Biochemistry Department's space requirements by adapting part of the existing building. Following the subsequent approval by the University Grants Committee, however, of a scheme to redevelop the present site of the Royal College of Science, these plans were changed and it was decided to house biochemistry in a completely new building, which was seen as the first stage of this redevelopment.

Prof. Chain was appointed to the vacant chair of biochemistry and took up his appointment on October 1, 1961. Demolition of the old building began early in 1962, and the new laboratories, having a total floor space of 63,000 ft.^{2} (not including the pilot plant), were completed in the summer of 1965 at an approximate cost of £1·2 million. The architects were Architects' Co-partnership and the main contractors Tersons Ltd.

The Department is designed and extremely well equipped for research covering a broad range of metabolic studies in animals and micro-organisms and to a lesser extent in plants. In addition to these more usual aspects of biochemical research, there is particular emphasis on the development of instrumentation such as the automation of analytical and fermentation processes.

The financing of the research is being greatly facilitated by three major sources of funds which are supplementary to the normal awards of the University Grants Committee. These are the Science Research Council, which is making a grant of £100,000 in support of research on fermentation biochemistry and technology and biosynthetic mechanisms in micro-organisms; the Trustees of the Fleming Memorial Fund for Medical Research, who have granted £50,000 over 5 years for basic medical research; and the Medical Research Council, which has established a Metabolic Reactions Research Unit under the honorary direction of Prof. Chain.

The research programme is primarily a direct extension of the various lines developed by Prof. Chain and his colleagues at the Istituto Superiore di Sanità during the past 15 years where, at the invitation of its director, Prof. Domenico Marotta, he had organized and directed the Biochemistry Department which included the International Centre for Chemical Microbiology.

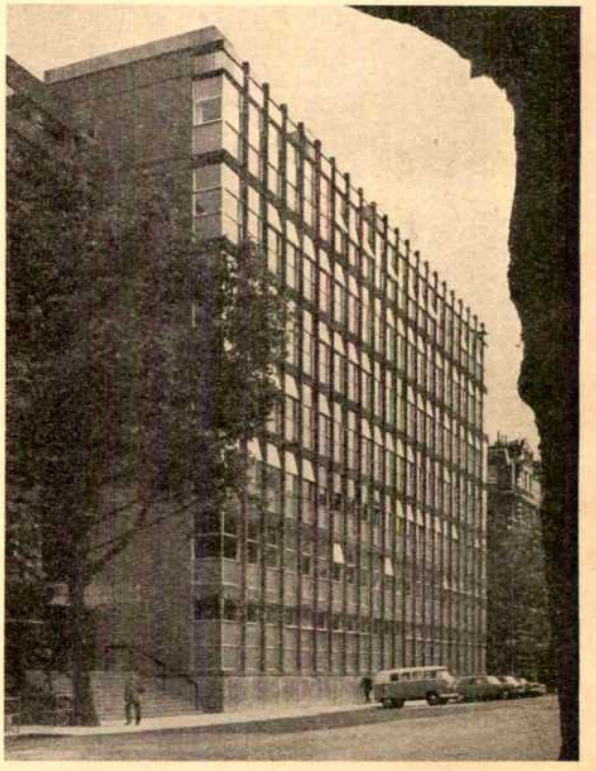


Fig. 1. Front view of the new biochemistry building, Imperial College of Science and Technology, London

The new Department contains 33 laboratories and three classrooms located on six levels, providing accommodation for about a hundred research workers and postgraduate students and the appropriate technical staff. An additional number of rooms, which are not occupied for prolonged periods and, therefore, do not require daylight, are located in the 'inner core' of the building; they are ventilated and house instruments, high-speed centrifuges, chromatography equipment, wash-ups, incubator rooms, store rooms and photographic equipment.

The basement contains a large mechanical workshop and air-conditioned and temperature-controlled animal houses. All the laboratories on the second level are devoted to research on animal biochemistry, the centre of interest being the study of hormonal regulatory mechanisms and of neurochemical problems. For the more physiological and pharmacological aspects of this research, special physiological laboratories are provided on the third level. The Metabolic Reactions Research Unit is housed in the biochemical and physiological laboratories on the second and third levels.

The laboratories on the fourth level are designated partly for research on problems of chemical microbiology and partly for isotope analysis by Geiger–Müller counting techniques, scintillation counting and mass spectrometry. A 'hot' laboratory for the preparation of ^{14}C- and tritium-labelled compounds is also available. The research problems under investigation include studies of biosynthetic pathways of microbial metabolites of biological or biochemical interest and the enzyme systems involved, the elucidation of the chemical structures of new compounds belonging to this category and some aspects of non-specific immunity.

In close conjunction with and in support of the biochemical studies, microbiological investigations are carried out in laboratories located on the sixth level. These are concerned with investigations of the morphology, physiology and genetics of the micro-organisms producing the metabolites under investigation, strain selection and improvement, some of the more botanical aspects of the interaction between fungi and plants and tissue culture.

For the development of new instrumentation required for the sections of animal biochemistry and chemical microbiology, as well as the fermentation pilot plant, a series of electronics laboratories is provided on the fifth level. The development of automatic scanning devices for the quantitative evaluation of bi-dimensional radiochromatograms and various kinds of column chromatography procedures, with the computerized evaluation of the results, is under active investigation; other problems under investigation include the automation of fermentation processes and analytical procedures. Adjoining the instrumentation section is a series of laboratories for the study of new methods of biochemical engineering, particularly in the fermentation field.

A special feature of the Department, and one that is unusual for a university biochemistry institute, is its fermentation pilot plant of semi-industrial dimensions for the bulk production of (non-pathogenic) micro-organisms and microbial metabolites of biochemical or biological interest, and the study of fermentation processes of industrial interest. It contains a series of stainless steel fermenters of 20, 50, 300 and 3,000 l. capacity which are run continuously 24 h a day on a shift basis, and is provided with all the filtration and extraction equipment required for working up the largest quantities of micro-organisms and culture media obtainable in the fermenters. Attached to the pilot plant is a series of microbiological laboratories for the preparation of the inocula and for routine microbiological and chemical tests. The pilot plant serves the needs of the chemical microbiology section of the Department in the first instance, but it is hoped that it will eventually be possible to meet at least some of the requirements for microbiological material frequently occurring in outside university and other biochemical research laboratories.

No undergraduate studies are planned, but a 2-year postgraduate course in general biochemistry, chemical microbiology and fermentation techniques leading to the award of Diplomas of Membership of the Imperial College of Science and Technology (D.I.C.) and M.Sc. degrees was initiated at the start of the present session. Teaching laboratories to accommodate forty postgraduate students and a seminar room are located on the third and fifth levels.

The underlying unification of the apparent diversity of research activities is seen as a consequence of the universal nature of most fundamental biochemical processes, in the detailed investigation of which it is obviously desirable to minimize any limitations on the choice of the most suitable experimental biological system. This thesis was strongly argued by Prof. Chain in his inaugural lecture, which he entitled "Landmarks and Perspectives in Biochemical Research".

In this lecture he outlined the major historical landmarks in biochemical research and detailed his own approach to the subject, while in his closing remarks he directed attention to what he considered to be important prerequisites of successful research. With regard to the immensity of United States contributions to biochemistry, it was emphasized that, although in Britain there must of necessity be a proportionately smaller number of research institutes, there is no reason why the individual units should not be at least as well equipped as their United States counterparts, and furthermore that there is much to be gained by close contacts with our colleagues in the United States involving regular visits by both senior and junior research workers. However, in addition to overcoming the frustration inherent in inadequate financial support for research, he emphasized a less frequently stated but equally important problem, namely, the lack of senior positions for young, promising biochemists who are consequently prone to lend sympathetic ears to the overtures of overseas laboratories.

In order to nurture the spirit and energy which are the birthright of any new department, it is vital to recognize and take all possible steps to avoid those frustrations which alone can prejudice the enthusiasm, dedication and congenial research atmosphere so essential to the successful study of the secrets of nature. ROBERT THOMAS

EVAPORATION OF LAVA AND ITS CONDENSATION FROM THE VAPOUR PHASE IN TERRESTRIAL AND LUNAR VOLCANISM

By DR. BERNARD VONNEGUT, ROBERT K. McCONNELL, jun., and RONALD V. ALLEN
Arthur D. Little, Inc., Cambridge, Massachusetts

STILL and motion pictures of eruptions on the volcanic island of Surtsey, taken in February 1964, show an interesting phenomenon associated with the fountains of molten lava. When the fountains are active, a dense cloud of dark smoke forms, which is carried off by the wind (Fig. 1). More recent observations of this volcano reported by Björnsson[1] in the summer of 1964 describe a similar phenomenon in which a cloud of dark smoke was observed to form down-wind of the quiet lake of molten lava that filled the crater.

These dark clouds, except for their colour, are quite similar in location and appearance to the clouds of white steam from erupting geysers and from heated pools in geothermal areas. The resemblance between the steam clouds and the volcanic clouds is so pronounced that in our opinion there is little doubt that they are formed by a similar mechanism. It appears that the hot lava has a high enough vapour pressure for appreciable quantities of it to evaporate. On mixing with the much cooler atmosphere the vapour will condense to form visible aerosol particles in much the same way that condensing water gas forms the visible aerosol particles called 'steam'. If one extrapolates from the known features of the condensation of water and other substances to form aerosols, it seems reasonable that the lava vapours may condense either as vitreous droplets or as small crystalline particles.

If particles can condense from the gas phase over lava fountains and lakes, similar processes might take place in a phreatic eruption; some gaseous components from the hot lava may be present in the gas phase of the high-velocity eruption which will condense to form an aerosol when cooling takes place by adiabatic expansion or by mixing with the atmosphere. While the larger particles of tephra in a volcanic eruption are undoubtedly formed by mechanical disruption of lava, it appears quite possible that some of the smaller tephra particles of an eruption result from the condensation and growth of aerosol particles from the vapour phase. Thus the cloud-forming process in the phreatic eruption may be similar to that occurring in the screening smoke generators developed by Langmuir and Schaefer[2]. In these a high boiling petroleum oil was vaporized along with water and then caused to escape at high velocity into the atmosphere where it condensed as an aerosol.

All the evidence suggests that the spectacular meteorological optical phenomena, such as vividly coloured sunsets and Bishop's rings, associated with volcanic eruptions are the result of very small particles. Since the optical phenomena extend over large portions of the globe and persist for months or even years[3], the particles must be so small that they settle very slowly. deBary and Bullrich[4] state that in order to have Bishop's rings it is necessary for these to be present: (*a*) an abnormally dense layer of aerosol particles with almost identical radii of 0·3–0·4μ at a constant altitude over a wide area in the vicinity of the observer; (*b*) a concentration of aerosol particles 5–10 times greater than usual. The aerosol particles are so small and so uniform that it appears unlikely that they result from mechanical disruption. It seems far more probable to us that this class of particulate matter is the result of condensation from the vapour phase.

Thus it seems likely that the formation and properties of the high-temperature clouds of the volcano are similar to ordinary atmospheric water clouds. It would therefore be worthwhile to obtain quantitative information concerning the partial pressures of the lava vapour during volcanic eruptions. If the vapour pressures are comparable with the partial pressures of water vapour in the atmosphere, a significant portion of volcano clouds and of the small tephra particles may be formed by a condensation and coagulation process similar to that which occurs in water clouds. Conceivably, if the partial pressure of the lava components is sufficiently high, the heat released by the formation of liquid or solid cloud particles may be of significance in the formation of nuées ardentes.

We have carried out some preliminary laboratory experiments that confirm that aerosols are produced when hot lava evaporates into the atmosphere. We have observed smoke to form when basalts are melted on an electrically heated wire filament or in a crucible. Basalt heated in air to about 1,400° C in a platinum crucible produced a light brown condensate on a water-cooled glass flask above the crucible (Fig. 2). A mixture of basalt and dunite heated to about 1,500° C under reducing conditions (in a graphite crucible in argon) produced a 'smoke' containing dark brownish-grey structures resembling cobwebs. X-ray diffraction of these 'cobwebs' indicated the presence of silicon, SiO_2, Mg_2Si and MgO. X-ray fluorescence of both deposits showed strong evidence of potassium, calcium, iron and silicon.

It is apparent that, in the processes we observed to occur over Surtsey, the ambient atmosphere is of dominant importance. The presence of an atmosphere affects the formation and deposition of aerosols in at least four ways: (1) it maintains a high enough concentration of the lava

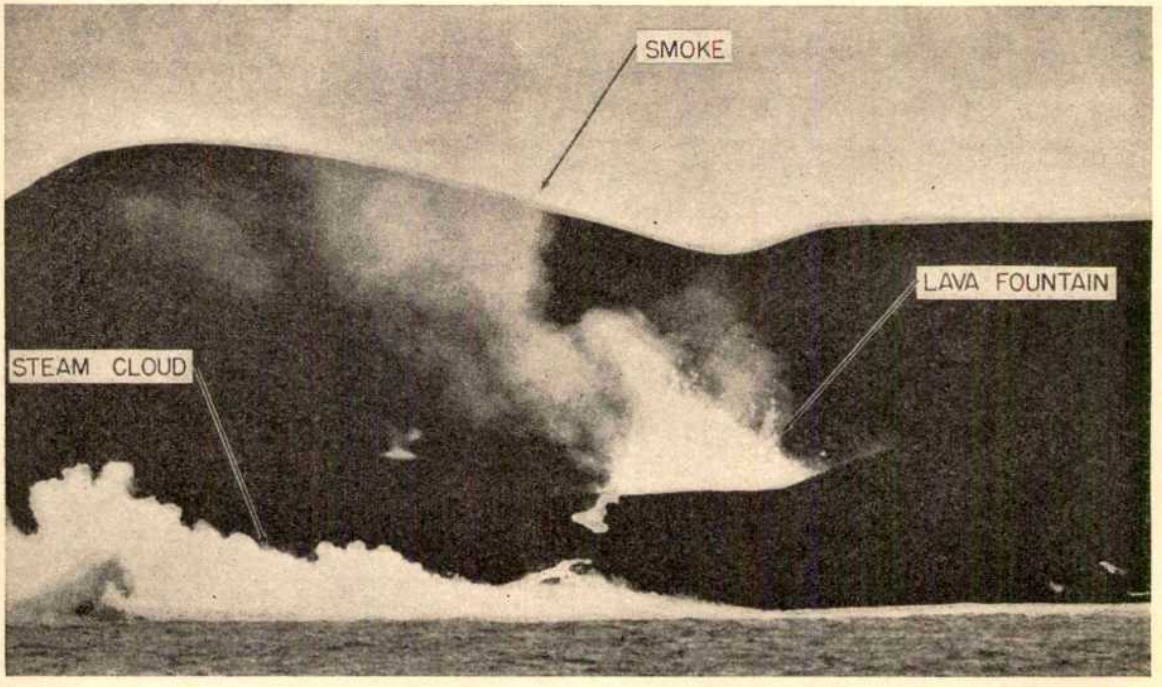


Fig. 1. View of the new island, Surtsey, with cloud of dark smoke above lava fountain. White plume in foreground is steam cloud caused by contact of lava flow with sea water. (Photo by Sigurgeir Jónasson)

gas to allow sufficient supersaturation for particle formation by condensation; (2) it causes sudden quenching of the hot gases by mixing and expansion; (3) it prevents rapid falling of the particles; (4) it transports the particles by the wind.

Because of the great influence of the atmosphere on the behaviour of hot lava vapours, it is interesting to consider what sort of processes might take place in Nature under conditions of very low atmospheric pressure such as those which exist on the Moon. Here, when silicates vaporize from volcanism or other causes, the results will be different. At these very low pressures the mean free path of the molecules will be large and the chances of their coming together to condense to form a particle will be negligibly small. Using an analysis similar to that of Watson *et al.*[5] it can be shown that on the Moon most of the species evaporating from the surface of a rock melt will move at less than escape velocity and will return to the Moon's surface. Here they may condense to form a structure that is frost-like or dendritic in character. Following their analysis it can also be shown that the average distance a molecule of metallic oxide evaporated from a melt will travel before landing on the Moon's surface will be of the order of several hundred kilometres. This ensures relatively uniform deposition over a substantial fraction of the surface from even a small area of melt.

To investigate effects of vacuum we performed a simple experiment by heating about 1/5 g of basalt to about 1,200° C in a vacuum of 10^{-4} torr for a few minutes. This produced a dark brown film several microns thick on a microscope slide suspended above the melt. Again X-ray fluorescence showed strong lines of potassium, calcium and iron. No good X-ray diffraction peaks were observed from the thicker parts of the deposit, but under the optical microscope small particles showing apparent crystalline outlines were observed on the thinner parts of the film (Figs. 3 and 4). Dark-field electron microscopy of thinner parts of the film from the slide showed the presence of crystals ranging in size from several hundred to several thousand angstroms.

The observed photometric properties of the surface of the Moon are consistent with the idea that vapour deposition may have played a part in its formation. Hapke[6] has summarized the photometric characteristics of the entire visible portion of the lunar surface, drawing particular attention to the low albedo, strong back-scattering, unusual polarization properties, and brownish-grey colour. He concludes that the only materials that would be photometrically consistent with the observations are some types of vegetation, non-metallic whiskers and dark, finely divided particles. There can be no vegetation because of the lack of atmosphere. He suggests that although whiskers might be formed by the combined effects of sputtering by the solar wind and condensation of material vaporized by micrometeorites, they are unlikely to survive under repeated meteorite bombardments. Hapke concludes that the surface must be covered with a layer of pulverized rock powder 10μ or so in size which has been "darkened by exposure to solar radiation or some other agent and arranged by micrometeorite bombardment into a porous material with a bulk density only one-tenth that of solid rock". A comparison of Fig. 2 with Hapke's[7] photographs of a fine powder with the required 'fairy-castle' packing shows a strong resemblance. Simple preliminary measurements indicate that our light-coloured air-deposited material also exhibits a strong back-scattering peak. The materials evaporated under less oxidizing conditions in a graphite crucible in argon or in a vacuum showed considerable darkening as would be expected.



Fig. 2. Photomicrograph of condensate formed on cold surface in air above crucible containing molten basalt at about 1,400° C

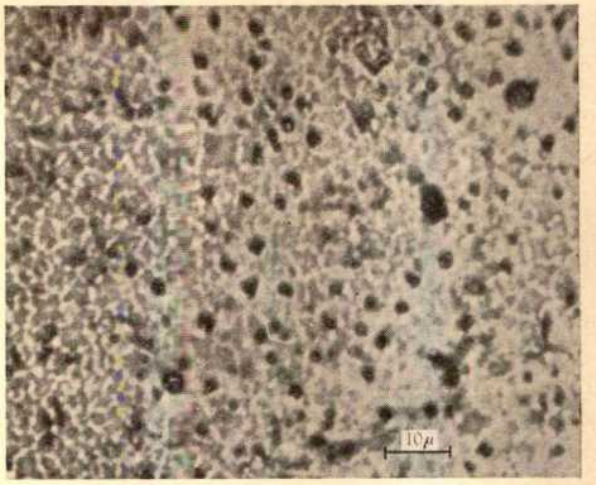


Fig. 3. Photomicrograph of thin condensate film formed on glass held for several minutes in a vacuum above molten basalt at about 1,200° C

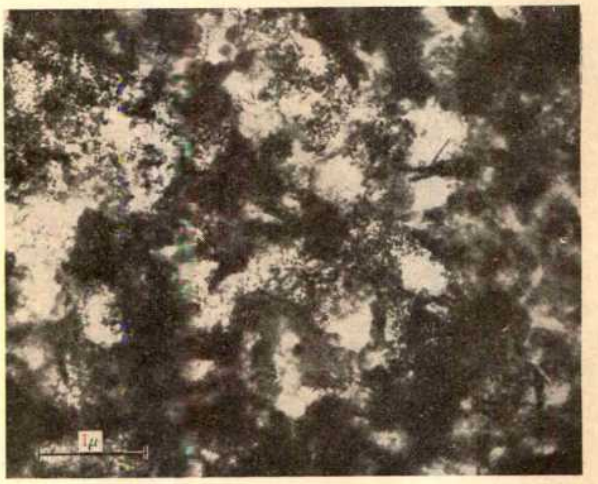


Fig. 4. Transmission electron photomicrograph of that area of the film shown in Fig. 3 from which electron diffraction pattern indicating crystallinity was obtained. Note the similarity between the fine granular structure shown in this figure and the much coarser structure of the air-deposited material of Fig. 2

It thus appears that the volcanic evaporation–condensation mechanism should be added to micrometeorite impact and solar wind sputtering as a possible factor contributing to the evolution of the visible portion of the Moon's surface.

From our observations at the volcano and in our laboratory we conclude that the formation of particulate matter by the condensation of vaporized components of hot lava is important in the origin of terrestrial volcanic clouds and atmospheric aerosols. The volcanic evaporation–

condensation mechanism may also be of importance in the formation of the surface of the Moon.

We thank the Manned Spacecraft Center of the National Aeronautics and Space Administration, the Geophysics Branch of the Office of Naval Research, the Atmospheric Sciences Program of the National Science Foundation and Arthur D. Little, Inc., for the support that made this work possible.

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A PACKAGED PLANT GROWTH CABINET WITH HIGH AND UNIFORM INTENSITY OF ILLUMINATION

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IT is becoming normal practice for a research institute or university department concerned in any way with the growth of plants to construct facilities for growing plants under controlled conditions. The trend in countries other than the United Kingdom has been to construct a phytotron[1,2]—a large complex of rooms, cabinets and glasshouses situated at one site, using natural and artificial light. In the United Kingdom, the Agricultural Research Council has adopted the policy of helping to set up controlled environmental facilities of limited size at existing institutes and universities. While it is recognized that certain types of research demand a 'walk-in' room, wind-tunnel or other specialized type of chamber, it was considered that a packaged 'reach-in' cabinet would fulfil a substantial proportion of the needs. The Agricultural Research Council has therefore provided support for an investigation at the National Institute of Agricultural Engineering of the design principles of an artificially-lit cabinet (Fig. 1). As a preliminary to this investigation, a survey was undertaken of more than 100 organizations considered to be potential users in order to find out the main requirements for controlled environment facilities. Several aspects of the final design of the cabinet, for example, the chosen range of operating temperatures, are a direct result of the survey. The present position is that the cabinet has been developed in conjunction with R. K. Saxton (Sax-Air), Ltd., and fifty are being made by this company. About thirty cabinets have so far been installed at eight research institutes and university departments.

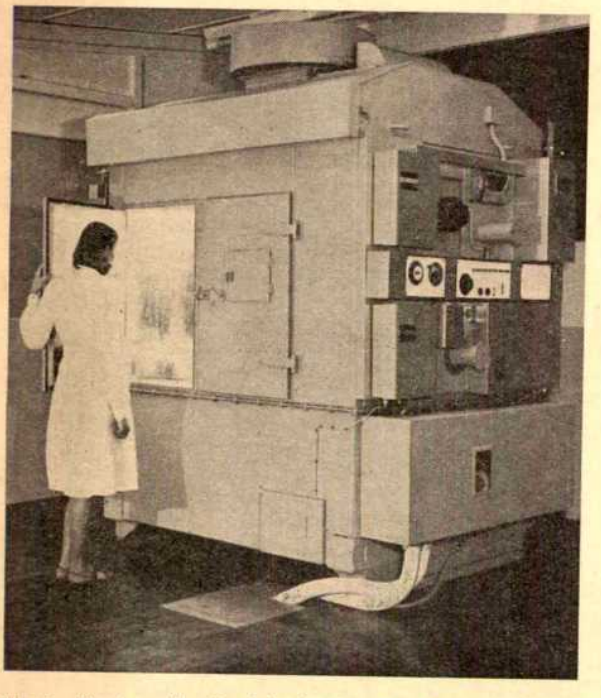


Fig. 1. Plant growth cabinet developed at the National Institute of Agricultural Engineering

In the past, designers of growth cabinets have used a constant temperature room as a starting point and have added illumination; the association of plant growth rooms with basements is partly a result of this expedient. More recently, there has been a tendency to use packaged cabinets or even rooms as free-standing entities having greater independence of the building structure. Such an approach gives more room for plant handling, lamp access and equipment maintenance, coupled with greater flexibility for major alterations or even entire removal to another building. Many of the problems in design apply equally to a cabinet or a room, these being best regarded as designs with different or complementary uses rather than rigid alternatives; for example, a room is particularly suited for prolonged measurements *in situ*, or for growing large arrays of plants.

For a growth chamber to be satisfactorily designed, it is essential for the designer to be aware of the needs of the user, but the latter should also be aware of the engineering, physical or economic implications of his requirements—in short, an understanding is needed between the engineer and the biologist. Examples of this can be given: should a user decide that temperatures below 5° C are not required in a cabinet, then, by avoiding de-frosting problems, a significant increase in operational simplicity is achieved. Similarly, a user, in specifying the accuracy of control of temperature and humidity he requires, must ensure that this is compatible with the dependence of relative humidity on temperature. In the present state of knowledge, the relative merits of certain aspects of the design of a growth chamber are still not resolved; for example, direction of air movement and its velocity.

It is possible to define an ideal growth chamber in terms of what occurs in natural conditions or semi-natural conditions such as in a glasshouse; however, this is misleading, because most natural environments tend to fluctuate widely and any predetermined environment is artificial since it can only approximate to a condition or combination of conditions that exists periodically in a natural climate. Three criteria appear to be important: conditions should be uniform in space and time; conditions should be defined and reproducible; and plants grown in this environment should not be too dissimilar from ones grown in a typical natural climate.

There are certain design problems which are peculiar to plant growth chambers. First, the highest possible uniform light intensity must be provided over the whole experimental area, thus eliminating use of either the ceiling or the walls or doors for any purpose likely to obstruct light, and also imposing a substantial heat load on the air-conditioning system. Secondly, plants modify the composition of the atmosphere by transpiration of water and exchange of carbon dioxide, thus necessitating

air conditioning and fresh-air exchange. Thirdly, plants exhibit a large change of size in a short time which calls for a design that permits the conditions to remain uniform without continual adjustment of floor-level.

In the cabinet to be described, first advocated by Morris[3,4], the problem of providing a high intensity of light with good uniformity in time and space was solved in two ways: by the provision of a self-contained lighting unit; and by using this lighting unit in combination with specularly reflecting walls on the plant chamber to produce a rectilinear-box, artificial, overcast sky.

The lighting unit consists essentially of a box containing fifty-six 5-ft. fluorescent tubes arranged in two tiers; this arrangement gives 35 per cent more light than a conventional close-packed single tier[5]. The lamps are surmounted by a white reflector. Under these conditions, plain lamps are superior to those with an internal reflector[6]. Lamps arranged in this manner require forced-draught cooling which is provided by an axial-flow fan above the unit, air being sucked in through filters at the end of the lamps and passing through holes near the centre of the overhead reflector to the fan and thence to the atmosphere. Because the light output of this kind of lamp is dependent on temperature, the lamps are cooled uniformly over the bank and compensation for variations in ambient air temperature is provided by a temperature-sensitive device in the top of the lighting unit which permits some of the air to by-pass the lamps for lower ambient temperatures. The lamps are enclosed beneath by a double-glazed 'Perspex' ceiling; a screen of some kind is essential in order to separate the air streams conditioning the lamps and plants, respectively, thus making possible the independent control of the temperature environment of both lamp and plant. In addition, if double glazing is used, long-wave heat radiation from the lamps is reduced by two-thirds and this reduces the load on the air-conditioning system in the plant chamber. 'Perspex' was chosen in preference to glass because it is light in weight and not easily broken; experience has shown that with reasonable care it does not become scratched, nor does it 'age' under this type of irradiation. Rather surprisingly, the loss of light transmission attributable to the double-glazed ceiling is only about 6 per cent, due to multiple reflexion between the ceiling and the light source.

A rectilinear-box, artificial, overcast sky is characterized ideally by uniform flux density of light in all directions in the upper hemisphere, although in practice the best reflecting materials do not achieve this[6] and approximate as regards sky luminance to a real overcast sky[7]. The cabinet dimensions were chosen so that the plant floor was a square of 1·37 m side with the lighting unit overlapping by 8 cm on all sides. The walls were lined with metallized polyethylene terephthalate up to the ceiling. Under these conditions, the light intensity over three-quarters of the floor area does not fall below 95 per cent of the value at the centre of the floor; the lowest value of 92 per cent occurred in the corners. The light intensity at floor-level (112 cm from ceiling) is 75 per cent of that at ceiling-level.

The quality of illumination raises several points of interest to botanists. First, considering only the wavelengths 0·3–0·75μ, the intensity of illumination is only one-third of midday summer sunlight. However, the daily integral of light energy incident on the floor of the cabinet, using an 18-h day-length and a realistic schedule of lamp group replacement[8], reaches the average daily integral of light energy in the open for the month of June in the United Kingdom—averaged over a period of 5 years (Fig. 2), namely, 198 cals cm^{-2} per day. Furthermore, the light in the growth cabinet is diffuse, coming from all directions above the horizon, and might be expected to be utilized by plants more effectively than the highly directional light occurring on bright days out of doors. Thus, although there is likely to be a demand for still higher intensities, the cabinet, in terms of growth-rates, can give a performance comparable with that out of doors in summer. Secondly, per unit of light energy in the photochemically active range (0·3–0·75μ), sunlight contains one and a half to two times the total radiant energy than does the lighting in the cabinet. Thus, for a given growth-rate, transpiration is likely to be considerably less under cabinet conditions than under natural illumination. Thirdly, the 'overcast sky' provides a directionally defined type of illumination which can be repeated and is uniform over the whole of the floor. Because of the reflecting walls, guard plants are unnecessary if the outer row of plants is a 'half-pot spacing' from the walls, an effect illustrated in Fig. 3.

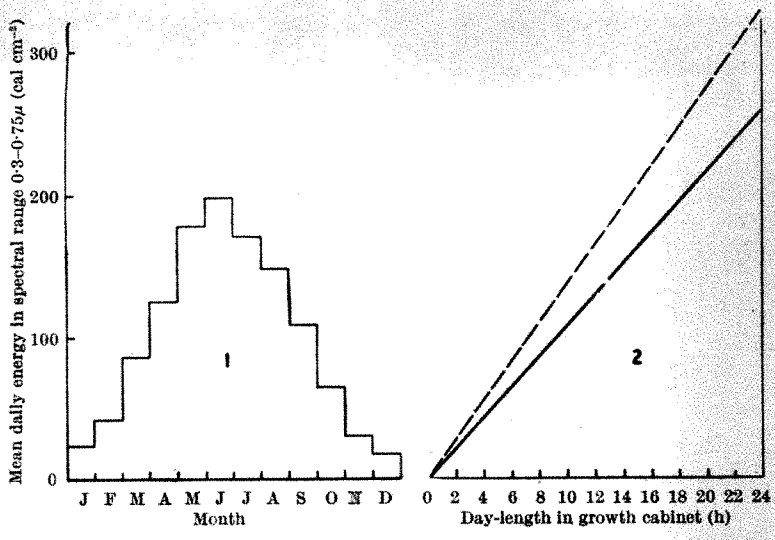


Fig. 2. (1) Variation of mean daily light energy with time of year at Kew Observatory. Average for 1956–1962 inclusive. Values are for total radiation × 0·47. (2) Variation of daily light energy with day-length in the plant growth cabinet. Broken line is for new lamps giving 0·23 cal cm^{-2} min^{-1} of light energy. Continuous line is for group replacement of lamps (1/5 every 1,000 h) giving 0·18 cal cm^{-2} min^{-1} of light energy. Measurements taken 112 cm from lamps

The aspects of design relating to air conditioning proved unexpectedly to present greater difficulties than for illumination. The area occupied by plants has to be a large proportion of the total floor area occupied by the cabinet; this results in severe problems related to the aerodynamics of turning and expanding the air flow. Furthermore, it is desirable to limit air velocity to below 100 ft./min and this produces unavoidable gradients of temperature and moisture content of the air as it passes through the chamber. However, with uniform distribution of air velocity, such gradients can be measured, calculated and controlled. Vertically upward air flow was adopted in preference to horizontal, since it ensures similar conditioning of all plants. It has advantages over vertically downward flow, since it permits the use of the space beneath the plant floor for obtaining a uniform velocity distribution through the plant canopy despite turning the air flow through 180° and expanding the duct area twenty-fold. It also means that the reflecting walls can be complete on all sides apart from a narrow exit slot along the top of one wall. Although Joffe[9] has expressed concern over the existence of stagnant air pockets above plants when using vertically upward flow, neither inspection of smoke trails nor the determination of velocity distribution in different horizontal planes produced evidence of stagnation. The characteristics of the air distribution system will have a bearing on this, and



Fig. 3. Visual illustration of the lighting principle used in the cabinet. 25 pots of *Helxine solieroliì* are shown inside a cube of size 68 cm with wall of glass mirror

there is little resistance to air flow inside the plant chamber. Covering the floor with close-packed 3·5-in. diam. pots has no detectable effect on volumetric air throughput, and streamline flow is maintained over the remaining unobstructed area of the floor, at a correspondingly increased linear velocity.

For a reasonably airtight cabinet operating on a closed air circuit, continuous release of moisture from plants in the chambers permits control of humidity by continuous condensation on a cooling coil of controlled temperature, thereby limiting the dew-point. If the moderate air-velocity, and thus the air circulation rate, is also taken into account, the cooling and heating loads imposed by cooling the air to dew-point and re-heating to dry-bulb are not prohibitively large. Such a system was therefore adopted in this cabinet. The need to control cooling-coil temperature made it essential to use secondary refrigeration and a proportioning controller. It is interesting to note that plant transpiration rate influences the design of the cooling coil. In principle, a silent pump on the cabinet continuously pumps coolant through a cooling coil, and coolant at 33° F is available from a reservoir remote from the cabinet. The temperature of the cooling coil depends on the position of a servo-operated valve which mixes coolant from the reservoir with warmer coolant from the coil. The air is re-heated by means of an electrical heater fed from the output of a servo-operated transformer. Both temperature and humidity depend for control on measurement of temperature, and the sensing elements are therefore completely trouble-free, being dry-bulb resistance thermometers. The controllers are two-term electronic instruments. The main fan is variable in speed. The refrigeration unit consists of a packaged chiller connected to the cabinet by two pipe-lines. Up to three cabinets can be supplied from a single refrigeration unit.

The variation of air temperature is less than ±0·5° C with time and ±0·75° C over a horizontal plane. When the lights are on, leaf temperature will be subject to a temperature gradient in a vertical direction. The workable temperature range of the cabinet is 4°–30° C. Relative humidity is uniform in time to about ±2 per cent provided adequate evaporating surface is provided either by plants or by a special wick; this figure will vary with actual temperature and humidity.

Although the cabinet consists basically of a steel structure of special design, most of the components used are available commercially. The special design, in addition to incorporating the features already mentioned, gives good accessibility to components, permits breakdown into five sections for installation in buildings with restricted access, and also gives scope for modifications by users. The cabinet also has a comprehensive built-in alarm system.

I thank Mr. L. G. Morris for his technical advice, and Dr. D. Rudd Jones for his sustained interest in this project. I also thank the Director-General of the Meteorological Office for permission to use records of total daily radiation.

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WHAT'S IN A VIRUS NAME?

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Inadequacy of Names in Use at Present

VIROLOGY has advanced greatly in recent years, but virologists have had little success in devising a satisfactory way of naming the entities they study. Most virus names now in use are essentially arbitrary and have to be learned by rote because they give little indication of the properties of the virus named. This applies to vernacular names such as 'tobacco mosaic virus' and even more so to semi-numerical names of the type '*Nicotiana* virus 1', which gives only half as much information as its corresponding vernacular name. There have been several, largely unsuccessful, attempts to introduce latinized binomial names for viruses. This type of nomenclature has been particularly effective for those animals and plants that reproduce sexually, but less so for those that reproduce mainly by other methods.

Some of the shortcomings of latinized binomials are indicated by the chaotic situation which has resulted from

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their use with a non-phylogenetic, but hierarchical, classification of bacteria. Indeed, when the basic unit in this classification, the 'microbial species', is considered by the curator of the British National Collection of Type Cultures to be a 'macromyth'[1], it is not surprising that latinized binomial names have proved unsatisfactory for such organisms. It is also becoming more widely recognized that Latin is no longer internationally well understood[2]. The latinized binomial system may have been unsatisfactory in practice for bacteria because of the rules that have governed its use, rather than because it is intrinsically bad, but we think that if applied to viruses it will give similar or even worse results.

What, then, can be done? Criticism of existing practice is most helpful when accompanied by suggestions for a better system, but before making our suggestions we wish to discuss the aims of nomenclature and its basis in classification.

Aims of Nomenclature

If the only purpose of virus nomenclature is to provide each virus with a specific label, then it is necessary only to devise a series of unique epithets "quaint enough to be unforgettable"[3]. However, a name would be far more useful if it indicated some of the properties and thus the affinities of the virus. Ideally a name should be: (1) simple, easily understood and usable by people speaking different languages; (2) indicative of the properties of the virus named; (3) unchanging. The main difficulty is to devise names that are unchanging, or rarely change, and yet indicate what is known of the properties of the virus named, when this is likely to increase greatly as the result of future research.

The solution we suggest is to use a name made up of two functionally different parts, one part a convenient and unchanging label, which would be the vernacular, or trivial, name, and the other part an internationally agreed code indicating the present state of knowledge of the properties of the virus. The idea of a code has recently been considered both for viruses[4] and for bacteria[5] but not developed in detail, and for enzymes a similar code has been in use since 1961. Such a code will best indicate the properties and thus the affinities of the virus if based on a virus classification, and so, before describing our ideas for a code, we shall discuss virus classification.

Classification of Viruses

The aim of virus classification is to make an ordered arrangement of viruses that will indicate their similarities and differences. Viruses can of course be classified on the basis of any features. However, if there is any point on which virologists are at all likely to agree, it is that we now need a general rather than a special classification. Such a classification has two main uses: (1) To predict the unknown properties of a virus by analogy with the known properties of similar viruses. This is of value both in virus identification, where the identity of a virus may be more readily predicted and more speedily established by studying the most convenient and most clearly differentiating properties suggested by a classification, and also, when a 'new' virus is found, to predict by analogy some of its properties. (2) To form a basis for virus nomenclature.

One of the great debating points of taxonomy in recent years has been whether or not more importance should be attached to some characters than others. 'Important' characters are supposedly recognized because they are either basic or indicate phylogeny. The phylogeny of viruses, however, is at present a matter for speculation only, and though nucleotide sequence may perhaps be the only functionally basic character of a virus, at present we neither know nor could interpret such a sequence. Alternative characters must therefore be used. The hierarchical systems for grouping viruses proposed by Holmes[6], Hansen[7], Cooper[8], and by Lwoff, Horne and Tournier[4], have required the classifiers to decide on the relative importance of these alternative features. We can think of no satisfactory way of estimating the relative importance of different characters, and therefore consider it essential to follow the principles of Adanson[9], who suggested that all known information should be used, and that all characters should be considered equally important. Using these principles we will obtain a phenetic classification of viruses, that is, one based on the similarity of their overt properties. This does not mean that the classification may not have some phylogenetic significance, for the classifications of higher plants and animals used at present were phenetic in origin and have only later been shown to agree, in parts, with phylogeny.

Now that electronic computers exist, classifications using Adanson's principles can be made easily and accurately[10]. The first attempt to classify viruses in this way[11] had limited success because, if this type of classification is to have much worth, it must be based on at least sixty equally weighted independent characters[12], and at that time too few characters were known. A quick survey of the most recent literature on 500 or so of the best-known viruses infecting vertebrates, insects, seed plants and bacteria shows that we still have less than 5 per cent of the information needed. Therefore, although a general classification of viruses on Adansonian principles can be begun now, it still can be only crudely quantitative. Plainly, we cannot yet classify viruses objectively and rationally, but we should expect to be able to do so one day. In the meantime, if we are to do anything at all, we must make do with some more subjective approach, but it is imperative, and this is one of our principal points, that the temporary measures (both for classification and nomenclature) should in no way prejudice the evolution of a more satisfactory classification.

The Problem of Grouping

When we use the existing information on characters of viruses (for example, Table 1) to compare the viruses phenetically, we find that many arrange themselves in groups, but the difficulty is to decide how best to delineate these groups. We may illustrate our approach to this problem by the four examples given in Table 2. Whereas we distinguish the turnip yellow mosaic group of viruses from the squash mosaic group because the two groups differ in several characters shared by viruses within each group, we might not place poliovirus 1 and acute bee paralysis virus in separate groups because in this case the differences seem few.

Table 1. Some Characters to be used in classifying Viruses

(1) Viral components	
Nucleic acid	— DNA or RNA
	— single or double stranded
	— molar base ratio
	— molecular weight
	— percentage in particles
Protein	— No of different proteins in virus particles
	— enzymes in particles
	— other virus-induced proteins
	— serological relationships, as revealed by precipitation, neutralization and complement fixation tests
	— haemagglutinating properties
Lipid, Polyamine, etc.	— composition and amount
(2) Particle form	— shape
	— size
	— structure and symmetry of nucleocapsid
	— accessory structures
	— density
	— sedimentation coefficient
	— accessory particles; types and properties
(3) Stability of infectivity and particles	
	— temperature; thermal inactivation point
	— effect of chemicals, for example, ether
	— effect of enzymes
	— photo-reactivability
(4) Interactions with hosts	— site of growth in cell
	— tissue tropisms
	— pathological effects
	— host range; host taxa; no. of susceptible species
	— response to inhibitors of multiplication
(5) Mode of natural spread	— vector or not; type of vector; behaviour of virus in vector
	— own infection mechanism (phages, etc.)

The members of a group differ most in the fine details of host range and pathological effects. It would seem that these biological characters are not correlated with the other characters we have used for grouping.

The status of serology in such a system of groups warrants special mention, for many people believe that serology is the most, if not the only, useful character in virus classification. This attitude has perhaps arisen because serological relationships have been found so far only between virus isolates that share most of their properties. Therefore we find that, in a phenetic classification, viruses known to be serologically related are in the same group, though any one group may contain viruses that seem serologically unrelated. Serological tests, therefore, help not only to identify and group viruses, but also to distinguish between the members of a group.

Nomenclature of Viruses : Our Suggestions

The first part of the name, the vernacular part (for example, the list of names of plant viruses used by the *Review of Applied Mycology*[13]), would have the advantage that it is already widely used. Most such names indicate the disease with which the virus was first associated, and could be translated into other languages where this has not already been done.

The second part, the code or cryptogram, would indicate current information about the virus and might therefore frequently change. A cryptogram could be devised with many symbols, one for every character of the virus; this would give a shorthand summary of all the known properties of the virus, and would thereby give each virus a unique specification, but though it might be useful for a reference book it would be too cumbersome for general use as part of the name of a virus. Therefore we suggest an abbreviated type of cryptogram with only four terms. This would be sufficient to separate viruses into the groups already discussed here, or small collections of such groups. Table 3 shows the form of this cryptogram, and gives examples of the cryptograms of a few well-known viruses. The symbols are intended (where possible) to be suggestive of the characters they represent.

Although we have selected characters for the cryptogram that conveniently separate the groups of viruses recognizable at present, other characters may have to be substituted from time to time to take account of changes in classification. We emphasize that the cryptogram is not a part of the classification; it is a part of the name of the virus that reflects some aspects of the classification and indicates the state of knowledge of some of the properties of the virus.

In general, bacterial and vertebrate viruses will be most clearly differentiated from one another by the first three terms, for few of these viruses have vectors, whereas viruses of higher plants will mainly be distinguished by the last three terms because many of them contain single-stranded ribonucleic acid.

'Four-letter words' are often printed with some of their characters disguised by the symbol '*'. We suggest that this symbol be used in the cryptogram when there is no information on the relevant property, both when the property has not been studied, and also when in spite of study the property is not known, for to distinguish between the two possibilities would be difficult. A further tentative suggestion is that bold type should be used to indicate that the property has been determined on the virus in question, and, when the property can reasonably be assumed by analogy with a closely related virus, normal type be used.

A few of the cryptograms will, as already mentioned, relate to more than one of the groups of viruses obtained using the whole range of known characters. Thus both the turnip yellow mosaic group and the squash mosaic group of viruses would have the cryptogram

$$\frac{\mathbf{R}}{\mathbf{1}} : \frac{\mathbf{2}}{\mathbf{35}} : \frac{\mathbf{S}}{\mathbf{S}} : \frac{\mathbf{S}}{\mathbf{B}} \quad \text{(Table 2)}$$

To distinguish the individual groups in such collections would require a range of additional features, and because

Table 2. Properties of some Small Viruses

Property	Turnip yellow mosaic group of viruses	Squash mosaic group of viruses	Poliovirus 1	Acute bee paralysis virus
Type of nucleic acid	RNA	RNA	RNA	RNA
Strandedness	single	single	single	—
Nucleotide ratio (moles G : A : C : U approx.)	16 : 20 : 40 : 24 or 16 : 22 : 33 : 29	22 : 30 : 17 : 31	25 : 31 : 19 : 25	—
Molecular weight of nucleic acid in particles	$1{\cdot}8 \times 10^6$	2×10^6	2×10^6	—
% nucleic acid in particles	37	35	30	35
Appearance of particles	'spherical', 25–30 mμ diam., 32 major morphological sub-units	'spherical', 25–30 mμ diam., distinct polygonal outline	'spherical', 25–30 mμ diam., rounded polygonal outline	'spherical', 25–30 mμ diam., rounded polygonal outline
Sedimentation coefficient (S)	106–121	103–125	158	160
Sedimentation coefficient of naturally occurring accessory particles (S)	49–56	84–100 and 54–60	—	80
Thermal inactivation point (10 min)	75° C	75° C	62° C	75° C
Serological relationships	The TYM group includes two sub-groups of viruses, with distant serological relationships between viruses within each sub-group, but none to viruses in the other sub-group	Distant serological relationship between all members of the SM group where this has been tested	†	†
Mode of spread	Beetle vector	Beetle vector	No vector	No vector
Host range	Seed plants: moderate range of species	Seed plants: moderate range of species	Vertebrates: primates only	Invertebrates: bees only
Pathological effects	Mostly mosaics	Mostly mosaics, or mottles, some hosts give necrotic local lesions	Inapparent; or necrosis of gut cells, more rarely of nerve cells	Inapparent; or necrosis of nerve tissue
Number of phenetically similar viruses	6	5	—	—

† Poliovirus 1 and acute bee paralysis virus are unrelated serologically.

Table 3. THE CRYPTOGRAM

ARRANGEMENT

$$\frac{\text{Type of NA}}{\text{Strandedness}} \cdot \frac{\text{M.W. of NA}}{\%\ \text{NA}} \cdot \frac{\text{Outline of particle}}{\text{Shape of N.C.}} \cdot \frac{\text{Hosts}}{\text{Vector}}$$

EXPLANATION AND SYMBOLS†

Type of NA	R, RNA; D, DNA
Strandedness	Strandedness of nucleic acid 1 = single, 2 = double
M.W. of NA	Molecular weight of nucleic acid in millions
% NA	Percentage of nucleic acid in infective particles
Outline of particle	S, Essentially spherical E, Elongated with parallel sides, ends not rounded U, Elongated with parallel sides, end(s) rounded T, Having a tail X, Complex or none of above
Shape of N.C.	Shape of nucleocapsid S, Essentially spherical E, Elongated with parallel sides, ends not rounded U, Elongated with parallel sides, end(s) rounded X, Complex or none of above
Hosts	Kinds of host infected V, Vertebrate; F, Fungus I, Invertebrate; B, Bacterium S, Seed plant; A, Actinomycete
Vector	Kinds of vector 0, Spreads without a vector; no vector known A, Aphid (Insecta, Hemiptera, Aphididae) B, Beetle (Insecta, Coleoptera) C, Mealy-bug (Insecta, Hemiptera, Coccidae) D, Fly (Insecta, Diptera) E, Mite (Arachnida, Eriophyidae) F, Fungus (Fungi) G, Tingid bug (Insecta, Hemiptera, Gymnocerata) I, Tick (Arachnida, Ixodoidea) L, Leaf-, plant- or tree-hopper (Insecta, Hemiptera, Auchenorrhyncha) N, Nematode (Nematoda) S, Flea (Insecta, Siphonaptera) T, Thrips (Insecta, Thysanoptera) W, White-fly (Insecta, Hemiptera, Aleyrodidae)

EXAMPLES

Herpes simplex virus	$\frac{D}{2} \cdot \frac{74}{*} \cdot \frac{S}{S} \cdot \frac{V}{0}$
Influenza virus *A*	$\frac{R}{1} \cdot \frac{2}{1} \cdot \frac{S}{E} \cdot \frac{V}{0}$
Coliphage *T2*	$\frac{D}{2} \cdot \frac{120}{30} \cdot \frac{T}{X} \cdot \frac{B}{0}$
Tobacco mosaic virus	$\frac{R}{1} \cdot \frac{2}{5} \cdot \frac{E}{E} \cdot \frac{S}{0}$
Clover wound tumour virus	$\frac{R}{2} \cdot \frac{*}{*} \cdot \frac{S}{S} \cdot \frac{IS}{L}$

† Bold type could be used to indicate that the property has been determined on the virus in question, and, when the property can reasonably be assumed by analogy with a closely related virus, normal type could be used.

we do not wish to indicate these in the cryptogram and make it more complex, we suggest that such groups should be distinguished in another way. One possibility would be to follow the four terms of the cryptogram with a name specific to the group, for example, 'TYM-group'. Names could be coined where they do not already exist, as for example with most plant or insect viruses. Alternatively, instead of a group name an arbitrary symbol, for example, a Greek letter, could be used (α, β, γ, etc.).

The cryptogram would thus specify the group to which a particular virus belongs. To specify individual viruses, an arbitrary number could be assigned to each member of each group. This would help to indicate synonymy between different vernacular names in any one language and between those in different languages, for example

Tobacco ringspot virus
Tobacco ring spot No. 1 virus
Tobacco virus 10
Annulus tabaci
Tabakringfleckenvirus

$$= \frac{R}{1} \cdot \frac{2}{35} \cdot \frac{S}{S} \cdot \frac{S}{N} : \text{NEPO group, 3}$$

Where such numbers already appear in the vernacular name, for example, human adenovirus 5, they would of course be unnecessary in the cryptogram.

Approval of Names

Although the four terms of the cryptogram would be decided simply on the basis of experimental work, decisions on which viruses should be included in any one group of phenetically similar viruses are less straightforward, and the numbering, if any, of viruses within a group is arbitrary. We therefore suggest that an international body should be responsible for these decisions. The proposed International Committee for Nomenclature of Viruses is the obvious choice. Possibly sub-committees could be set up to consider which viruses should be grouped together, and as the result of their recommendations, approved lists of vernacular names, together with the appropriate cryptograms, group names and numbering within the groups, distributed at regular intervals.

The advantages and disadvantages of the system of nomenclature which we propose (vernacular name together with a cryptogram based on an Adansonian classification; VAC system) are best shown by comparing it with another, such as the one using latinized binomials based on a non-phylogenetic hierarchy of 'basic' characters (LB system).

One advantage of the LB system would be that the names could be relatively short and easy to memorize, whereas the VAC system would produce a more cumbersome 'name', but this is no great disadvantage, for in the usual type of scientific research paper the code part would be used only once to define a virus. The LB system would introduce yet another set of names to be learnt, for the vernacular names would undoubtedly still be used. In our proposed system, existing names would continue to be used, and only a few symbols would have to be learnt to interpret the cryptogram, and these symbols would, moreover, be used for all viruses.

The LB system is rigidly based on the characters chosen to determine the form of the hierarchy of taxa: divisions, orders, families, etc. If one of these characters should be found to be unsatisfactory for some reason, then part or all of the hierarchy might collapse, depending on the 'importance' of the character, and names would have to be changed or redefined, whereas in the more flexible VAC system only one symbol in the cryptogram would change.

With the VAC system a 'new' virus can be named as soon as it is found, and its vernacular name need never change. Its cryptogram will become more complete and perhaps change as its properties are determined, and the cryptogram will clearly indicate some of its known properties, those assumed by analogy and some of those not known. By contrast, in an LB system, someone will have to make the purely subjective decision about whether enough is known about a virus for it to be placed in the hierarchy and invested with a name, and once the virus has been named only the expert will know the adequacy or inadequacy of the information on which the name was based. Therefore with an LB system we may find many viruses, which are named on inadequate information and which will later have to be renamed, and/or many viruses will remain unnamed because not enough is known about them.

It is perhaps true that the VAC system is primarily a system helpful to the practising virologist, whereas the main outcome of adopting the LB system may be that virus taxonomists are kept happy and occupied.

Our main points can therefore be summarized as follows:

(1) The names of viruses in use at present are inadequate.

(2) The disadvantages of latinized binomials based on a non-phylogenetic hierarchical classification greatly outweigh their advantages.

(3) Virus nomenclature should be based on a general classification made using the principles of Adanson. Any system adopted now should not prejudice future developments.

(4) We propose a nomenclature in which an unchanging vernacular name is, where necessary, accompanied by a cryptogram indicating present knowledge of several of the properties of the virus, and its affinities.

(5) As a basis for discussion, a cryptogram using four terms is described.

(6) The new names should be subject to approval by an international body.

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[3] Bawden, F. C., *Plant Viruses and Virus Diseases*, fourth ed. (Ronald, New York, 1964).
[4] Lwoff, A., Horne, R., and Tournier, P., *Cold Spring Harbor Symp. Quant. Biol.*, 27, 51 (1963).
[5] Cowan, S. T., *J. Gen. Microbiol.*, 39, 143 (1965)
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[7] Hansen, H. P., *Proc. Conf. Potato Virus Diseases*, Lisse-Wageningen, 1957, 218 (1958).
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[12] Sneath, P. H. A., in *Microbial Classification*, edit. by Ainsworth, G. C., and Sneath, P. H. A., 289 (Cambridge University Press, 1962).
[13] Anon., *Rev. App. Mycology*, 35, Suppl. (1957).

NEWS and VIEWS

Nobel Celebrations in Sweden: Sir Lawrence Bragg, O.B.E., F.R.S.

The visit of Sir Lawrence and Lady Bragg to Stockholm for the Nobel Festival on December 10, 1965, at the invitation of the Nobel Foundation, was a unique event in the history of the Foundation. Sir Lawrence received his prize 50 years ago, and his visit was celebrated by a number of dinner parties and receptions. On December 9, Sir Lawrence gave the first Nobel Guest Lecture, taking as his subject the development of crystallography from its beginning to the latest crystallography of proteins. The Nobel Guest Lecture is a new innovation of the Foundation and is to be given each year by a distinguished laureate. Sir Lawrence's presence at the celebrations was very favourably commented on by many speakers at the celebrations, including the acting president of the Nobel Foundation, Mr. G. Söderlund, and the permanent secretary of the Swedish Academy, Dr. K. R. Geirow. In addition, on December 14, Sir Lawrence and Lady Bragg visited Uppsala University, where Sir Lawrence lectured on "Recent Advances in Protein Analysis". [A recent biographical note on Sir Lawrence was published on p. 353 of the July 24, 1965, issue of *Nature*.]

Civil Engineering in the University of Aston in Birmingham: Prof. M. Holmes

Dr. M. Holmes has been appointed professor of civil engineering and head of the new Department of Civil Engineering in the University of Aston in Birmingham (designate). In 1950 Dr. Holmes graduated from the University of Leeds with a B.Sc. degree in civil engineering, and in 1958 he was awarded a Ph.D. degree by the University of Bristol. He served for two years with the Royal Air Force as a flying officer, and then, after a short period in industry, he joined the staff of the University of Bristol as a lecturer in civil engineering. In 1960 he was appointed reader in civil engineering at the Bradford Institute of Technology, and since 1962 he has been on the staff of the Department of Civil Engineering at the University of Leeds, where he is at present a senior lecturer. Dr. Holmes has conducted research in plastic design methods for tall steel buildings and also into the study of beam to column connexions in precast concrete. He has published a considerable number of papers on a wide variety of civil engineering subjects. Dr. Holmes's appointment takes effect from May 1.

Physical Chemistry in the Bradford Institute of Technology: Prof. M. W. Roberts

Dr. M. W. Roberts has been appointed to the new chair of physical chemistry in the Bradford Institute of Technology. Dr. Roberts was educated at the Amman Valley Grammar School, Ammanford, and then at the University College of Swansea, University of Wales. He graduated in 1952 and carried out postgraduate research with Prof. K. W. Sykes in the field of surface chemistry. From 1955 until 1958 he was seconded from the United Kingdom Atomic Energy Authority as a Post-doctoral Fellow to the Imperial College of Science and Technology, where he collaborated with Prof. F. C. Tompkins in investigations of the nitridation of calcium. After this, Dr. Roberts held an appointment as senior scientific officer at the National Chemical Laboratory and worked on the mechanism of metal oxidation. In 1959 he was appointed lecturer in physical chemistry at the Queen's University of Belfast, where he continued his studies of the mechanism of surface processes and also collaborated with Prof. C. Kemball in catalytic investigations. In 1964 he was invited by the Czechoslovak Academy of Science to lecture at the Institute of Physical Chemistry, Prague.

Mathematics in the College of Aeronautics, Cranfield: Prof. A. Stratton

Mr. Andrew Stratton has been appointed professor and head of the Department of Mathematics at the College of Aeronautics, Cranfield. He will take up the appointment on March 1, 1966. Mr. Stratton is at present head of the Research and Assessment Group of the Weapons Department of the Royal Aircraft Establishment, Farnborough. He has served continually at the Establishment since he graduated in 1939; initially he was concerned with radio proximity fuses and in 1954 was made superintendent of the Instruments Division (later the Inertial Navigation Division), where he was responsible for research and development work in the fields of flight instrumentation and simulation, airborne digital computers, advanced navigation and attack systems and the theory and technology of inertial navigation. As head of the Research and Assessment Group of the Weapons Department, he has been responsible for the theoretical assessment of weapon systems and for an intra- and extra-mural programme of research in weapon technology. Mr. Stratton is the author of many publications dealing particularly with the presentation of information in aircraft and inertial navigation, and his mathematical interests are concerned with the integrating function of mathematical analysis in engineering and applied science. His external activities include the offices of chairman of convocation at the University of Exeter and member of the Council of the Institute of Navigation.

Thames Barrage Scheme

In a written answer in the House of Lords on December 20, Lord Lindgren stated that while a Thames Barrage Scheme would always have attractions, no decision on such a matter could be final. The Government's view was that the potential advantages of such a scheme, in respect of such matters as flood prevention and amenity, were heavily outweighed by its disadvantages, most notably in regard to the grave interference which a barrage would impose on the operation of the Port of London.

Computers for Research

In a written answer in the House of Commons on December 21, the Secretary of State for Education and Science, Mr. A. Crosland, stated that he had invited the Council for Scientific Policy and the University Grants Committee to set up a Working Group to undertake a full-scale review of the computer requirements of universities and Research Councils, which would lead to a new 5-year programme of procurement. The Working Group, of which Prof. B. H. Flowers was chairman, reported in July, and its proposals are designed to meet the growing need for digital computing facilities for research. The Group recommended that there should be regional computing centres at the Universities of London, Manchester and Edinburgh, with very large computers to which research workers from other universities and research institutions would have access. It recommends better computing facilities for individual universities and research establishments which would be compatible with the proposed regional computers, and the system would thus form an integrated whole. At present prices, implementing these proposals for new machines, equipment, buildings and extra operating staff is estimated to cost £20·5 million for universities and £9·3 million for Research Councils in 5 years, compared with an average rate of approved orders for computers from universities over the past 3 years of less than £500,000 a year (£1·2 million in 1964). The Government has approved generally the proposals of the Group, but it is proposed to implement the programme over 6 years instead of 5. In the first 3 years expenditure will be at the rate of about £3 million a year and orders will be placed as soon as possible. Most of the recommendations of the Group for Research Councils had high priority within the programmes of the Councils. Mr. Crosland added that he proposed to set up, in consultation with the University Grants Committee and the Council for Scientific Policy, a Computer Board, which would keep the programme under review and be responsible for ensuring the full utilization of the facilities provided in both the centres and the universities generally. The programme formed part of the plans announced by the Minister of Technology for developing the use of computers as an instrument of modernization and of economic advance. The success of this plan called for a wider use of computers in universities so that many more graduates could become aware of the capabilities of computers and be trained in their use.

University of the Air

In a written answer to a question in the House of Commons on December 21 asking the Secretary of State for Education and Science for his plans for founding a 'university of the air', Miss Jennie Lee, Joint Under-Secretary of State for Education and Science, stated that a university of the air would provide opportunities for obtaining degrees and other technical and professional qualifications through special television and radio programmes, reinforced by correspondence courses, residential courses, and tutorial groups. It would have its own administrative centre, but would utilize existing organizations, such as local education authorities, universities and the broadcasting authorities. Costs, channels and other implications were now being considered by the Government.

Mass Spectrometry Data Centre for Aldermaston

The United Kingdom Atomic Energy Authority, with support from the Office of Scientific and Technical Information, is to set up a mass spectrometry data centre at the Atomic Weapons Research Establishment, Aldermaston. The centre will undertake an investigation into the use of computers for chemical analysis by matching and evaluating mass spectra, which will start immediately. In a 6-month research period, different matching techniques will be tried in conjunction with the relevant computer programmes to find the most suitable system. Another investigation which will start immediately will be into modern methods of classifying and handling information with the view of producing a spectra reference and retrieval system. This should produce a method of quickly recognizing compounds from their spectra. During the course of 1966 a world-wide index to compounds and materials already examined by mass spectrometry will be developed. For a given compound or material the index will give information such as where to find its spectrum and possibly a bibliography, its molecular weight and molecular formula. A mass spectra holding and information service will also be developed during the coming year. These last two services will, in due course, become available to mass spectrometry users throughout the world. Aldermaston has one of the largest mass spectrometry units in Britain, together with advanced computing and information facilities, which will contribute to the establishment of this mass spectrometry data centre.

Royal College of Science : Research Report

The *Research Report of the Royal College of Science, 1962–65*, summarizes the main lines of scientific research in progress during the past 3 years in the seven departments of botany and plant technology, chemistry, history of science and technology, mathematics, meteorology, physics, zoology and applied entomology (Pp. 87. London: Imperial College of Science and Technology, University of London, 1965). Emphasis is laid on the increasing cost of research brought about by use of specialized and often costly instruments. There are no references to published work. (The Royal College of Science is one of the three constituent institutions of the Imperial College of Science and Technology: the other two being the Royal School of Mines and the City and Guilds College.)

Research and Development in the Electronics Industry

The main feature of *The National Institute Economic Review* (No. 34; November 1965) is a long article on research and development in the electronic capital goods industry, with substantial appendixes on sources and methods, statistical tables, references and lists of firms, organizations and individuals who assisted in the enquiry. Although the industry is one of the most labour-intensive, with a high ratio of fixed assets per man, about 60 per cent of world exports of electronic capital goods are from the United States, compared with 20 per cent from world exports of all types. This lead is attributed less to original inventions than to more rapid development of greatly improved models and more efficient technical service and marketing. The effect of the enormous scale of the military and space research and development programmes on this United States lead is questioned: some European countries also have large military programmes, and the ratio of total research and development expenditures to turnover in many European firms is as high as that of their United States competitors. There is some evidence that firms which have been heavily dependent on military work have a less satisfactory performance in exports and in the civil market. For some products at least, the scale of development work in European firms has been below the minimum 'threshold' necessary for effective competition on the world market. A high ratio of research and development expenditure can still be a low absolute figure if sales are low, and any number of firms with a scale of effort below the 'threshold' will be ineffective as they will be late in developing the new products essential for survival. Military and space programmes gave United States firms an advantage for some products where management was able to exploit and adapt the consequent experience and technique for the differing requirements of the civil market, but similar military programmes in

Europe are not necessarily the best way to counteract this competition. The selection of the major research priorities is a political choice, and if the main priority is economic growth, the United States advantage can probably be offset by much more ambitious civil research and development projects in such fields as industrial automation, a computer 'grid', and electronic teaching aids.

Biochemistry and Microbiology of Reproduction

World Health Organization Technical Report Series No. 313 embodies the report of a World Health Organization scientific group on the *Biochemistry and Microbiology of the Male and Female Genital Tracts* (World Health Organization. Technical Report Series, No. 313. Pp. 15. Geneva: World Health Organization; London: H.M.S.O., 1965. 3*s*. 6*d*.; 0.60 dollars; 2 Sw. francs). The report summarizes our knowledge of the chemistry and enzymology of the uterus; sperm transport, capacitation and the acrosome reaction; nidation; placentation and the chemistry and enzymology of semen. It also discusses the effects of compounds of cadmium, zinc and selenium on reproduction, and the effects of infection on reproductive activity. Research needs are indicated, with emphasis on determination of the time of ovulation in the primates and the reaction of the uterine tissues during the reproductive cycle and pregnancy. Emphasis is also laid on the importance of viewing the male and female components in reproduction as an integrated whole and on the need to extend investigations to a wider range of species.

The Royal Society and Nuffield Commonwealth Bursaries Scheme

Awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Dr. A. T. Casey, senior lecturer in organic chemistry, University of Melbourne, to enable him to use new techniques in the study of metal–metal bonding in inorganic compounds, particularly those of the transition metals, at University College, London, for a year from December 1965; Dr. A. R. H. Cole, reader in physical chemistry, University of Western Australia, to enable him to gain experience in high-resolution electronic spectroscopy, in Ottawa between April and September 1966; Prof. E. O. Hall, professor of metallurgy, University of Newcastle, New South Wales, to enable him to gain experience in the use of thin-film electron microscopy, at Cambridge for a year from February 1966; Dr. F. T. Last, of the Glasshouse Crops Research Institute, Littlehampton, to enable him to examine, in New Zealand, from April to September 1966, methods for elucidating the part played by different fungi in the brown root rot complex of tomatoes; Prof. E. R. Love, professor of mathematics, University of Melbourne, to assist him to continue work on integral equations and integral transforms, at Cambridge for three months during 1966; Dr. C. R. K. Murty, reader in physics, Andhra University, to enable him to learn experimental techniques for measuring relaxation time, at Nottingham for 4 months from April 1966; Mr. B. S. Newell, of the C.S.I.R.O. Division of Fisheries and Oceanography, Cronulla, New South Wales, to enable him to study the development and improvement of analytical methods for nitrogen compounds in the sea, at Plymouth from January to December 1966; Dr. N. Okafor, lecturer in microbiology, University of Nigeria, to enable him to learn techniques of food irradiation, at Cambridge for 4 months from June 1966; Dr. J. G. Ramsay, reader in geology, Imperial College of Science and Technology, London, to enable him to study the structural and metamorphic history of part of the Himalayan mountain chain, working at the University of Baroda, with field work in the Gujarat region and in the Kumaon Himalayas, between March and June 1966; Dr. B. P. Setchell, senior research scientist, C.S.I.R.O. Division of Animal Physiology, Parramatta, New South Wales, to enable him to work on the development of a technique for perfusing a mammalian testis isolated from the body, at Cambridge for a year from November 1966; Mr. M. D. Sutherland, reader in organic chemistry, University of Queensland, to assist him to become familiar with the techniques of tracer work as applied to biogenesis, at Manchester, and also to work at Cambridge, between February and August 1966.

European Molecular Biology Organization Grants

The European Molecular Biology Organization (EMBO) has received a grant of £240,000 from the Volkswagen Foundation in support of European co-operation in molecular biology. The grant is intended for both short- and long-term fellowships, for a small number of visiting professorships and for advanced courses and study sessions. The most novel feature will be the award of short-term, quickly available fellowships to allow investigators to collaborate with colleagues in other laboratories. For example, when a problem urgently requires a specialized technique available in only one particular laboratory, which may be in another country, the Organization will award a fellowship allowing the investigator to go and work there for a period of a few weeks. Post-doctoral fellowships will generally be awarded for a year. They will be given to young and promising research workers to allow them to work under the guidance of leaders in the field of molecular biology. Senior fellowships may be awarded at anything up to professorial level; for example, in order to help universities to stimulate the development of molecular biology. According to the rules of the Organization, applications will be evaluated solely from the point of view of the scientific excellence of the project, regardless of the nationality of the applicant or of his membership of the Organization, but exchanges between scientists working in different European countries will generally be preferred to projects involving a single country only. This is because the aim of the Organization is to promote molecular biology at a European, rather than a national, level. Further information can be obtained from the executive secretary of the Organization, Dr. R. K. Appleyard, University of Brussels, 67, Paardestraat, St.-Genesius-Rode, Belgium.

International Clearing House for Medical Terminology and Medical Lexicography

An International Clearing House for medical terminology and medical lexicography has been established, following a meeting of the Bureau of the Council for International Organizations of Medical Sciences. The establishment of such a House was recommended on November 18, 1965, by an international group of experts, convened in Paris by the Council, under the chairmanship of Prof. John E. Gordon. The World Health Organization and the United Nations Educational, Scientific and Cultural Organization were represented at the meeting. This group strongly emphasized in its report that medical terminology is in a confused state; in certain branches of medicine the situation is approaching chaos. Certain diseases have been discussed by medical scientists under no less than thirty different names, and many diseases are known, in the same language, under as many as ten or more entirely different names. Under such conditions, scientists cannot be certain whether or not they are talking about the same thing, and communication becomes more and more difficult. Research that costs time, effort and money may be largely in vain if the necessary information cannot readily be located in the scientific literature. Misunderstandings, potentially dangerous to the public, also arise from the confusion prevailing in medical terminology. The main task of the International Clearing House will be to stimulate learned medical societies to examine the problems of medical language, prune from

it unnecessary words, and adopt internationally acceptable terms and definitions. The Clearing House will also encourage proper scientific bodies to prepare medical dictionaries and will do everything possible to facilitate their publication and dissemination. It will collect information from all over the world on all activities regarding medical terminology and make it available on request to those interested. It will deal only with the five languages most commonly used in medicine at the international level, which were agreed to be English, French, German, Russian and Spanish. Further information can be obtained from Prof. M. Florkin, Council for International Organizations of Medical Sciences, 6 rue Franklin, Paris 16.

New Chromophoric Reagents for Labelling the Thiol Groups of Proteins

S. F. CHANG (Research Division, Parke-Davis and Co., Ann Arbor, Michigan) and Prof. I. E. Liener (Department of Biochemistry, University of Minnesota) have written to the Editor as follows: "We would like to correct our recent communication which described two new chromophoric reagents for labelling the thiol groups of proteins (*Nature*, 203, 1065; 1964). In preparing 4-phenylazo-2-chloromercuriphenol (compound I) it was stated that 1·14 g of *O*-chloromercuriphenol was dissolved in 30 ml. of 15 per cent potassium hydroxide prior to coupling with diazotized aniline. This should be corrected to read: '1·14 g of *O*-chloromercuriphenol was dissolved in a minimum amount of 15 per cent potassium hydroxide at room temperature and diluted to 30 ml. with distilled water'. The remainder of the procedure is unchanged. Although compound II (4-(*p*-nitrophenylazo)-2-chloromercuriphenol) may also be obtained by this procedure, the following alternative method is recommended: Dissolve 1·0 g of *O*-chloromercuriphenol in 100 ml. of 70 per cent ethanol, filter, and add 15 g of sodium acetate to the filtrate. Cool to −2° C. To a solution of 0·42 g of *p*-nitroaniline, 4 g of cracked ice, and 12 ml. of concentrated HCl was added dropwise 0·21 g of sodium nitrite dissolved in 3 ml. of distilled water. The diazonium salt solution was cooled to −2° C and added dropwise to the solution of *O*-chloromercuriphenol. After the mixture had been stirred for 1·5 h in an ice bath, and diluted to 400 ml. with cold distilled water, the orange-yellow precipitate was collected by filtration and dried over $CaCl_2$ *in vacuo* overnight. This compound could be further purified by recrystallization from hot ethyl acetate or by passage through a column of acid-washed alumina (Merck) with ethyl acetate as the solvent. A red-coloured impurity was retained on the column, whereas the orange-yellow band corresponding to compound II could be eluted with the same solvent. The analysis of compound II is the same as that previously reported."

Announcements

SIR NIGEL POETT has been appointed director of the British Productivity Council on the retirement of Sir Charles Norris.

THE third international Biomagnetic Symposium will be held in the University of Illinois during March 22–23. Further information can be obtained from Prof. M. F. Barnothy, College of Pharmacy, University of Illinois, 833 South Wood Street, Chicago, Illinois.

THE eighteenth technical exhibition of the Oil and Colour Chemists' Association will be held at Alexandra Palace, London, during March 14–18. Further information can be obtained from the General Secretary, Oil and Colour Chemists' Association, Wax Chandlers' Hall, Gresham Street, London, E.C.2.

A CONFERENCE on "Exploiting Instrument Development", arranged by the British Scientific Instrument Research Association, will be held in Eastbourne during March 22–23. Further information can be obtained from Miss A. E. S. Mills, Scientific Instrument Research Association, South Hill, Chislehurst, Kent.

A CONFERENCE, convened by the Organization for Economic Co-operation and Development, on "The Effects of Automation and Technical Change on Labour" will be held in Zurich during February 1–4. Further information can be obtained from the Organization for Economic Co-operation and Development, Château de la Muette, 2 rue André-Pascal, Paris 16.

A SYMPOSIUM on "Planning Mechanical Engineering Departments in Universities", arranged by the Education and Training Group of the Institution of Mechanical Engineers in association with the Royal Institute of British Architects. will be held at the Institution during March 15–16. Further information can be obtained from the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1.

A DISCUSSION meeting of the Royal Society on "The Structure and Function of Lysozyme" will be held at the Royal Institution on February 3. An ordinary meeting of the Society will follow the discussion. Further information can be obtained from the Executive Secretary, the Royal Society, Burlington House, London, W.1.

THE NIGHT SKY IN FEBRUARY

All times are in Universal Time

MOON

New Moon	20d 11h
Full Moon	5d 16h

CONJUNCTIONS WITH THE MOON

Venus	17d 12h,	12° N.
Mars	21d 16h,	4° N.
Jupiter	2d 00h,	2° S.
Saturn	21d 17h,	3° N

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_g (10^6 miles)	Zodiacal position
Mercury	*S*	Unfavourable		19h 20m	−1·2	123	–
Venus	*R*	6h 35m	5h 20m	4h 45m	−4·1	30	–
Mars	*S*	18h 30m	18h 40m	18h 50m	+1·5	214	–
Jupiter	*S*	4h 55m	4h 00m	3h 00m	−2·1	431	Taurus
Saturn	*S*	19h 55m	19h 05m	18h 20m	+1·3	980	Aquarius

D_g is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
247 B. Tau	*D*	1d 01h 14·5m	+5·7
57 Gem	*D*	3d 18h 19·7m	+5·1
x Gem	*D*	4d 03h 49·2m	+3·7

(*D*, disappearance; *R*, reappearance)

OTHER PHENOMENA

22d 13h Mars 1·1° N. of Saturn.
23d 16h Mercury 1·7° N of Saturn.
24d 13h Mercury 0·7° N. of Mars.

Saturn and Mars become too close to the Sun during the month for favourable observation.

PROGRESS OF INDUSTRIAL RESEARCH IN BRITAIN

*RESEARCH for Industry, 1964**, the report of the Industrial Grants Committee of the Department of Scientific and Industrial Research, covers the first 3 months of 1965 in addition to 1964, and this year comes from the Ministry of Technology. It includes progress reports from thirteen research associations, whose grants came up for renewal in 1964, together with articles by Dr. N. F. Astbury on "Research Associations, Education and Industrial Training", and by S. S. Carlisle on "Automation Research and Development in Research Associations". It also contains the usual directory information on the research associations. The total annual income is now £11·3 million with £2·6 million in grants from the Department of Scientific and Industrial Research, compared with £170,000 and £61,000 respectively in 1927.

At its final meeting the Committee considered an application for a grant from the Machine Tool Industry Research Association, which is growing at a satisfactory rate and doing a most important job. The Committee considers that the Association should be given every possible encouragement and recommends a continuation of the generous terms of this grant.

On grants policy generally, the Committee now favours a revenue grant, usually for 5 years, as a fixed percentage of grant-earning subscriptions over a range of industrial income, and in 1964 seventeen of its eighteen recommendations took this form.

Two ear-marked grants recommended during the year were £34,325 over 3 years, to the Water Research Association to investigate the application of de-salting brackish and sea-waters to British water supply, and up to £39,865, also over 3 years, to enable the Ceramic, Steel Castings and Cast Iron Research Associations to extend their investigations into measuring dust in the working environments of their industries.

A grant announced in 1963, to the Cotton, Silk and Man-made Fibres Research Association, has been increased to £74,600 over 3 years to study new methods of spinning. The British Baking Research Association is also to receive an ear-marked grant of £26,800 towards the cost of a 5-year study of the staling of bread; the Electrical Research Association up to £17,400 for basic research into electrical conduction in dielectrics; and the Timber Research and Development Association half of the £45,000 cost of an operational research study of timber handling over 2·5 years, especially of the feasibility of importing timber into Britain in packages of standard size. A 50 per cent grant not exceeding £38,000 was awarded to the Rubber and Plastics Research Association for an investigation of the possibility of controlling a rubber factory engaged in batch production by a computer, programmed to plan the production in the most efficient way, with regard to utilization of plant and materials. A 75 per cent grant, not exceeding £54,300, is to be awarded to the British Industrial Biological Research Association for research into the injection method of testing food abstracts.

The British Cast Iron Research Association carried out an extensive programme of experimental work to obtain more basic data on cupola melting and to develop improved techniques of melting and metal treatment; much effort was devoted to the development and application of non-destructive testing techniques for iron castings.

One outcome of the Electrical Research Association's work on high-temperature plasmas has been a novel form of plasma furnace which can spheroidize highly refractory particles and may have other important applications. During the past 18 months, the British Hat and Allied Feltmakers' Association has discovered a new method of preparing hatter's fur (this has already reached the stage of production), and also a method of dyeing felt bodies, using the faster classes of dye without recourse to pre-neutralization. In the work undertaken by the Furniture Industries Research Association on comfort of chairs, all existing anthropometric data relating to sitting were surveyed. Furthermore, research is now proceeding on the use of resilient urethane foams for upholstered furniture, on the structural design of chair frames and the joints used in them, and on the avoidance of lacquer waste in spraying furniture.

The inter-firm comparison of the performances of furnaces melting container glasses, which the British Glass Industry Research Association started in 1962, has proved so successful that all British manufacturers are now included, as are more than half the American container glass industry and almost all the Western European firms making glass containers. Considerable contributions have been made towards explaining the basic mechanisms of the corrosion of refractory materials by molten glass and the materials carried in the waste gases, as well as towards the more accurate prediction of the service life of refractories and ways of assessing the effects of changes in refractories on the economics of furnace operation. The Association is also attempting to fill the gap in available knowledge of the process of forming commercial glass by a fundamental investigation, using computer techniques, of the various parameters involved and by direct measurements on the forming machines in the works of members.

The Hosiery and Allied Trades Research Association's basic studies of the factors involved in producing baulked nylon yarns have led to the development and widespread application of two control tests; the crimp rigidity test and the equivalent steam-setting temperature test. A lateral stretch device has been developed for checking the width-wise extension of ladies' hose, as has a quality-control gauge for latch- and bearded-needle flat machines which enables the number of loops in a fixed length of yarn to be determined.

The British Launderers' Research Association has reduced the amount of water required for rinsing without adversely affecting the quality of the work produced. Investigations are proceeding into the application of heat-transfer fluids other than steam to the drying and ironing equipment of laundries, with a view to increasing productivity by the use of higher operating temperatures. Two effective procedures have been developed for disinfecting wool blankets—one involving boiling and the other the use of chemical disinfectants.

The British Leather Manufacturers' Research Association has completed its investigation of the drying rates of different kinds of leather in air at various temperatures, humidities and rates of flow and normal pressure, as well as a 5-year research project on the resistance of leather to heat, moisture and perspiration.

An important achievement has been the introduction of 'Procion' dyes to the leather industry and development of a 'Procion' resist. Addition of benzyl and isopropyl alcohols increased the range of dyes that can be used in spray-dyeing leather and also increased their penetration.

Recent activities of the British Scientific Instrument Research Association in developing industrial measurement techniques have included the development of an emissivity-compensated service pyrometer, which is now undergoing trials in several industries, and a nucleonic gas-flow meter and density gauge. Research is being carried out on techniques of automatic surface inspection, and the development of more efficient detecting screens

* Ministry of Technology. *Research for Industry, 1964.* (A Report on work done by industrial research associations in the Government scheme.) Pp. iii + 72 + 20 plates. (London: H.M.S.O., 1965.) 8*s*.

for high-energy X-ray fluoroscopy. Work in thin-film technology has been considerably developed and has concentrated on the electrical application of thin films.

The British Steel Castings Research Association is developing special methods of applying materials and has shown that chromite sand is a valuable substitute for zircon sand. Tests have shown that, using this method, austenitic manganese steels can be produced from scrap more economically than by previous methods. Trials are at present proceeding to assess the efficiency of deoxidizers which could lead to the production of superior steels at a lower economic cost.

Research in plant engineering has included the wet reclamation of clay-bonded sand, and investigations of the efficiency and rate of cleaning of steel-castings have shown how shot and other abrasives can be used more efficiently and can provide a method for removing very hard and tenacious cores. In welding technology, the use of a gas shield around a welding arc has proved of great value during the past few years in solving such problems as the welding of thin aluminium alloy sheets, the positional welding of steel and the welding of a whole range of alloys in which metal transfer in the ordinary gas-shielded welding is difficult and erratic. The British Welding Research Association has also overcome difficulties due to resistance in spot-welding by developing a monitoring device which gives a reliable indication of the occurrence of defective welds during the welding operation. Progress already achieved in welding high-tensile steels made it possible for welding to be used in constructing the Forth Bridge, and this is estimated to have cut construction costs by £1 million. A completely new method has been developed for the hydrogen analysis of metals, and methods and equipment developed by the Association to test very large steel plates are now used in every industrial country.

The Welwyn Hall Research Association was formed on October 1, 1964, by the amalgamation of the Whiting and Industrial Powders Research Council and the Chalk, Lime and Industries Research Association. During the past 5 years the Council has advanced over a broad front, rather than in particular fields. The latter Research Association, however, has investigated particularly the problems of the heat balance of shaft lime-kilns and their operation, and this has led to a better understanding of the factors affecting fuel efficiency. An investigation of the factors affecting the quality of calcium silicate bricks has also been undertaken, which has led to the manufacture of stronger bricks with greater dimensional stability.

SPACE AGE SCIENCE AND TECHNOLOGY

APART from what the Earl of Bessborough said in his opening speech, the debate on space age science and technology in the House of Lords on November 3 is of interest mainly for what Lord Shackleton said about Britain's effort in this field and for what Lord Bowden and Lord Snow, who replied to the debate, said about the organization of our research effort and the choice of priorities. The Earl of Bessborough asked some searching questions about the extent of our effort in space research, with which he was dissatisfied. He did not suggest that this effort was necessarily more important than that concerned with other scientific and technological needs, particularly in the less developed countries. The research effort was considerable, but he was concerned that the overall proportion of our research expenditure in the main fields should be right. Lord Shackleton referred to the brilliant report of the Bondi Committee, but said that it could not be published for security reasons. The original estimate of the cost of the European Launcher Development Organization was £70 million, of which our share was 38·8 per cent. When the studies for further programmes were available early next year we should have to consider the estimate very carefully in relation to the technological advantages to be derived from the present and possible future programmes. Concerning satellite communications, he said that the United Kingdom, France and several European countries established in 1963 the European Conference on Satellite Communications and, in the following year, negotiations with the United States, Canada, Australia and Japan led to the drafting of two interim agreements. These covered the establishment of a global satellite system under the Interim Committee on Satellite Communication on which the United States was represented by its Communications Satellite Corporation.

At present there was no alternative to the use of United States launchers and satellites. The *Early Bird* satellite, which is now in regular service and carries part of our transatlantic telephone service, was commissioned before the agreements were signed. It is expected that we will have a global service and that this will render an economic return in due course, but it will take some time for the system to reach a level of development which will be economic. Satellite *Early Bird* was primarily an experiment to determine, first, whether a satellite of this type would provide an acceptable telephone service and, secondly, to gain operating experience. The specification for future satellites which has now been drafted aims at an average life of five years, and it is hoped that, in the future, ten years will be achieved. Although the United Kingdom and the European Launcher Development Organization have the range and supporting facilities of Woomera, there are great advantages in equatorial orbits, and some consideration is being given to the possibility of an alternative launching site near the Equator. Work in Government establishments and in industry on the basic satellite-technology programme has covered design and technological problems relating to satellites, including studies for astronomical satellites and communication satellites and research and development on solar cells, the effect of space environment on electronic components, altitudes sensing, and control systems. Defence Research Establishments had been working on methods of space communication without using man-made satellites. Notable advances in weather forecasting had already been made through the use of space techniques. In the current year the level of expenditure in this field looked like being about £20 million.

In a maiden speech, Lord Ironside referred to the European Space Research Organization Convention, which came into force on March 23, 1964, in which ten countries were now participating, Austria and Norway having dropped out. Much expenditure was at present capital, but the emphasis was slowly shifting to project work. Contributions to the European Space Research Organization, at present something like £7 million, were expected to approach £20 million a year in two or three years' time. Lord Ironside said he thought the return to this country would come first through the return of technologists, after service abroad, and through the placing of contracts in this country by the Organization, which would strengthen United Kingdom industry in space technology and stimulate advances in related technologies, such as telecommunications, vacuum techniques and cryogenics. Lord Bowden's speech was essentially concerned with the organization of civil research and the choice of priorities, and he claimed that the new Council on Science Policy constituted a notable advance. He asked whether the Government proposed to publish Prof.

Flower's survey of the requirements of the universities, and he again expressed his concern that our choice of priorities might be wrong. Lord Sherfield thought manned space research was an example of the fields in which we could not compete. He urged that in the choice of priorities we should build on success and give priority in resources to advanced fields in which we were either still in the lead or among the leaders, instancing nuclear research and technology, fusion research, radiochemistry, radio astronomy and important areas of the biological sciences, as well as some sectors of aviation. He also emphasized the importance of international co-operation, either with single countries as with France in the *Concord* project, or with groups of countries as in the organizations already instanced. He suggested that some countries might tend to support their national programme rather than their share in an international programme to which they were also committed and for this reason he preferred a national effort. He thought our organization of research was soundly based, but regretted that the Industrial Research and Development Authority recommended by the Trend Committee had not been adopted; he believed that the establishment of a Ministry of Technology was a mistake.

Lord Todd also offered a warning against excessive commitment to space research and thought that the advantages coming from technological fall-out were exaggerated. Britain should either support technology with a definite economic aim or support primary scientific research rather than projects in which advantages were reaped from technological fall-out. She must beware of putting too much manpower into scientific research and too little of it into pursuit of technological ends; it was imperative that some hard choices be made which would lead to Britain opting out of some fields of science. He thought we would undoubtedly have to double our present expenditure on scientific research during the next five years, that is to say, there should be a growth rate of about 15 per cent per annum during that period; any cut in this would seriously endanger Britain's position as a leading nation.

EARNINGS OF CHEMISTS

AN article in *Chemistry in Britain* for October 1965 commenting on the remuneration survey, 1965, conducted by the Royal Institute of Chemistry, gives some interesting comparisons of the earnings of chemists with those of other professional people and scientists. Figures for 10 professions in 1964 are given by J. Graham in a series of articles for *The Scotsman*. The average median earnings of chemists in Great Britain between the ages of 30 and 65 are shown by the survey as £2,650 in 1965 compared with £1,600 in 1956. These figures compare with figures for university teachers in 1964 and 1955 of £2,350 and £1,500, respectively. For engineers the corresponding figures are given as £2,200 and £1,210; for graduates in industry £2,575 and £1,570; for doctors (consultants) £4,000 and £3,130, and for general practitioners, £2,765 and £2,160. Figures obtained in a survey by the Institute of Physics in 1964 showed that salaries of its Fellows and Associates had risen by just over 72 per cent in the nine years from 1956, compared with a corresponding rise for chemists over the same period of 66·3 per cent. A typical Fellow of the Institute of Physics can expect to reach a peak of £3,400–£3,500 a year, compared with £3,100–£3,200 for a Fellow of the Royal Institute of Chemistry. Looking at the earnings of chemists in various occupations, private industry no longer leads the field; its highest median salary of £3,100 in the 56–60 age group coming third, behind universities and colleges of advanced technology (£3,460) and £3,150 in the Atomic Energy Authority. Starting salaries in the Scientific Civil Service appear to be outstandingly high, presumably because of more recruitment direct into the Senior Scientific Officer grade, and the subsequent prospects also seem to be better than in most other categories of employment. This closing or narrowing of the gaps between median incomes in industry and those in other occupational categories is particularly interesting in view of current discussions on the possible shortage of scientists. It should be remembered, however, that chemists are not a particularly unified group and are engaged in a very wide range of diverse activities, which sometimes have only a tenuous connexion with their qualifications.

A further article in the November 1965 issue of *Chemistry in Britain* comments that chemistry appears to be about middle of the professions surveyed and that it would appear that the median income levels in all the professions are tending to move closer together: a relatively small change of pace in relation to the general forward movement of incomes could drastically alter the position. Chemists still do considerably better financially in some fields of employment than in others, but such differences also seem to be diminishing. The very low proportion of chemists (0·5 per cent) reporting they were unemployed does not suggest a surplus, and of the 55 out of 10,459 without employment, only 29 had been out of work for more than 3 months, 13 of whom were under 40. While the earnings of older chemists have not increased sufficiently to preserve the differential between them and their younger colleagues, the survey encourages the belief that most chemists whose employers regard them as 'too old at forty' have a good chance of finding one who does not. The survey also shows that any movement of chemists away from industry has been comparatively small, although most of the decline, from 59·6 per cent in 1956 to 55·5 per cent in 1964, occurred in 1962–65.

'O'-LEVEL EXAMINATIONS

FOUR years ago R. W. Crossland and R. Amos presented an analysis of 'O'-level papers of the General Certificate of Education (G.C.E.) in biology of four Boards over the period 1948–59. The analysis was an attempt to quantify the emphasis placed on four main outcomes of biology teaching in the contents of the question papers.

These outcomes are: (1) the acquisition of facts, (2) the interpretation of facts and the drawing of conclusions from experiments, (3) the application of scientific principles to new situations, (4) the designing and planning of experiments.

Late in 1962 a similar analysis of the corresponding papers was made for the period 1960–62 and more recently the analysis was brought up to date by including the papers for 1963 and 1964*. The results of the three analyses are here shown separately, and, for comparison, the corresponding figures for the other science subjects are also shown (Table 1).

The analyses show the relative proportions of acquisition of facts and scientific method for the various sciences at 'O' level. Biology papers devote about 90 per cent of questions to facts. There are those who hold that one needs to know a good many biological facts before one can do much reasoning. The authors suggest that the Nuffield Foundation Teaching Project and similar pro-

* *Biol. Hum. Affairs*, 36, No. 3 (1965).

Table 1. Comparison of the Mean Percentages of Each Outcome in Biology, General Science, Chemistry and Physics Papers, 'O'-level G.C.E. of Four Boards, 1948–64

	Northern Universities			Welsh			Cambridge			Oxford		
	'48	'60	'63	'48	'60	'63	'48	'60	'63	'48	'60	'63
Biology	'59	'62	'64	'59	'62	'64	'59	'62	'64	'59	'62	'64
(1) Acquisition of facts	78	84	93	90	91	92	84	89	91	85	85	85
(2) Interpretation	2	1	2	0	0	0	0	1	5	0	7	7
(3) Application	1	1	2	0	0	5	1	4	0	4	3	3
(4) Experimental	19	13	3	10	9	3	15	7	4	12	6	5
General Science												
(1) Acquisition	58	53	60	78	—	82	66	63	70	66	71	—
(2) Interpretation	14	25	13	5	—	3	7	9	7	6	10	—
(3) Application	19	13	19	9	—	12	12	19	18	15	16	—
(4) Experimental	8	9	8	8	—	3	13	10	5	14	4	—
Chemistry												
(1) Acquisition	52	68	73	59	53	60	48	48	64	64	57	57
(2) Interpretation	8	9	3	4	10	1	12	—	—	5	2	2
(3) Application	17	11	16	11	18	32	12	37	25	14	21	26
(4) Experimental	24	13	8	26	20	7	28	16	11	17	20	15
Physics												
(1) Acquisition	43	41	54	40	36	36	39	34	30	40	52	46
(2) Interpretation	8	10	1	5	7	2	3	10	—	5	4	2
(3) Application	38	36	38	39	50	58	34	45	62	38	33	45
(4) Experimental	12	14	7	16	9	4	25	12	8	18	12	7

jects in Britain and the United States will develop new teaching techniques which will also show how to give young pupils an understanding of the nature of the scientific process.

To-day the goal of mastering the factual content of a subject is not sufficient; there are other goals and needs at all levels of learning. The teaching of facts and method may go hand in hand. It is how the facts are learned and organized that makes a difference. Science is most effectively taught and learned when both pupils and teacher practise scientific investigation—when they devise experiments to solve problems and improvise simple apparatus for carrying them out.

One would expect general science, chemistry and physics papers to have smaller percentages of factual questions, because it has long been the standard practice in the teaching of physical sciences to apply scientific principles in numerical problems. About 20 per cent of the general science and chemistry papers and 40 per cent in physics are devoted to 'Application'. But, in general, these problems are formal and stereotyped and amount to little more than a memory test. There is no real, new scientific problem to solve.

About 20 per cent of chemistry papers and slightly less percentages in the other scientific subjects deal with experimental work. Here again, this is rarely more than memory work, dealing with hoary experiments and demonstrations which have been thoroughly rehearsed. Rarely is a candidate expected to show imagination and ingenuity in devising experiments to test hypotheses which are new to him.

In their article, Crossland and Amos provide further evidence to show that scientific method is virtually ignored in 'O'-level science courses. They discuss the implication of this deficiency for the education of all pupils and make suggestions for a shift in emphasis from mere memory work towards some appreciation of scientific methods in 'O'-level examinations.

The syllabuses in biology, general science, chemistry and physics of the nine General Certificate of Education Examining Boards were also examined. Apart from one Board, the Oxford and Cambridge, references to scientific method in biology, chemistry and physics are limited. In biology they are confined to a training in observation and recording; "Biological knowledge should be based on observation of living plants and animals both in the laboratory and out of doors" is typical. Practical work in physical sciences is meant to give a training in skills and techniques, and in carrying out directions. In general science there are clearer signs that scientific method should be taught; for example, in the Cambridge syllabus we read " . . . the student should be led to some appreciation of scientific principles and methods by which they have been established". But the one Board which comes nearest to the treatment of scientific method is the Oxford and Cambridge. In its biology syllabus it states: (1) " . . . (candidates) expected to draw conclusions from simple experiments which they perform". (2) " . . . to understand the necessity for control experiments". (3) " . . . questions . . . which test powers of observation and reasoning of candidates". The general conclusion to be drawn from a study of the syllabuses is that only a limited amount of scientific method is expected at 'O'-level.

FUEL UTILIZATION

FUEL utilization is often regarded as the Cinderella of industry. Industries such as aircraft, motor cars, electronics and computers regard the designers of combustion chambers, boilers and furnaces as belonging to a comparatively primitive and unenterprising sector of industry. Such a view is only justified to the extent that the very small profit margins of old-established industries, which were in existence at the very beginning of the industrial revolution, allow only a small fraction of their annual turnover to be spent on research and development. In the case of units such as power station boilers or blast furnaces, each of which costs hundreds of thousands, if not millions, of pounds, the difficulties of developing a radically new and better design are immense, and unless there is a co-ordinating national development programme it is impossible to undertake the development of a greatly improved new type of appliance. Work with small pilot plant for such a new idea costs tens of thousands of pounds, and work with large pilot plant, which is essential to give answers to the economic questions of capital cost, reliability and required quality of constructional materials, costs in the range of hundreds of thousands of pounds.

It is, therefore, particularly timely and of national importance that the Institute of Fuel should have devoted the whole of a four-day conference to fuel research and development*.

The first paper in the conference was a directory of organizations carrying out research and development. This covers industrial firms and research organizations of the fuel industries, and ranges from small individual burner-makers up to organizations like Imperial Chemical Industries, Ltd., and Shell. Strangely enough, it does not, however, include any work done by universities and technical colleges. a number of which are doing valuable work at the small pilot plant and bench scale stages of applied research. The authors of the directory conclude that about 2,900 graduates and 5,000 non-graduates are working in the organizations covered by their survey and that the annual expenditure on fuel research and development excluding nuclear energy is just under £20 million. If we take it that the national annual fuel bill is of the order of £2,000 million, this comes out at 1 per cent, a very much lower figure than would be found in the more rapidly changing industries.

The first main session of the conference dealt with the organization of a research department, and the papers covered a number of interesting points, including French,

* Institute of Fuel. *Proceedings at the Conference on Fuel Research and Development held at Eastbourne, 4th to 8th October, 1965.* Vol. 1: Papers. Pp. 294. *Conference Handbook.* Pp. 14. (London: The Institute of Fuel, 1965.)

American and Hungarian organizations. My criticism of this section would be that it does not give sufficient emphasis to the long-term practical objects of fuel research and development, namely, how to obtain the desired output of the product with improved quality and regularity and reduced capital and running costs.

The next three papers dealt with the approach to research and its assessment and communication. The special problem of fuel research is that it does not attempt to attract the most original-minded youngsters in the schools, but its advantage is that people are prepared to discuss comparatively openly the new ideas which arise in these fields. The paper by the National Research and Development Council is very authoritative, as they have been working for many years on the best way of assessing new projects. Their particular fuel interests at the moment are connected with an automatic oil drill rig, fuel cells, high temperature combustion and new materials. Most new developments in fuel technology pose new problems for materials either in terms of chemical resistance or strength at high temperatures. This is well illustrated by the fact that Lord Fisher wrote to Charles Parsons in 1909 suggesting that a Parsons turbine should be operated on combustion gases, whereas it was not for another 30 years that steels were available to provide the necessary strength under the required temperature conditions.

The remaining sessions of the conference follow very logically the stages of development; the bench stage is illustrated by studies on pulverized coal, shock tubes and electrically augmented flames. These last two have not yet found any commercial application, but have interesting possible applications in the field of high-temperature endothermic reactions or the production of reaction intermediates. The small and large pilot plant work is illustrated by work on flow patterns and a general account of the work of the International Flame Research Foundation on pulverized coal flames. The Ijmuiden experimental furnace of the International Flame Research Foundation can be regarded as a large pilot plant for the study of new kinds of flames for boilers and furnaces. Apart from this there is a great dearth of work with large pilot plant in the fuel field which makes it very difficult for any radically new process to be developed. This is because the cost of building a large pilot plant is at least one-tenth of the cost of the final equipment.

The final section dealing with applications is particularly satisfactory since it contains two examples of the successful application of two quite new processes, namely: (1) the reforming of naphtha to make town gas; (2) the development of the fuel for the advanced gas-cooled nuclear reactor together with some work on minor changes such as the injection of fuel into the tuyères of a blast furnace and the high intensity gas burner.

The discussion at the conference was very lively and there is no doubt that the conference provided a valuable stimulus to this very important branch of applied science.

M. W. Thring

ATMOSPHERIC CLEAR AIR TURBULENCE

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THE purpose of this article is to report on a matter of considerable interest to aircraft operations. A new approach has been made to the problem of clear air turbulence from the point of view that energy is provided by the transfer from large (or synoptic) scale flows toward the smaller-scale flow in the gravity wave spectra. Although the exact mechanics of such transfer have not been computed, the synoptic conditions favourable for the rapid transfer of energy have been predicted from inviscid flow theory.

Synoptic evidence given by previous investigators, including Endlich[1], Reiter[2], and George[3], from examination of weather maps has confirmed the suggestion that there are certain well-recognized, large-scale patterns associated with the major outbreaks of clear air turbulence.

Endlich[1] and Reiter[2] noted that large vertical shears with strong backing of wind with height are frequently found in regions of clear air turbulence. The synoptic maps show a westerly polar jet stream beneath a strong south-westerly sub-tropical jet stream. How, precisely, an instability arises in these large-scale flow configurations is not understood, and hence the precise modes of energy transfer from the larger to the smaller scales are as yet little known.

In most investigations of clear air turbulence the emphasis has been on the use of local values of parameters such as the Richardson number (Endlich[1]) or the Scorer parameter (Reiter[2]). It is a basic premise of this article that these, or any other, parameters are only important in the region where turbulence is created, and in that region are only important at the time before inviscid flow breaks down into turbulence. Thus, once a turbulent eddy is excited it may be too late to look at these parameters since the flow field will be changed because of the onset of turbulence.

In our investigations the object is to explain clear air turbulence in terms of the following hypotheses: (*a*) Shear is generated rapidly in some region of the atmosphere to be called the source region. (*b*) The shear reaches a critical value and the flow field becomes turbulent. (*c*) The turbulent flow field is carried away from the source region and gradually decays.

The shear is measured in terms of the vorticity vector, $\mathbf{W}$, which is expanded in a Taylor's series, assuming the convection and diffusion of vorticity are small:

$$\mathbf{W}(t) = \mathbf{W}(0) + \left(\frac{\partial \mathbf{W}}{\partial t}\right)_0 t + \left(\frac{\partial^2 \mathbf{W}}{\partial t^2}\right)_0 \frac{t^2}{2} + \ldots \qquad (1)$$

In order to test the importance of effects which are non-linear with time, we have examined, on weather maps, values of $\partial^2\mathbf{W}/\partial t^2$ calculated from a formula derived from the following theoretical approach (details of which have been over-simplified for the purpose of discussion).

The equation of motion in a fixed frame of reference for an inviscid fluid is:

$$\frac{d\mathbf{V}}{dt} = -\frac{1}{\rho}\nabla p - g \qquad (2)$$

p is the pressure, ρ is the density; $-g$ is the gravitational force. $d\mathbf{V}/dt$ is the acceleration. The operation of $\frac{\partial}{\partial t}(\nabla \times)$ on equation (2) yields:

$$\frac{\partial}{\partial t}\left[\nabla \times \frac{d\mathbf{V}}{dt}\right] = -\frac{\partial}{\partial t}\left[R\nabla T \times \nabla \ln p\right] \simeq R\nabla T \times \nabla(\nabla.\mathbf{V}) \qquad (3)$$

The last step follows from the equation of continuity and the neglect of $\partial T/\partial t$. (*Note.* A more rigorous derivation by Moore[4], which is too long for this article, has shown that if diabatic heating is neglected, the relation is:

$$\frac{\partial^2 \mathbf{W}}{\partial t^2} + \frac{\partial}{\partial t}\left[(\mathbf{V}.\nabla)\mathbf{W} - (\mathbf{W}.\nabla)\mathbf{V} + \mathbf{W}(\nabla.\mathbf{V})\right] = \nabla\gamma RT \times \nabla(\nabla.\mathbf{V}) + \nabla RT \times \nabla(\mathbf{V}.\nabla \ln p) + \nabla\{(\gamma-1)\,RT\,\nabla.\mathbf{V} + \mathbf{V}.\nabla\,RT\} \times \nabla \ln p$$

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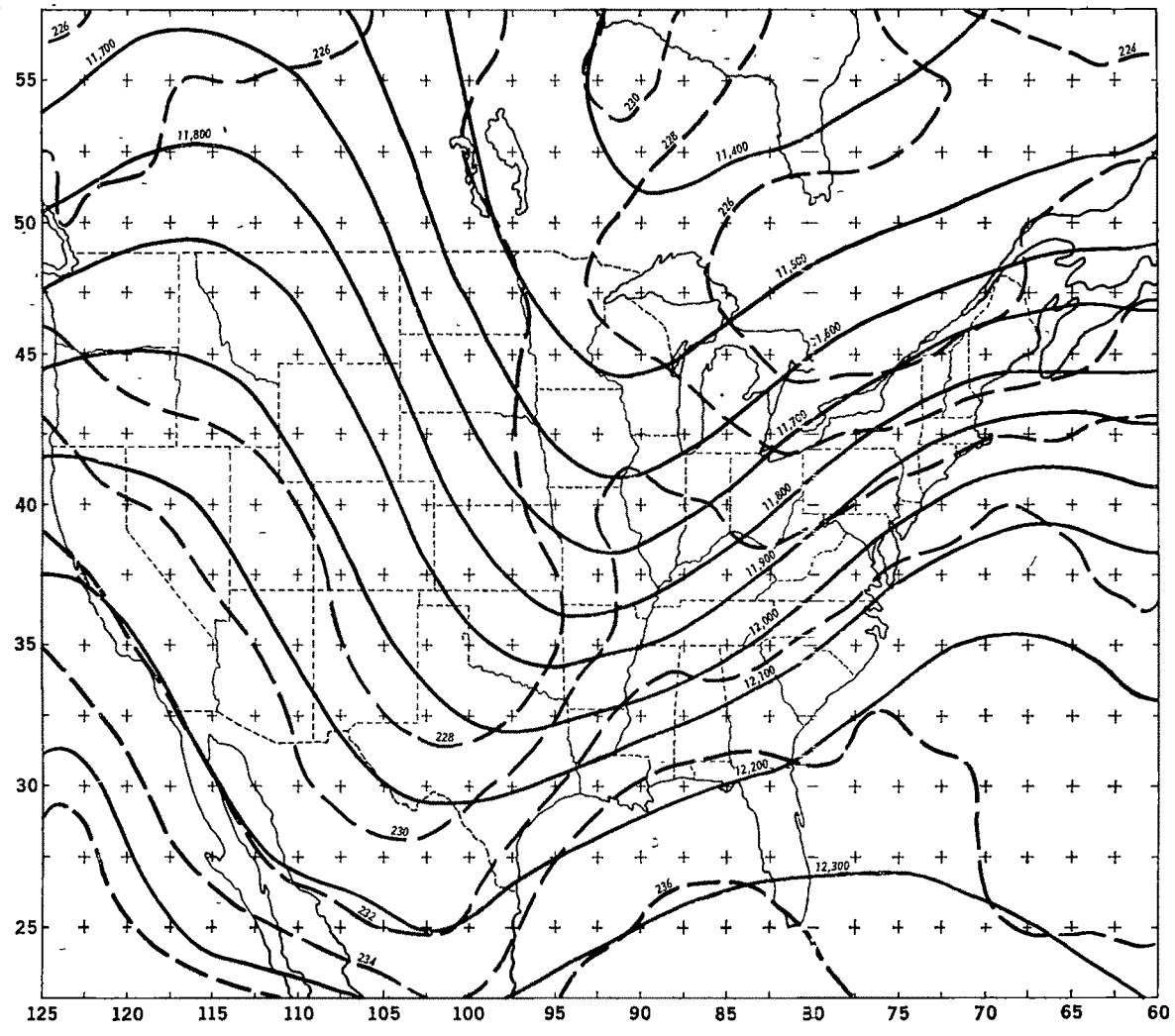


Fig. 1. High-altitude pressure and temperature field, April 13, 1962, 00Z. Solid lines are contours in metres of the 200-mbar pressure surface. Dashed lines are the isotherms at the 300-mbar level (in °K)

If the velocity normal to a surface of constant pressure is small, the 'vertical' component of the right-hand side of this equation is the component of $\nabla (\gamma RT) \times \nabla(\nabla.\mathbf{V})$ normal to the constant pressure surface.) On neglecting the partial derivative with respect to time of the convective terms involving the vorticity (the second term on the left side of the equation in the foregoing *Note*), the left side of equation (3) becomes $\partial^2 W/\partial t^2$. Thus the first non-linear term in equation (1) is proportional to $\nabla T \times \nabla (\nabla.\mathbf{V})$.

Application to a synoptic situation. As a test of the foregoing hypotheses (*a*) and (*b*), the vertical component of $\nabla T \times \nabla(\nabla.\mathbf{V})$ has been computed and plotted on a series of weather maps which correspond to the well-documented case study published by Reiter[2]. The values for the parameter already mentioned have been compared with the distribution of clear air turbulence also reported by Reiter.

We used the following method to compute the vertical component of $\nabla T \times \nabla(\nabla.\mathbf{V})$ in equation (3). It is easily

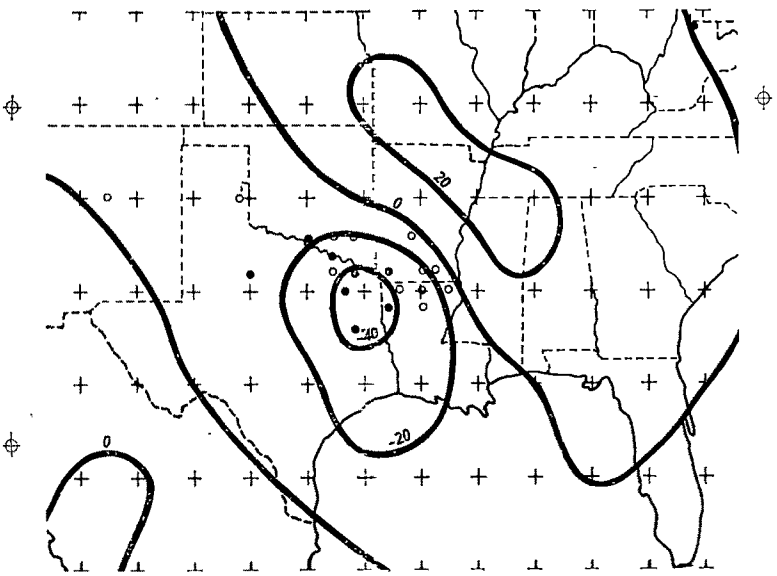


Fig. 2. Curves of constant value of the Jacobian of temperature and divergence of the velocity on the 300-mbar surface. Values in units of 10^{-17} deg sec^{-1} m^{-2}. Solid circles mark location of severe clear air turbulence. Open circles correspond to moderate clear air turbulence. Clear air turbulence data due to Reiter (ref. 2)

shown that $\nabla T \times \nabla(\nabla . \mathbf{V}).k$ is identical with the Jacobian of T and $\nabla . \mathbf{V}$ which is easily computed. $\nabla . \mathbf{V}$ on a constant pressure surface can be shown to equal

$$-\frac{1}{\gamma p}\frac{dp}{dt}$$

which is evaluated from the well-known quasi-geographic dynamical equation (see Thompson, ref. 5).

As an indication of the validity of the theory, Fig. 1 shows the temperature field at 300 mbars and flow field at 200 mbar for 00Z on April 13, 1962. Fig. 2 shows the vertical component of $\nabla T \times \nabla(\nabla . \mathbf{V})$ over the central part of the United States.

The open circles represent moderate turbulence, and the solid circles represent severe turbulence reported by pilots within 6 h of the time of observation. It is obvious that in this case the shear has been generated rapidly in a source region located near the north-east border of Texas, and that the flow field has also become turbulent, thus agreeing with our hypotheses (*a*) and (*b*). The increase in the number of the reported cases of moderate turbulence in the down-wind direction does not disagree with the hypothesis that the turbulence is carried away by the winds from the source region and gradually decays. A more complete analysis of this situation is in the process of preparation.

George[3] has previously pointed out the importance of the temperature gradient from an empirical analysis of synaptic situations associated with clear air turbulence. Similarly, Reiter[2] pointed out the importance of the convergence or divergence of the flow field; Stinson[6] has recently made a qualitative investigation of this factor. The probability of the validity of the theory of this note appears to be enhanced because it combines both the temperature gradient and the divergence of the velocity in one numerical factor, and provides a theoretical justification of the foregoing experimental deductions.

We thank Mr. J. J. George for his advice in this work, which was supported by the Douglas Aircraft Company Independent Research and Development Program.

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EFFECT OF CARBON DIOXIDE ON THE SHATTERING OF FREEZING WATER DROPS

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THE production of numerous ice particles by the shattering and splintering of freezing drops has been widely accepted as an important process which occurs under natural conditions in supercooled clouds. Braham[1], for example, has invoked this phenomenon in order to explain the high concentration of ice particles found in summer cumulus clouds over Missouri, while Latham and Mason[2] have proposed a theory of thunderstorm electrification which is based on the charge separation that accompanies the production of ice splinters.

Direct evidence for the shattering and splintering of freezing drops rests on laboratory experiments made by a number of workers (Langham and Mason[3], Mason and Maybank[4], and Stott and Hutchinson[5]). The most complete investigation of the effect is probably that of Mason and Maybank[4], and this work appears to show quite clearly that when the temperature at which a drop is nucleated falls below about −4° C, a sharp decrease occurs both in the probability of shattering and in the number of ice splinters which are thrown off from the freezing drop. For example, Mason and Maybank found that for drops of 1 mm radius nucleated at −1° C, 47 per cent shattered and, on average, 54 splinters were produced per drop; however, with a nucleation temperature of −7° C, only 6 per cent of the drop shattered and the average number of splinters fell to about 13. For nucleation temperatures below about −9° C, not one drop was observed to shatter and the average number of splinters was about 4.

The method normally used to nucleate supercooled drops at temperatures above −4° C is to drop a small piece of solid carbon dioxide into the cold chamber in which the drop is situated. The passage of the cold carbon dioxide through the air results in the production of numerous small ice crystals, some of which collide with the surface of the drop and initiate freezing. Of course, carbon dioxide may be used to cause nucleation below −4° C, and very often is, but nucleation at these lower temperatures may also be achieved using an artificial ice nucleus such as silver iodide. In the experiments of Stott and Hutchinson[5], carbon dioxide was used exclusively. Langham and Mason do not mention the method used for nucleating the drops, but it is likely that carbon dioxide was used on occasions; Mason and Maybank[4] used both carbon dioxide and silver iodide, but for nucleation above −4° C only carbon dioxide could have been used. During the course of an investigation in this laboratory into the crystallographic structure of freezing drops, it became obvious that the presence of carbon dioxide gas could have a profound effect on the manner in which a drop froze. Accordingly, experimental work was undertaken to determine the effect of carbon dioxide on the shattering of freezing drops.

The experimental arrangement was as shown in Fig. 1. By pumping methanol, previously cooled in a liquid nitrogen bath, at various rates through the hollow walls of a small chamber (approximately a cube of side 2 in.), the interior of the chamber could readily be maintained at any temperature from 0° to −40° C. Changes in the temperature of a drop during the cooling and freezing process were measured continuously by suspending the drop on the tip of a thermocouple 0·005 in. in diameter which was connected through an amplifier to a chart recorder. An additional thermocouple was placed in the chamber and close to the drop in order to record the temperature of the environment. Double glass windows in two of the sides of the chamber allowed for illumination and visual observation of the drop. Ice crystals, needed to nucleate the supercooled drops, were introduced without the use of solid carbon dioxide by momentarily lowering into the chamber a small metal rod cooled by liquid nitrogen.

In a series of preliminary experiments a number of drops were frozen in the chamber in an environment of ordinary air. The drops varied in diameter from 0·6 to 1·6 mm and were nucleated with ice crystals introduced

into the chamber either by using the metal rod already described or by placing a small amount of liquid nitrogen into the chamber. The rates of cooling used varied from 5° C/min to 160° C/min and the nucleation temperatures ranged from 0° to −20° C. In all, 150 drops were frozen in this manner, but not one drop was observed to shatter. However, about 50 per cent of the drops developed small spikes (approximately 0·2 mm in length) during freezing, and about one drop in every ten developed a long spike (approximately 1 mm in length). Following these experiments, a number of drops were nucleated with ice crystals produced by dropping a small piece of solid carbon dioxide into the chamber. Under these conditions about one-third of the drops shattered. These experiments suggested quite strongly that the shattering of the freezing drops was associated with the presence of carbon dioxide in the cooling chamber.

In order to investigate the effect of carbon dioxide on the shattering of freezing drops in a more quantitative manner, an addition was made to the experimental apparatus which allowed definite concentrations of carbon dioxide to be introduced into the chamber. This addition consisted of two flowmeters and associated cooling coils (Fig. 1). Into one of the flowmeters pure air was introduced at a known rate and into the other pure carbon dioxide gas; after passing through the cooling coils this mixture was allowed to enter the cooling chamber. With this experimental arrangement the following procedure was adopted. A drop of singly distilled water, usually of about 1 mm diameter, would be suspended on the top of the thermocouple and then lowered into the upper part of the chamber, where the temperature was about 2° C. A known mixture of carbon dioxide and air was passed continuously into the chamber at a constant rate of 200 ml./min independent of the concentration of carbon dioxide; shortly afterwards ice crystals would be produced in the lower part of the chamber by introducing the metal rod cooled by liquid nitrogen. After the drop had been exposed to the air–carbon dioxide mixture for 2 min at 2° C, it would be lowered quickly to the −10° C level in the chamber and its mode of freezing observed through a telescope. Prior to changing the concentration of carbon dioxide in the mixture, the chamber would be flushed thoroughly with clear air. The foregoing procedure was repeated for twelve different concentrations of carbon dioxide, and at each concentration ten drops were frozen. The temperatures at which the drops nucleated in these experiments varied from 0° to −5° C.

The manner of freezing, and the probability of a drop shattering, were found to change markedly as the concentration of carbon dioxide to which the liquid drop had been exposed was increased. For very low concentrations of carbon dioxide (less than 1 per cent) only minor bulges and small spikes formed, and some small cracks were observed in the surfaces of the frozen drops. When the concentration of carbon dioxide in the mixture reached about 25 per cent, however, long ice spikes of up to 2 mm in length were forced out from the freezing drop and violent shattering and cracking occurred. As the concentration of carbon dioxide was increased above 75 per cent huge protuberances formed containing large quantities of gas; these protuberances usually ruptured at their ends as the gas was forced out, and the drop sometimes shattered completely.

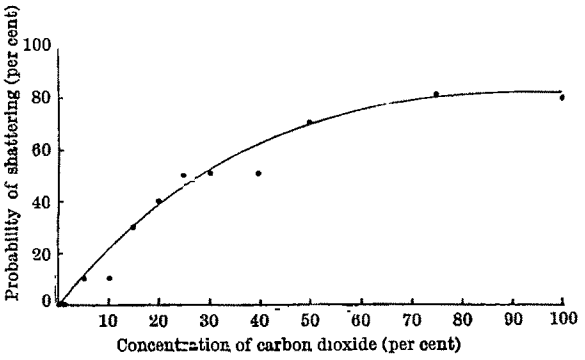


Fig. 2. The probability of a drop shattering on freezing as a function of the concentration of carbon dioxide in the environmental gas (for details see text)

The results of these experiments are shown in a quantitative manner in Fig. 2, where the probability of a drop shattering is plotted against the concentration of carbon dioxide gas in the mixture to which the liquid drop is exposed at 2° C. These results show quite clearly that the probability of shattering increases rapidly as the concentration of carbon dioxide in the mixture increases. For concentrations of carbon dioxide less than 1 per cent the probability of shattering is effectively zero. In this figure the term 'shattering' includes the complete disintegration of a drop or a spike, the breaking off of part of a drop, or noticeable fragmentation of the surface of the ice when a gas bubble emerges from the end of a protuberance.

In order to determine whether, even after being subjected to relatively high concentrations of carbon dioxide gas, drops shatter only when nucleated above −5° C, twenty liquid drops were subjected to an air–carbon dioxide mixture containing 25 per cent carbon dioxide, and these drops were then nucleated at approximately −12° C in the manner already described. Six of the twenty drops shattered—four disrupting completely and two forming spikes which later shattered. Although this gives a probability of 30 per cent, as compared with a probability of 50 per cent for drops subjected to 25 per cent concentration of carbon dioxide and nucleated above −5° C, it demonstrates that, provided a liquid drop has been situated in an environment containing a relatively high concentration of carbon dioxide, it may shatter during freezing even though it is nucleated below −5° C. Indeed, drops subjected to high concentrations of carbon dioxide have been observed to shatter when nucleated at temperatures as low as −19° C.

The effect of carbon dioxide on the shattering of freezing drops may be understood in general terms as follows. Carbon dioxide is approximately one hundred times more soluble in water than is nitrogen and nearly fifty times more soluble in water than is oxygen. The solubility of carbon dioxide in ice, on the other hand, is very small compared with that in liquid water. Therefore, as a drop freezes radially inwards, the carbon dioxide in the liquid water will be forced out as gas bubbles, and this will result in the build-up of large stresses in

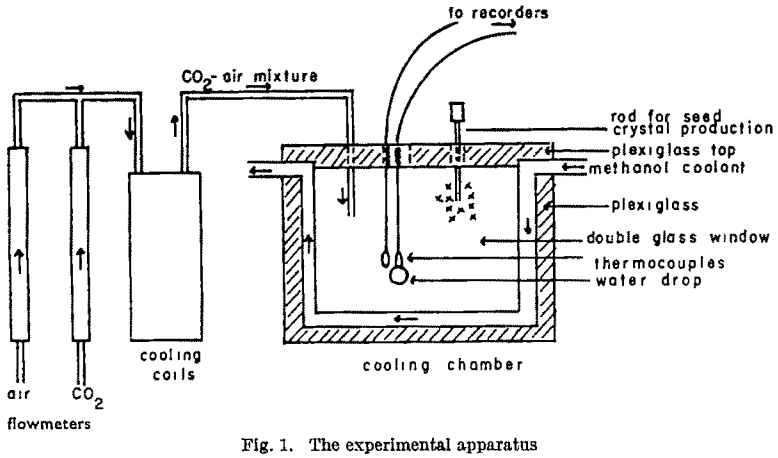


Fig. 1. The experimental apparatus

the surrounding ice shell. Under these stresses water may be pushed out from the interior of the drop to form spikes and bulges on the surface of the sphere, and, if this is not sufficient to accommodate the increasing pressure in the interior, complete shattering of the drop will occur. Since the concentration of carbon dioxide in liquid water increases linearly with the partial pressure of carbon dioxide in the environmental gas, the increase in the probability of shattering with increasing concentration of carbon dioxide (Fig. 2) is readily understood. The expansion of the water as it freezes in the interior of a drop will also contribute to the tensions in the ice shell, but our experiments show that this effect alone is insufficient to cause shattering.

In the light of these results we suggest that the shattering of freezing drops observed in the laboratory by previous investigators was in all cases probably due to the presence of carbon dioxide in solution in the liquid water. Moreover, we would ascribe the apparent effect of nucleation temperature on shattering, observed by Mason and Maybank[4], to the use of carbon dioxide for seeding at temperatures above −4° C. From the appearance of the long spike shown in Fig. 2 of Mason and Maybank's paper, we would judge that the liquid drop had been exposed to an environmental gas containing about 25 per cent carbon dioxide gas. If this were the case, according to our Fig. 2 the probability of shattering would be approximately 50 per cent and, for nucleation temperatures above −4° C, this is in good agreement with the statistics for shattering given by Mason and Maybank.

It follows also from our results that under natural conditions in the atmosphere, where the concentration of carbon dioxide is always less than about 0·06 per cent, the shattering of freezing drops probably never occurs. However, the introduction of sufficient quantities of carbon dioxide gas into a natural cloud could give rise to the shattering of droplets during the freezing process. In fact, we would suggest that the seeding of supercooled clouds with silver iodide might be more effective if large quantities of carbon dioxide gas were introduced into the cloud just prior to seeding.

It should be noted that the results reported in this article refer only to the shattering of freezing drops and not to the production of splinters. However, shattering and splinter production are intimately connected, for both phenomena derive from the build-up of stresses in the freezing drop. It therefore seems likely that the number of splinters thrown off from a freezing drop will increase as the concentration of carbon dioxide in the liquid drop rises. Measurements of the effect of carbon dioxide on splinter production are now being undertaken and the results will be published in due course.

This research was supported by the Atmospheric Sciences Section of the U.S. National Science Foundation under grants *GP*–2607 and *GP*–3809.

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A COMPARISON OF TOTAL MAGNETIC FORCE SECTIONS ACROSS NORTHERN CALIFORNIA AND SOUTHERN NEW ZEALAND

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GRANTZ and Zietz[1] have compared magnetic profiles across "belts of moderately deformed sedimentary rocks" in Alaska and California. These profiles record broad highs in total magnetic intensity over the Jurassic and Cretaceous marine sedimentary rocks underlying the Cook Inlet and Copper River lowlands in Alaska, and over similar rocks underlying the northern and central Great Valley, California. These highs are parallel to the major geological features in each area, and are absent over parallel belts of more severely deformed sedimentary rocks of similar age which occur in the bordering Chugach Mountains and Alaska Range in Alaska, and in the Coast Ranges in California.

Similar geological situations exist in New Zealand and California. In Southland, New Zealand (Fig. 1), the old land mass of Fiordland is bordered on the east by a syncline of gently deformed marine and freshwater sediments (Hokonui facies) of great thickness (~ 50,000 ft., according to Wellman[2]). This syncline is known as the New Zealand marginal syncline[3]. Farther east still are the severely deformed parallel greywackes (Alpine facies) and schists that were deposited in the New Zealand geosyncline. Between the marginal syncline and the New Zealand geosyncline are found ultramafic and ultrabasic rocks.

Fig. 2 compares the aeromagnetic total force profiles across the Sacramento Valley and Coast Ranges in California with the seaborne total force profiles round the south-east coast of the South Island, New Zealand. Because the rock sequences are from west to east in New Zealand and east to west in California, the New Zealand profile has been reversed for easier comparison.

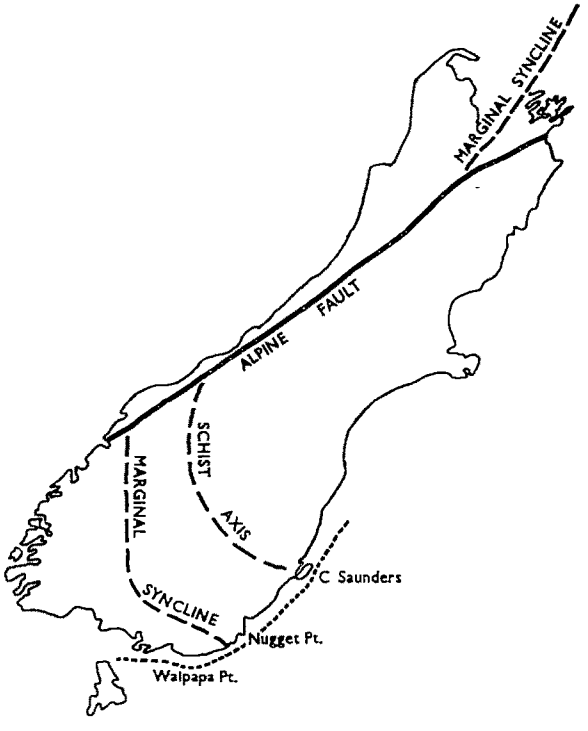


Fig. 1. South Island of New Zealand, showing principal structural features, and track of magnetic observations (dotted line)

The following parallels can be drawn, going from right to left in Fig. 2.

(1) At the western end of the New Zealand profile the many narrow peaks are due to the underlying pyroclastics, pillow lavas and shallow intrusives of gabbro, diorite and biotite granite of the Longwood complex. The comparable eastern part of the profile in California was not recorded by Grantz and Zietz, but they state that "available magnetic data over the Jurassic slate and greenstone belt of the Sierra Nevada, which borders the Great Valley on the east, record so many magnetic features of shallow origin that it is difficult to determine whether or not broad magnetic highs occur there".

(2) Between Waipapa Point and Nugget Point on the New Zealand profile is a broader anomaly 35 miles in width and of amplitude varying from 500 to 1,000 gammas. The anomaly over the northern and central Great Valley is about "30 miles wide and ranges in amplitude from a few hundred to more than 1,000 gammas"[1]. The crests of the magnetic highs lie respectively a few miles west and a few miles east of the thickest parts of the sedimentary prisms in Southland and the Great Valley.

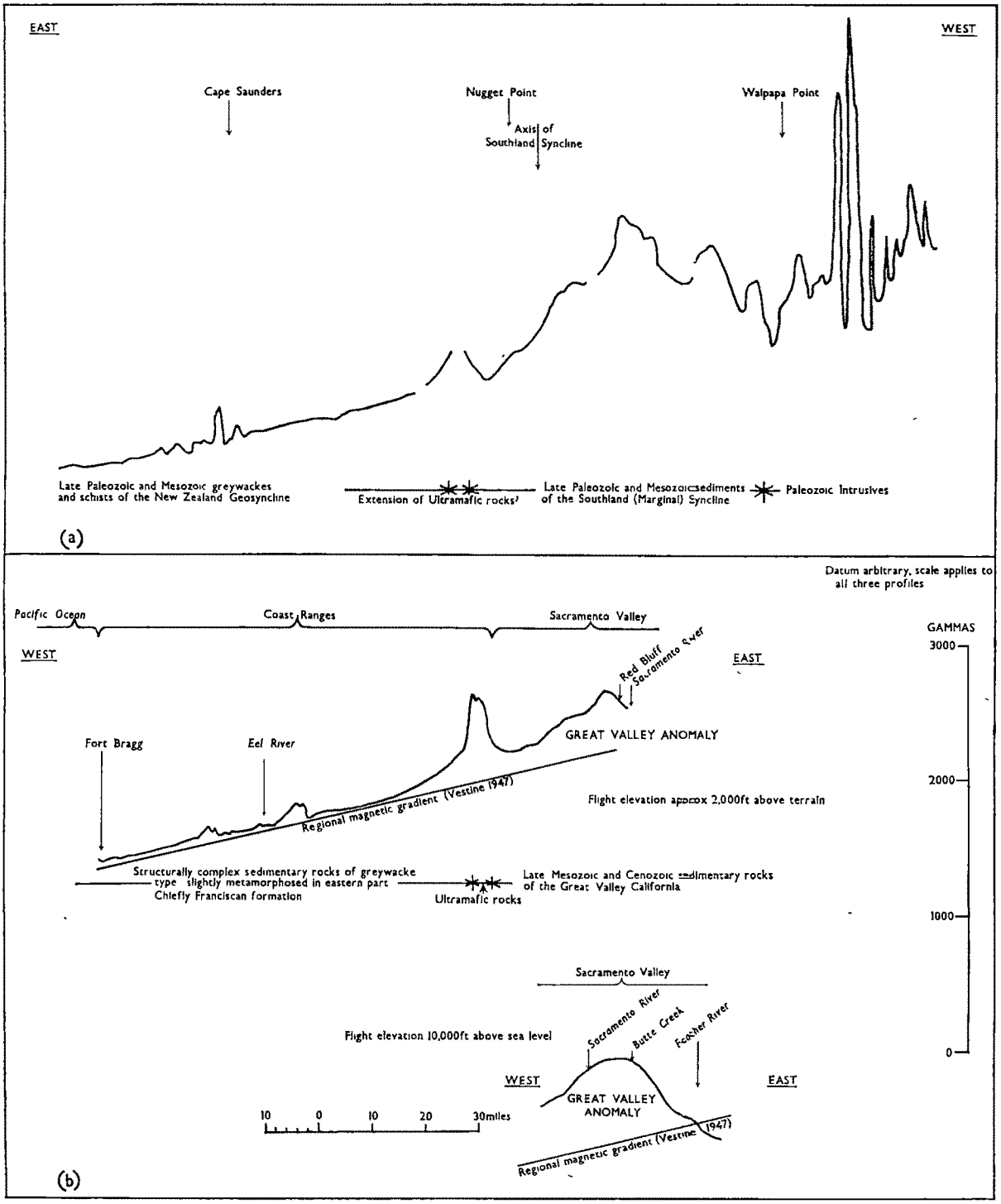


Fig. 2. (*a*) Total force magnetic profile around south-east coast of South Island, New Zealand. Observations at sea-level (*b*) Total force magnetic profiles across northern California. Observations at elevations given in Figure. (After Grantz and Zietz (ref. 1))

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That is, in both cases, the highs are on the continental sides of the marginal synclines.

(3) Between the overturned north-eastern flank of the New Zealand marginal syncline and the schists to the north-east is a single narrow anomaly of about 300–400 gammas (though reaching 1,200 gammas elsewhere). This can be traced north-west along the junction of the marginal and geosynclinal facies until it joins exposures of volcanic and ultramafic rocks 80 miles away at Mossburn[4]. At a similar position in the Californian sequence a single 600-gammas peak coincides with exposed ultramafic rocks.

(4) Over the New Zealand geosyncline magnetic anomalies are small except where late Tertiary volcanics (Cape Saunders–Taiaroa Head) are exposed. Over the Coast Ranges of California there are minor anomalies which are due to basic volcanics or ultramafics.

The most interesting sections of these profiles are the broad magnetic highs over the marginal synclines. The Bouguer gravity anomaly associated with the magnetic high in the Great Valley of California is about 20–40 milligals. There is no significant gravity anomaly associated with the magnetic anomaly in Southland, New Zealand[4]. Thus in the latter case it has been assumed that the density of the disturbing magnetic rock is about 2·65 g/cm³ which is the density of the Late-Palaeozoic to Mesozoic sediments of the syncline. Permian sediments of this density and with magnetic susceptibilities of up to 4,000 × 10^{-6} c.g.s. units occur within the area of the magnetic high, but it is unlikely that these are extensive enough to cause the major high, though they may cause local noise[4]. Of igneous rocks, only serpentine and perhaps andesite appear to have the relatively high susceptibility and low density to explain the high magnetic anomaly and lack of gravity anomaly. The Southland syncline is separated from its supposed extension, the Nelson syncline, by the transcurrent Alpine Fault. Hatherton[5] has suggested that the magnetic and gravity anomalies in the Nelson–Cook Strait region can be best explained by a dunite-serpentine system. Such a system generally shows an inverse relationship between density and magnetic susceptibility[5].

As Grantz and Zietz pointed out after studying the California and Alaska profiles, "there may be a causal relationship between the existence of the rocks that produce the broad magnetic highs and the structure and lithology of the sedimentary prisms that overlie them". The addition of the New Zealand results increases the probability that the broad magnetic highs resulting from apparently deep-seated igneous rocks are a generic characteristic of the stable synclines adjoining geosynclines.

I thank Prof. George A. Thompson of Stanford University for bringing the results of Grantz and Zietz to my notice.

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DETECTION OF HYDROGEN EMISSION LINES $n_{159} \rightarrow n_{158}$ AND $n_{157} \rightarrow n_{156}$ IN GALACTIC H II REGIONS

By Prof. A. E. LILLEY, Prof. D. H. MENZEL, H. PENFIELD and B. ZUCKERMAN
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In 1959, Kardashev[1] predicted that spectral lines of excited hydrogen of the $n \rightarrow n - 1$ type could be observed in galactic H II regions. In particular, he showed that the most readily observable lines should appear in H II regions having a high continuum temperature and that lines of the type $n \rightarrow n - 2$, $n \rightarrow n - 3$, etc., should be less intense than those of type $n \rightarrow n - 1$.

The detection of lines of this type was first announced by two groups of Russian observers[2,3], who found the line $n_{105} \rightarrow n_{104}$ (ν = 5,762·9 Mc/s) in *M*17 and the Orion Nebula, and the line $n_{91} \rightarrow n_{90}$ (ν = 8,872·6 Mc/s) in *M*17. More recently, Hoglund and Mezger[4] observed the line $n_{110} \rightarrow n_{109}$ (ν = 5,008·9 Mc/s) in a number of galactic H II regions.

We have detected two new hydrogen lines corresponding to the transitions $n_{159} \rightarrow n_{158}$ (1,651·544 Mc/s) and $n_{157} \rightarrow n_{156}$ (1,715·676 Mc/s) in two galactic sources, *M*17 and *W*51. Local radio interference prevented a search for the intermediate transition $n_{158} \rightarrow n_{157}$ (1,683·203 Mc/s). Our data were obtained with the Harvard 60-ft. telescope equipped with a 10-channel cavity maser radiometer with a resolution of 80 kc/s. The antenna half-power beam-width was 52 min of arc and its aperture efficiency was 0·38 at the wave-length of operation. The total noise temperature of the system was approximately 100° K. Figs. 1–4 show the profiles obtained with this system, and Table 1 summarizes our results.

To interpret their observational results, Hoglund and Mezger assumed a Gaussian line shape and a Maxwellian distribution of electron velocities at temperature T_e. The total energy observed in a line is then:

$$\Delta\nu_L T_L = 1{\cdot}67 \times 10^6 T_e^{-3/2} E \text{ deg/sec} \qquad (1)$$

where $\Delta\nu_L$ is the line width at half power in cycles per sec and T_L is the excess of brightness temperature in the line above that in the continuum. E is the 'emission measure' in parsecs (pc) cm^{-6}. As noted by Hoglund and Mezger[4], equation (1) should be a reasonable approximation for an optically thin line of Gaussian shape. From Oster's[5] equation (162) we obtain the free–free continuum radiation. If we assume that the continuum is optically thin and set ν = 1,680 Mc/s and T = 10^4 in the Gaunt factor,

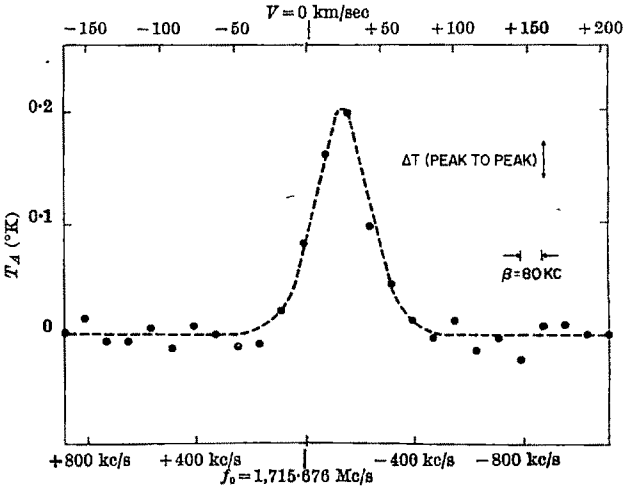


Fig. 1. Line spectrum for *M*17 ($n_{157} \rightarrow n_{156}$). The ordinate is antenna temperature. The abscissae are frequency and velocity with respect to the local standard of rest. A baseline slope of 0·10° K/Mc/s has been removed

Table 1. Summary of Observational Results

	$M17$ $\{\alpha=18h\ 17{\cdot}8m,\ \delta=-16°\ 09'\}$ 1950		$W51$ $\{\alpha=19h\ 20{\cdot}8m,\ \delta=+14°\ 08'\}$ 1950	
ν (Mc/s)	1,715	1,651	1,715	1,651
λ (cm)	17·5	18·2	17·5	18·2
T_A (°K)*	0·202 ± 0·024	0·260 ± 0·026	0·130 ± 0·030	0 088 ± 0·026
T_l/T_c	1·01 ± 0·17%	1·15 ± 0·17%	1·30 ± 0·36%	0·73 ± 0·25%
$\Delta\nu_L$ (kc/s)	240	230	310	200
$\Delta\nu_L$ (corrected) (kc/s)	219 ± 60	209 ± 60	291 ± 80	172 ± 80
V (km/s)†	25 ± 5	21 ± 5	56 ± 6	64 ± 6

* Peak antenna temperature of the line above the continuum.
† The radial velocities are with respect to the local standard of rest.

we find for T_c, the brightness temperature of the continuum:

$$T_c = \frac{3{\cdot}13 \times 10^{17} E}{T_e^{1/2}\nu^2} \qquad (2)$$

Although the observed hydrogen lines are almost certainly optically thin, the continuum radiation at the centre of $M17$ may be optically thick at ~ 1,700 Mc/s. When the regions responsible for the continuum and line radiation originate in the same source (subtend the same solid angle), then the antenna temperatures are proportional to the brightness temperatures.

Eliminating E between equation (1) and equation (2) and solving for T_e in terms of the observed quantities, we obtain for $M17$ at 1,715 Mc/s, $T_e = 6{,}800° \pm 3{,}000°$ K, and at 1,651 Mc/s, $T_e = 5{,}500° \pm 2{,}400°$ K. The estimated errors include those in T_L, T_c and $\Delta\nu_L$. These temperatures were calculated with Gaunt factors adjusted to the respective temperatures rather than to the 10^4 °K used in the first approximation. If the continuum radiation originates in a region that is optically thick, the foregoing electron temperatures are too high. The electron temperatures for $M17$ are less than the value of 10^4 °K usually given for H II regions and greater than the value for $M17$ calculated by Hoglund and Mezger[4].

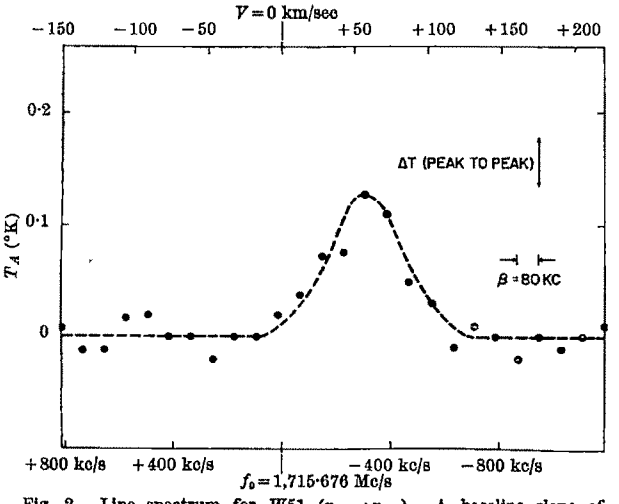


Fig. 2. Line spectrum for $W51$ ($n_{157} \rightarrow n_{158}$). A baseline slope of 0·20° K/Mc/s has been removed

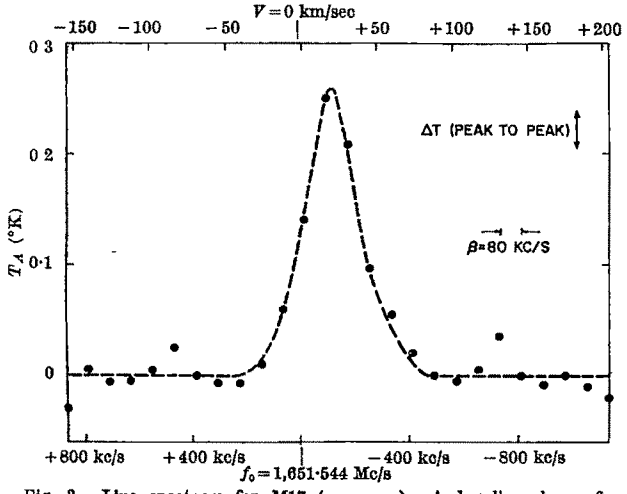


Fig. 3. Line spectrum for $M17$ ($n_{159} \rightarrow n_{158}$). A baseline slope of 0·25° K/Mc/s has been removed

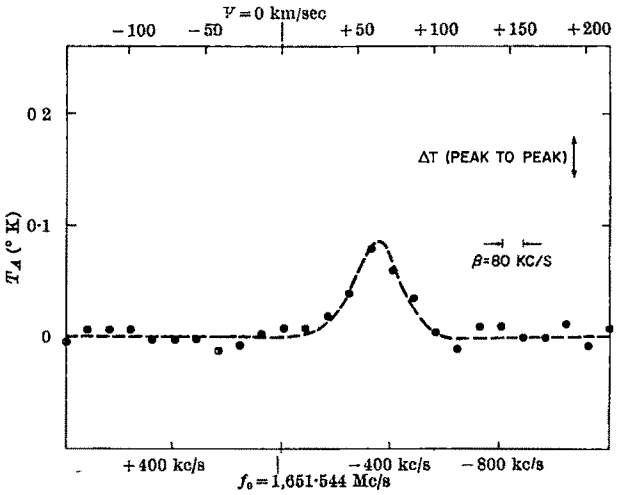


Fig. 4. Line spectrum for $W51$ ($n_{159} \rightarrow n_{158}$). A baseline slope of 0·12° K/Mc/s has been removed

We have not calculated T_e for $W51$ because of the small signal-to-noise ratio and, since the source is complex, we cannot determine what part of the continuum comes from the H II region responsible for the spectral line.

Kardashev gives formulae for broadening of the excited hydrogen lines by Doppler motion, turbulence, the linear Stark effect, and the statistical Stark effect. The relationship of the observed line-widths to the line broadening predicted by Kardashev is shown in Fig. 5. The two lines reported from the U.S.S.R., the line detected at the National Radio Astronomy Observatory, and the lines detected at Harvard University are plotted. The line broadening as predicted by Kardashev is marked $\Delta\nu_L$ and increases rapidly with increasing wave-length. We have obtained $\Delta\nu_L$ using Kardashev's equations 6, 7 and 8, $T_e = 10^4$ °K, $N_e = 10^2$ cm^{-3} and no turbulence. With these typical values assumed, the direct application of the Stark equations given by Kardashev predict a line width of an order of magnitude greater than that observed at $\lambda \sim 18$ cm.

Two additional reference lines are drawn for comparison with the observational data in Fig. 5. These are the straight lines which represent Doppler broadening only; the lower line for $T_e = 10^4$ °K (no turbulence) and the upper line for either $T_e = 10^5$ °K (no turbulence) or $T_e = 10^4$ °K and 40 km/sec turbulence. The observational data in Fig. 5 suggest that at least to $n \sim 160$ ($\lambda \sim 18$ cm) a combination of thermal broadening and a modest amount of internal turbulence within the nebula will entirely account for the observed line-widths. Moreover, there is observational evidence for internal motions within H II regions[5].

If the Stark equations as given by Kardashev were actually valid, we could find an electron density to generate the observed line-width at lower frequencies. If this procedure is followed for the source $M17$ we obtain $n_e = 10$ cm^{-3} (for $T_e = 10^4$ °K) and $n_e = 9$ cm^{-3} (for $T_e = 6 \times 10^3$ °K). With the electron density evaluated in this manner, we may use equation (1) to determine the size of the region, since $E \sim n_e^2\, l$. Using the observables in equation (1), an aperture efficiency of 0·38, and $n_e \sim 10$ cm^{-3}, we find a value of $l \sim 700$ pc to account for the observed T_L. This size and electron density may be compared with $l \sim 13$ pc and $n_e = 125$ cm^{-3} for the optical H II region given by Westerhout[7]. However, taking $n_e \sim 10$ cm^{-3}, $l \sim 700$ pc, and a distance to $M17$ of 1,700 pc[6], we can use equation (2) to evaluate the

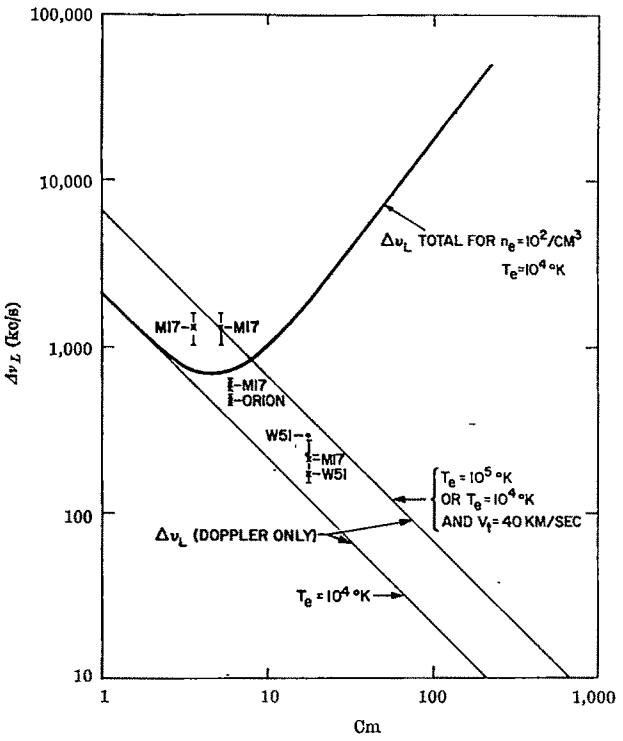


Fig. 5. Plot of line width ($\Delta\nu_L$) versus wave-length for all observed $n\rightarrow n-1$ lines. The error bracket shown for the Harvard data on *M*17 ($\lambda=18{\cdot}2$ cm) is typical of all four spectra

characteristics implied for the continuum. According to such a model, *M*17 would appear as a continuum source of very broad angular extent, but observations show no significant broadening in either the Harvard beam (52 arc min) or the Dwingeloo beam (34 arc min).

Consequently, the direct application of the Kardashev line-broadening theory leads to a contradiction with continuum observations, and grossly over-estimates the line-width. The fact that the theory was developed for a plasma having a much higher density than H II regions may be responsible for the over-estimate of the Stark effect. The importance of undertaking further observations at wave-lengths longer than 18 cm is evident in Fig. 5, and should help to clarify the problem of line broadening.

The successful detection of the $n\rightarrow n-1$ lines in thermal sources will allow astronomers to determine the galactic distribution of H II regions by methods similar to those developed for the 21-cm hydrogen line, bringing both ground state and excited hydrogen within the purview of radio telescopes.

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EFFECT OF GLASS CONTACT ON THE ELECTROPHORETIC MOBILITY OF HUMAN BLOOD PLATELETS

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An important property of blood platelets is their ability to stick to each other, *in vivo* during thrombus formation and *in vitro* during the process of platelet aggregation. However, little is known about the actual mechanism of platelet to platelet adherence. Platelets carry a negative charge[1] and it has been suggested[2] that changes in this surface charge might be related to platelet aggregation. The surface charge of a cell can be investigated by measurement of the electrophoretic mobility of the cell, but previous electrophoretic investigations of platelets have been directed towards the determination of the ionic groups responsible for the charge[3], or to estimations of mobility in patients with various clinical conditions[4,5].

We have investigated the electrophoretic mobility of human platelets and have shown (unpublished) that changes can be induced by the agents known to cause platelet aggregation. During these investigations we found that platelet mobility also changes in the absence of aggregating agents, and that this change was markedly affected by glass contact. This article sets out the results of our investigations of these changes in platelet mobility which occur in the absence of exogenous aggregating agents.

Platelet-rich plasma (PRCP) was prepared from venous blood as described by Emmons and Mitchell[6], and platelet-poor plasma (PPP) was prepared by centrifuging PRCP in siliconized tubes at 750*g* for 15 min. Mobility measurements were made on a mixture of 9 vol. PPP and 1 vol. PRCP, using the horizontal capillary electrophoresis apparatus developed by Bangham *et al.*[3]. All estimations were carried out at 25° C, using a potential gradient of 2·66 V/cm. Only platelets in the stationary layer[3] were studied and, to eliminate the effect of drift, five transits in each direction were timed. The eyepiece graticule was calibrated against a stage micrometer under water, and the mean transit times were converted into mobility rates, expressed in μ/sec/V/cm.

When platelet electrophoretic mobility was measured twice on freshly separated plasma samples, it was found that the first measurement always gave a faster result than the second, and that subsequent measurements gave decreasing mobilities until a stable figure was reached. The rate of decrease in mobility varied from subject to subject, but all reached a stable minimum mobility within 1 h. Thereafter the mobility did not change and duplicate estimations on 36 samples in this stable state gave a mean difference between measurements of 1·16 per cent. It was also found that bringing the fresh plasma samples into contact with a non-siliconized glass surface immediately reduced the platelet mobility to this minimum, and that ageing thereafter had no effect. Samples were brought into contact with non-siliconized glass by placing them in a test-tube which had been washed only with tap water and distilled water, covering the tube with 'Parafilm' and gently inverting twelve times. This process will be referred to as 'glass contacting', and the treated mixture as 'post-contact plasma', while a PRCP/PPP mixture which had not been so treated and which had only encountered siliconized surfaces will be referred to as 'pre-contact plasma'. The reduction in mobility which we observed after glass contacting will be referred to as the 'contact effect'.

(1) *Effect of ageing on pre-contact mobility.* The pre-contact mobility of one aliquot of platelets was measured. Between 15 and 20 min later, the pre-contact mobility

of a second aliquot which had been kept at room temperature was measured and was shown to be reduced. Both samples were then brought into contact with non-siliconized glass in the way already described and their post-contact mobilities measured: they were found to be slower. The mean mobilities from six experiments are shown in Table 1: the mean values and their significance-levels were calculated from the transit times, so that each figure in Table 1 represents the mean of sixty transit times. Once the stable, post-contact state had been reached either by glass contact or by ageing, a second exposure to a glass surface had no effect, nor did further ageing for several hours.

If the manoeuvres involved in the glass contacting process were performed in a siliconized tube the decrease in mobility did not occur. It is therefore the contact with the non-siliconized glass surface, and not agitation or contact of platelets with air, which causes the decrease.

(2) *Effect of siliconized and non-siliconized glassware.* Blood samples were collected by a two-syringe technique from a single venepuncture. The first syringe was siliconized, while the second was not, and the samples were afterwards handled in siliconized and non-siliconized glassware respectively. The pre-contact mobility of the platelets handled in the non-siliconized system was equal to the post-contact mobility of those handled in the siliconized system. Glass contacting of the platelets from the non-siliconized system in the usual way caused no change in mobility. The mean results of six experiments are shown in Table 2.

(3) *Microscopical appearance of platelets.* If the viscosity of the suspending liquid remains constant, a change in the size of a particle will cause a change in its electrophoretic mobility. It was therefore possible that the reduction of platelet mobility on glass contact might be due to a change in the configuration of the platelet, or to pairs or small clumps of platelets developing. Platelets were examined by phase-contrast microscopy before and after glass contact and there was no difference in the frequency with which pairs or small clumps were seen in the pre-contact and post-contact samples, nor could any difference be detected in platelet morphology. The change in mobility must therefore be due to a change in the surface charge of the platelets, or to a change in the viscosity of the suspending medium.

(4) *Effect of contacting the platelet-poor plasma.* Instead of contacting the PRCP/PPP mixture in the usual way, the PPP alone was contacted and was then used to dilute pre-contact PRCP. The mobility of the platelets was unaffected and the usual slowing occurred when the whole PRCP/PPP mixture was contacted. The reduction of platelet mobility on glass contact is not due, therefore, to a change in viscosity of the platelet-poor plasma; it is also unlikely to be related to activation of the early stages of the clotting process, for this would have occurred when the platelet-poor plasma alone was contacted.

(5) *Suspensions of platelets in saline.* PRCP was prepared in the usual way, but, instead of using PPP as the diluent, 1 ml. portions were diluted with 8 ml. 0·85 per cent (w/v) saline and 1 ml. 3·8 per cent (w/v) trisodium citrate. The pre-contact mobility of the platelets was measured; the platelet–saline mixture was then 'glass contacted' in the usual way and the post-contact mobility of the platelets determined. The mean values obtained from six experiments were: pre-contact, 1·34 μ/sec/V/cm; post-contact, 1·26 μ/sec/V/cm (P for difference 0·01). It will be noted that both pre- and post-contact mobilities are higher than those found when the PRCP is diluted with PPP; this results from the lower viscosity of the saline mixture. Nevertheless, the contact effect still occurs when the platelets are suspended in citrate-saline.

(6) *Effect of ethylenediamine tetraacetic acid (EDTA).* Blood was collected in a siliconized syringe and 9 ml. portions were transferred to centrifuge tubes containing either citrate or 0·15 ml. of 10 per cent (w/v) dipotassium EDTA and 0·85 ml. normal saline. The contact effect in the EDTA tubes did not differ from that observed when citrate was used as the anticoagulant.

(7) *Effect of heparin.* 9-ml. aliquots of blood were collected into either 1 ml. sodium citrate or into 1 ml. normal saline containing varying concentrations of heparin; these were such as to give concentrations in the final mixture of 1, 2, 5, 10 and 50 units per ml. It was found that with concentrations of 2 units/ml. of heparin and above the contact effect was abolished: the pre-contact mobility was the same as the post-contact mobility of the citrate sample. With 1 unit/ml. of heparin the pre-contact mobility was lower than that of the pre-contact citrate sample, but fell to the same level after contact as the post-contact citrate sample. When heparin was given intravenously in a dose of 750 N.I.H. units, it abolished the contact effect in a blood sample taken 5 min later.

(8) *Effect of enzyme poisons.* A variety of enzyme poisons was added to a fresh PRCP/PPP mixture in siliconized tubes; control tubes containing saline were prepared at the same time. Each was incubated at 25° C for 5 min and their pre-contact mobility measured. Both series were then contacted and their post-contact mobilities measured. It was found that *N*-ethylmaleimide in concentrations up to 10^{-2} M and *p*-hydroxymercuribenzoate 10^{-3} M (inhibitors of reactions involving sulphydryl groups), potassium cyanide $1{\cdot}5 \times 10^{-3}$ M (inhibitor of cytochrome oxidase), sodium fluoride $2{\cdot}5 \times 10^{-4}$ M (inhibitor of synthesis of phosphopyruvate), and sodium monofluoroacetate 10^{-2} M (inhibitor of aconitase, and so of the tricarboxylic acid cycle) had no effect on the pre-contact mobility, its reduction on glass contact, or the post-contact mobility of the platelets.

However, when the enzyme poisons were added to the citrate into which the blood was collected a different pattern emerged. *N*-ethylmaleimide at 10^{-3} M abolished the difference between pre- and post-contact mobilities, although the post-contact mobility was the same as that of a parallel sample taken into citrate alone. *N*-Ethylmaleimide 10^{-5} M had no effect, and 10^{-4} M an intermediate effect. *p*-Hydroxymercuribenzoate 10^{-3} M similarly abolished the difference between pre- and post-contact mobilities, while potassium cyanide, sodium fluoride and sodium monofluoroacetate had no effect.

(9) *Effect of pyruvate kinase.* In view of our finding that low concentrations of adenosine diphosphate (ADP) increase the electrophoretic mobility of platelets (unpublished), it seemed possible that the reduction of platelet mobility on glass contact might be due to loss from the platelet surface of ADP. ADP can also be removed from a system[7] by the enzyme pyruvate kinase (PK) acting through the reaction:

$$\text{Phosphoenolpyruvate (PEP)} + \text{ADP} \overset{\text{PK}}{\rightleftharpoons} \text{Pyruvate} + \text{ATP}$$

In this reaction the equilibrium lies far to the right. Blood was collected in the usual way and 8-ml. portions were transferred to centrifuge tubes containing 1 ml. sodium citrate and 1 ml. of a PEP–PK mixture in saline. Control tubes containing citrate and saline alone or citrate plus saline solutions of PEP or PK were also prepared.

PEP in a concentration in the final mixture of 0·005 μg/ml. did not have any effect on the pre- or post-contact

Table 1. Effect of Age and Glass Contact on Platelet Mobility

	Mean pre-contact mobility (μ/sec/V/cm)	Mean post-contact mobility (μ/sec/V/cm)	P for difference
Fresh platelets	1·18	1·10	< 0·001
Platelets 15 min old	1·14	1·11	*N.S.*
P for difference	0·03	*N.S.*	

Table 2 Effect of Siliconized and Non-siliconized Glassware on Platelet Mobility before and after Glass Contact

	Mean pre-contact mobility (μ/sec/V/cm)	Mean post-contact mobility (μ/sec/V/cm)	P for difference
Siliconized	1 18	1·03	< 0 001
Non-siliconized	1·02	1·03	*N S.*
P for difference	< 0 001	*N.S*	

mobility of the platelets. With 0·05 and 0·5 μg/ml. a progressive reduction in both pre- and post-contact mobility was produced, but the difference between the two was the same as in the control sample; the contact effect was thus unchanged. PK alone in a concentration of 0·1 mg/ml. in the final mixture had no effect on either pre- or post-contact mobility.

When both PEP and PK were present the difference between pre- and post-contact mobilities was abolished: this occurred with concentrations of PEP as low as 0·0005 μg/ml. and PK 0·01 mg/ml. The post-contact mobility was not, however, different from that of the control sample.

Contact with a glass surface causes a reduction in the electrophoretic mobility of platelets: this could be due to a reduction in their surface charge, to an increase in their size, or to an increase in the viscosity of the plasma in which they are suspended. A change in plasma viscosity is unlikely to be the cause of the change in platelet mobility since glass contact of platelet-poor plasma before it is mixed with the platelet-rich plasma has no effect; a viscosity change is also unlikely to be important because platelets are still affected by glass contact when one volume of PRCP is diluted with 9 vol. of saline. Microscopy shows that there is no apparent change in the size or shape or degree of aggregation of the platelets, and so it seems most likely that the reduction of mobility on glass contact is due to a decrease in the surface charge of the platelet. Since most previous investigators have used washed platelets it is not surprising that this difference in mobility before and after glass contact should not have been noticed before: Zucker and Levine[5] found that the mean mobility of washed platelets was lower than that before washing, but they did not consider the difference significant.

Initially it seemed possible that the change in mobility on glass contact was due to adsorption by the platelet of factors XII or XI which are activated by glass surfaces. This stage of blood coagulation is not calcium dependent, so the failure of EDTA to abolish the contact effect would be acceptable. However, our inability to produce the effect by contacting the PPP on its own makes this explanation unlikely and it is clear that it is contact of the platelets themselves with glass which mediates the effect.

The reduction of platelet mobility on glass contact is apparently related to reactions involving sulphydryl groups, for it is these reactions which are inhibited by *N*-ethylmaleimide and *p*-hydroxymercuribenzoate. Since the blood has to be collected into the enzyme poisons for them to have any effect, it seems possible that their action is to inhibit some change, which occurs during the production of PRCP, and which results in an increase in mobility. This increased mobility can then be reduced by glass contact.

When blood is collected into the ADP-removing PK/PEP system the contact effect is abolished; this suggests that ADP is in some way involved. The addition of low concentrations of ADP to a PRCP/PPP mixture causes an increase in platelet mobility: ADP is negatively charged and it seems likely that it is adsorbed on to the platelet surface increasing its net negative charge, and so causing an increase in electrophoretic mobility. Bringing the platelet into contact with glass may then remove the ADP; we have shown that ADP is affected by glass contact, for if a solution of ADP is brought into contact with an unsiliconized glass surface its effects on platelet mobility are much diminished. We therefore suggest that during the production of PRCP, ADP or some similar substance becomes adsorbed on to the platelet surface: possibly it is released by red cells or by the platelets themselves as a result of centrifuging. When the platelets come into contact with unsiliconized glass the ADP is either removed or inactivated; a similar process occurs at a slower rate if the platelets are in siliconized glassware.

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INTERCELLULAR SPACE IN NORMAL HUMAN STRATUM CORNEUM

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HISTOLOGISTS have disagreed on the question of whether or not there is an intercellular space in the stratum corneum. Pernkopf and Patzelt[1] assumed the existence of an intercellular space containing a substance which could be stained with silver nitrate. According to other authors, such as Weidenreich[2], Hoepke[3] and Lever[4], such an intercellular space does not exist. Modern histochemical research has also been unable to give a definite solution to this problem. Wislocki *et al.*[5] have demonstrated a weak persistence of periodic acid–Schiff-positive intercellular substance in the horny layer of plantar skin. Kligman[6], on the other hand, could not discern any intercellular substances in isolated sheets of the stratum corneum.

In order to elucidate the function of the horny layer as a barrier, investigations were undertaken of the horny cell itself. The cell membranes have been allocated a central part in this research effort, mainly following the comprehensive investigations of Unna[7] on the different degrees of solubility of the horny substances. When the stratum corneum is treated with nitric acid or cold alkaline solutions a membrane-like component insoluble in these chemicals is all that remains of the stratum corneum[7]. The investigations initiated by Unna have been extended, and his hypothesis that the cell membrane constitutes the most resistant component of the horny layer (Unna's keratin *A* (ref. 7)) has been generally accepted[1–3,6,8–10].

The assumption that the membrane-like structure demonstrated in the light microscope represents the cell membrane of the horny cell is, however, incompatible with electron-microscopical investigations of the plasma membrane in the stratum corneum. The plasma membrane has a thickness of about 200 Å (to be published), which is below the capabilities of the light microscope. Furthermore, no peripherally located intracellular component is discernible in the electron microscope which, as a consequence of the preparation technique, might fuse with the plasma membrane to form a membrane-structure corresponding to that observable in the light microscope. Thus the inference, therefore, is that the structures considered by histologists to be cell membranes either constitute

components located in a space between the cells or are artefacts due to the preparation technique for light microscopy.

An intercellular space has been definitely demonstrated by means of electron microscopy[11–17]. Only few observations of intercellular components have been made[13–17]. Usually the intercellular space appears electron-optically empty. A more detailed electron-microscopic investigation of the intercellular space was therefore deemed necessary, in order to localize the aforementioned membrane-like structure in the normal human stratum corneum. The problem of the possible dissolution of structural components during the process of tissue preparation for electron microscopy was considered to be of particular importance in this connexion. For this reason the time factor with respect to fixation, rinsing and dehydration has also been made the subject of electron-microscopic investigation. The findings will be reported here and discussed in relation to the aforementioned histological view concerning the localization of the membrane-like structure. Furthermore, in the light of the results obtained, the barrier function of the horny layer has been re-evaluated and an important role of the intercellular space has been emphasized.

Normal skin from the lateral abdominal and sacral regions from four men and four women, aged 30–50 years, was used. The tissue was fixed in a 1 per cent osmium tetroxide solution buffered with veronal-acetate to *p*H 7·2–7·4 (ref. 18) and made isotonic to blood by the addition of sodium, potassium and calcium chloride[19]. The temperature during fixation, rinsing in Tyrode's solution and dehydration to the stage of 95 per cent ethyl alcohol was about 5° C. The treatment of the tissue was carried out according to the following schedule:

Osmium tetroxide	30 min
Tyrode's solution	5 ,,
Ethyl alcohol (70 per cent)	20 ,,
Ethyl alcohol (95 per cent)	10 ,,
Ethyl alcohol (abs.)	60 ,,
Propylene oxide	60 ,,

The tissue was embedded in 'Epon'[20] and cut perpendicularly to the skin surface with an LKB 'Ultrotome'. All sections were stained with a saturated water solution of uranyl acetate at 60° C for 20 min[13] and with lead tartrate according to Millonig[21]. The sections were examined in an RCA EMU 3 A electron microscope.

The width of the intercellular space. In the cross-sections of skin from both the abdominal and sacral regions the intercellular space between the desmosomes shows a conspicuous variation in width from 300 Å to 20,600 Å. This variation occurs very irregularly and is not restricted to any particular sub-layer of the stratum corneum (Figs. 1–3).

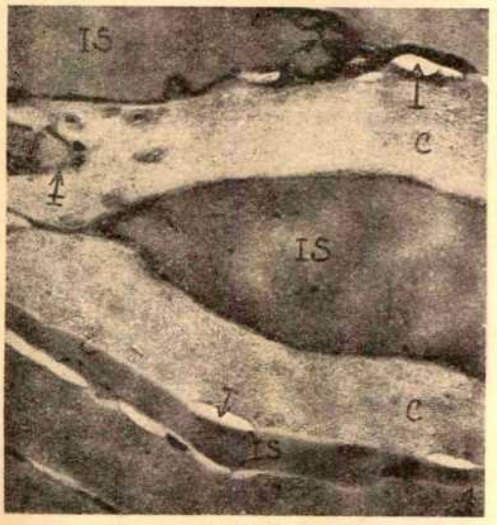


Fig. 1. Part of the basal sub-layer in normal human stratum corneum. The intercellular space (*IS*) varies considerably in width. It is filled with a non-homogeneous substance. A highly opaque membrane-like component (⟶) appears mainly at the periphery of the intercellular space. Small electron-optically empty areas (|⟶). The cytoplasm of the cells (*C*) shows fibrils with a keratin pattern. Granule of unknown nature (||⟶). (× 32,000)

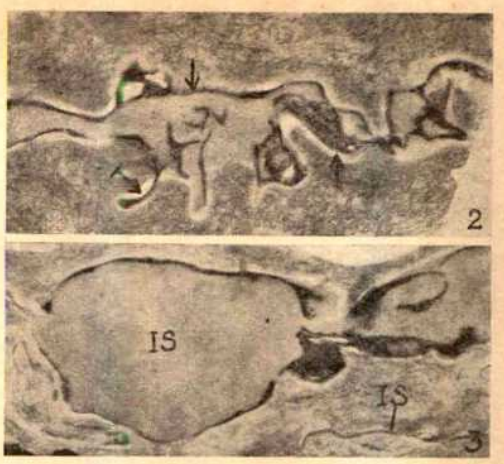


Fig. 2. Part of the intermediate sub-layer. The intercellular space is filled with a non-homogeneous substance. At the periphery of the intercellular space appears the highly opaque membrane-like component with its incorporated bodies (⟶). Small electron-optically empty areas close to the cell surface (|⟶). (× 16,000)

Fig. 3. Part of the superficial sub-layer. The intercellular space (*IS*) varies distinctly in width. It is filled throughout with a non-homogeneous substance. The highly opaque membrane-like component with its incorporated bodies appears at the periphery of the dilated parts of the intercellular space. (× 11,200)

The cells display a longitudinal, flattened shape. At either end they taper off in projections of different length, interlocking like cog-wheels. In the regions where the adjacent cells dovetail with each other the intercellular space generally seems to be narrow, but here, too, distinct variations in the width are observable.

Intercellular components. In the horny layer the intercellular space is filled throughout with a non-homogeneous substance showing moderate opacity (Figs. 1–3). In higher magnification this substance displays a pattern with indications of fine, opaque filaments. Small electron-optically empty areas are occasionally seen, for the most part close to the cell surface. Embedded in the non-homogeneous substance are a membrane-like component and granules of different shape, size and ultrastructure.

The membrane-like component displays a high opacity (Figs. 1–5). It appears mainly at the periphery of the intercellular space close to the cell surface, but is distinctly separated from the plasma membrane (Fig. 5). It may be observed along the length of the cells or may occur only fragmentarily. In some areas it displays a triple-layered pattern and in others it appears as a single, blurred layer. The membrane varies here between 100 and 400 Å in thickness. In other parts, again, bodies with a length of 2400–7300 Å and a breadth of 450–5100 Å are incorporated in the membrane-like structure. These bodies occur with greater or lesser frequency and contain both a highly opaque material which occasionally displays a laminated arrangement, and a less opaque substance (Figs. 2–4).

Granules containing triple-layered membranes are often observed (Fig. 6). It is, however, not yet clear whether the outer, bounding membrane is single- or triple-layered. The granules have a length of 2700–5200 Å and a breadth of 1500–2200 Å.

Another larger type of granule seems to be at least partly constituted of a system of triple- and multi-layered membranes (Fig. 9). In the present investigation only certain parts of these granules could be observed, and hence it is accordingly not possible to give any more exact indication of their size.

A typical granule is shown in Fig. 7. Its ultrastructure is diffuse. It seems to be bounded by an opaque, single layer. It has a rather low opacity, but some parts appear

to be more, and others less, opaque. It is about 3600 Å in length and about 1300 Å in breadth. Close to such a granule smaller, dense opaque granules are often observed.

Another type of granule appears as mainly dense corpuscles with a round or oval shape (Fig. 8). These vary in size and opacity[16]. They have a length of 170–1300 Å and a breadth of 170–900 Å. A granule or vesicle bounded by a rather broad, opaque membrane adjacent to which appear smaller granules can also be seen in Fig. 8. It has a length of about 3200 Å and a breadth of about 2300 Å.

Effect of the Time Factor with Respect to Fixation, Rinsing and Dehydration

The width of the intercellular space. As clearly emerges from this investigation, the intercellular space varies considerably in width. This variation is distinct in all sub-layers of the stratum corneum. Similar observations have been made in other investigations[11–17]. Variations in the time of fixation, rinsing and dehydration do not appear to affect the width of the intercellular space.

Since in earlier investigations the intercellular space usually appeared to be electron-optically empty, the widened parts have been considered as simply "enlarged and emphatic artefacts" due to the preparation technique for electron microscopy[6]. Without adducing any experimental foundation it has been asserted that the intercellular space is of a sub-microscopic order of magnitude[6]. Thus Kligman[6] points out that "if one knows the thickness of the horny layer and the number of cell layers, the thickness of the individual cells can be indirectly calculated". However, the present investigation shows quite clearly that the intercellular space constitutes an essential part of the stratum corneum and that it is not possible to get away from this when determining the thickness of the latter. Throughout the horny layer the cells show a variation in height of between 1,200 and 23,400 Å. The width of the intercellular space is 300–20,600 Å. There is no doubt that the intercellular space and its substances are affected by the preparation technique to the same extent as the rest of the tissue. However, throughout the stratum corneum the intercellular space is filled with a non-homogeneous substance irrespective of whether the intercellular space is wide or narrow. In this substance are embedded different structures, some of them of a size discernible under the light microscope. These observations may very well support the assumption that the intercellular space *in vivo* is also subject to wide variations in width.

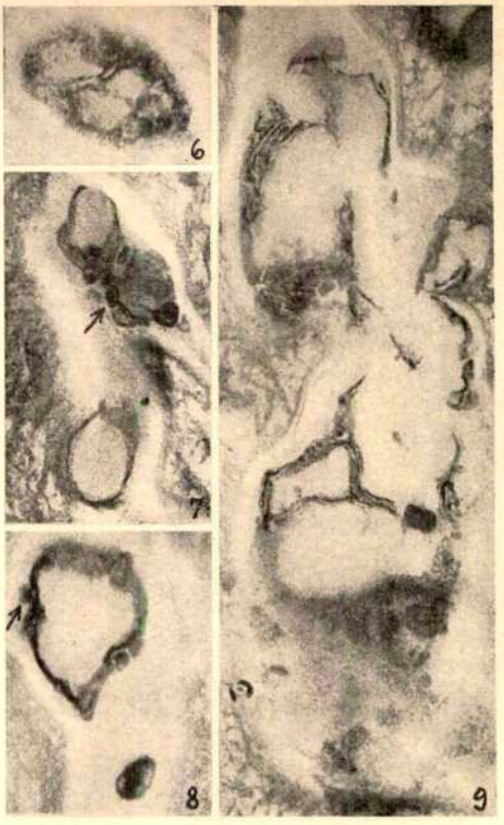


Fig. 6. Granule displaying a triple-layered inner membrane. The outer, limiting membrane is indistinct. (× 84,000)

Fig. 7. The granules seem to be partly bounded by an opaque, single-layered membrane. Some parts of the granules are more, and others less, opaque. Small, opaque corpuscles (⟶) occur close to the longitudinally sectioned granule. (× 60,800)

Fig. 8. At the bottom a small, oval granule with a varying opacity. In the left upper corner of the picture a granule or vesicle bounded by a rather broad, opaque membrane. Small corpuscles (⟶) appear close to the membrane. (× 70,400)

Fig. 9. Disrupted granules which partly consist of triple- and multi-layered membranes. (× 60,800)

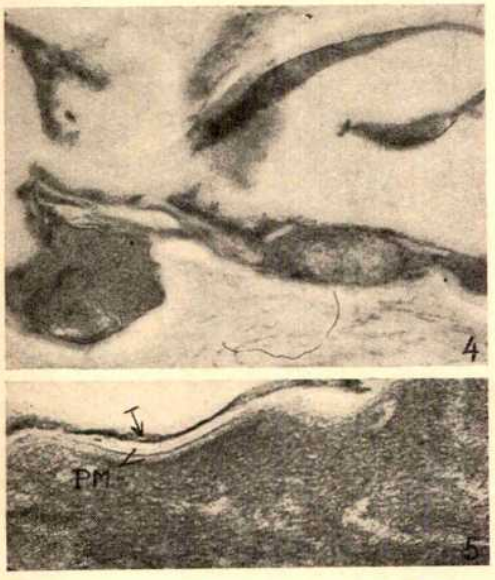


Fig. 4. Higher magnification of Fig. 3 showing bodies incorporated in the membrane-like component. The bodies partly display a laminated arrangement. (× 35,200)

Fig. 5. Part of a cell in the superficial sub-layer. The cytoplasm consists of rather opaque keratin fibrils and small areas with a less opaque substance. The cell is bounded by a plasma membrane (*PM*). This consists of a broad cytoplasmic zone, a narrow intermediate, and a narrow outer layer. The highly opaque membrane-like component (⟶) is located in the intercellular space distinctly separated from the plasma membrane. (× 127,200)

The non-homogeneous substance. In earlier investigations of the horny layer the intercellular space usually appeared electron-optically empty. However, small remains of a non-homogeneous substance were observable in the intercellular space in the basal and intermediate sub-layers, but never in the superficial layer[13–15]. In these investigations the tissue has been fixed, rinsed and dehydrated for long periods according to the following schedule: osmium tetroxide, 4 h; Tyrode's solution, 1 h; ethyl alcohol (70 per cent), 3 h or overnight; ethyl alcohol (95 per cent), 1 h; ethyl alcohol (abs.), 1–2 h.

From the present investigation it emerges that the non-homogeneous substance is not a material that is broken down in the stratum corneum to facilitate the flaking off of the cells at the skin surface but fills the intercellular space throughout the horny layer, that is, also in the superficial sub-layer. It is, on the other hand, sensitive to the preparation technique and is best retained with short fixation, rinsing and dehydration periods. However, even when the time factor for fixation, rinsing and dehydration has been considerably reduced (as in this investigation), empty areas are still electron-optically visible in the intercellular space. Further investigations are being carried out to elucidate which stage in the

processing for electron microscopy is most deleterious for this substance.

A non-homogeneous substance has also been distinctly observed in the intercellular space in parakeratotic horny layers, for example, in psoriasis[15,22,23] and in atopic dermatitis (to be published). In these investigations of parakeratosis, however, long fixation, rinsing and dehydration times have been used according to the schedule already given. This would support the assumption that the non-homogeneous substance is less easily dissolved in the parakeratotic horny layers than that in the normal stratum corneum. The different solubility of the intercellular non-homogeneous substances in ortho- and parakeratosis might be interpreted as an expression for differences as to their chemical composition.

The membrane-like component and granules. In electron-microscopic investigations in which the schedule for long periods of fixation, rinsing and dehydration was used, components similar to the membrane-like structure and the different granules described here appeared in the intercellular space; this was irrespective of whether the non-homogeneous substance was retained or not[13,15–17]. In an investigation of the horny layer in guinea-pig epidermis[13], two types of intercellular bodies were observed. One type displayed a diffusely outlined ultrastructure with a laminated arrangement. This represents what was there assumed to be a desmosome. The other type showed vesicular structures with an outer, highly opaque membrane. These components were also interpreted to be desmosomes, but they may actually correspond to some of the granules observed in this investigation. Their length is between 750 and 5700 Å and their breadth between 550 and 4600 Å.

The observations of the aforementioned components in the intercellular space even in the superficial sub-layer of the normal human stratum corneum, where the non-homogeneous substance has always been dissolved when long fixation, rinsing and dehydration times have been used, favour the idea that they suffer less from the preparation technique than does the non-homogeneous substance.

Electron-microscopic Localization of Unna's Keratin A

Optical microscopy may be used for the study of structures down to about 2000 Å (ref. 24). Several of the bodies incorporated in the membrane-like component and several of the different granules are of such an order of magnitude that they may be seen in the light microscope, and may give the impression of a diffuse membrane-like structure if they occur closely enough. Granules and bodies the size of which are close to the lower limit of the resolution of the light microscope may also be discernible by means of optical microscopy if they are numerous and occur densely in the intercellular space.

Matoltsy and Parakkal[9] treated plantar human skin and bovine oral epithelium with 0·1 N sodium hydroxide and after centrifugation obtained a membrane-like structure which they examined in the electron microscope. They assumed that this structure represented isolated envelopes of the horny cells, in accordance with the view earlier suggested by histologists[1–3,6–10]. However, this structure is very similar in arrangement to the membrane-like component described in the present investigation. The thinnest parts of their membrane seem to be about 160 Å and the thickest about 5400 Å, that is, it rather closely agrees, also in point of thickness, with the membrane-like component in the present investigation, also where the aforementioned bodies occur.

The membrane-like component shown here should not be confused with the plasma membrane of the cells. The former is definitely located in the intercellular space and is distinctly separated from the plasma membrane. The membrane-like component varies in thickness between 100 and 5150 Å, whereas the plasma membrane in all its parts is about 200 Å thick (to be published). There are, furthermore, distinct ultrastructural differences between these two structures. Thus the plasma membrane displays an asymmetric triple-layered pattern consisting of a broad, opaque cytoplasmic zone, an intermediate narrow layer with low opacity and an outer, opaque narrow layer facing the intercellular space (Fig. 5). Finally, the membrane-like component has a much higher opacity than the plasma membrane.

These different observations seem to justify the suggestion that the diffuse membrane-like structure observed in the light microscope and retained in the horny layer after skin has been treated with cold alkaline solutions or nitric acid does not represent the cell membrane as has generally been assumed[1–3,6–10], but is constituted by a complex of opaque components in the intercellular space. These are the only structures which are of a size recognizable in the light microscope, whereas the plasma membrane itself is of a submicroscopic order of magnitude. If this were the case, the membrane-like component and the different granules demonstrated in the present investigation would represent what histologists have considered as the most resistant material in the horny layer, or what Unna[7] has called 'keratin *A*'.

In this connexion, it is of interest to mention the investigations by Szodoray[25] and Matoltsy and Matoltsy[26] on the non-cornified epidermis. Szodoray treated skin sections with trypsin and obtained a membrane-like structure in the upper Malpighian layer, which, he assumed, represented the cell membrane. Matoltsy and Matoltsy[26] extended the investigations of Szodoray[25], confirmed his results and gave them the same interpretation. These authors exposed 2-day-old rat skin to the effect of 1·0 per cent trypsin. They pointed out "that trypsin attacks and disperses all components of the granular cells except the keratohyaline granules and cell membranes". It is difficult to measure exactly the membrane, which they thought to be the cell membrane, but it seems to vary between 300 and 2300 Å in thickness. This structure thus differs distinctly in thickness from that of the plasma membrane. The plasma membrane of the non-cornified cells is about 80 Å (ref. 9). On the other hand, important changes of the cell surfaces and the intercellular space are observed in the stratum intermedium as compared with the strata below. Whereas the intercellular space is mainly electron-optically empty and the cell surfaces display protrusions in the strata basale and spinosum, the cell surfaces in the stratum intermedium show invaginations[14,27–29]. The invaginations and the rest of the intercellular space in the stratum intermedium are filled with a non-homogeneous substance and granules[9,14,27–30]. Some of these granules agree in ultrastructure with the intracellular so-called Odland's bodies[9,14,27–33]. The granules in the invaginations have a size of 1000–5000 Å (ref. 9). They have a size similar to that of the bodies incorporated in the membrane-like component shown in the present investigation. Thus, in accordance with the aforementioned discussion on the horny layer, it is only the intercellular material that is visible in the light microscope, and not the 80-Å-thick plasma membrane. There is, therefore, no electron-microscopic support for the theory first proposed by Unna[7] that the cell membrane represents the most resistant component in the horny layer, nor for the hypothesis based on the investigations by Szodoray[25] and Matoltsy and Matoltsy[26] to the effect that the formation of these resistant cell membranes commences in the stratum intermedium.

On the basis of the present investigation it may be suggested that the keratinization process in the epidermis implies not only the transformation of a stratum basale cell into a horny cell with the formation of keratinous and non-keratinous material and of a modified plasma membrane, but also—which may be equally important—the appearance of structures in the intercellular space. These

structures first appear in the intercellular space in the stratum intermedium[9,14,27–30]. The hypothesis has been put forward that the so-called Odland's bodies are excreted from the cells into the intercellular space in the stratum intermedium[9,30]. The changes of the cell surfaces, with protrusions in strata basale and spinosum and invaginations in stratum intermedium[28], may support this view. The intercellular granules are not, in contradistinction to the findings of Matoltsy and Parakkal[9], restricted to the stratum intermedium, but, as is shown in the present investigation, distinctly discerned in the whole stratum corneum.

Barrier Function of the Horny Layer

The discussion of the stratum corneum as a barrier has mainly pivoted on the question as to whether the barrier is located in the basal part of the horny layer[34–37], or includes the whole stratum corneum[6,38]. The route of penetration of substances from without into the skin and vice versa was earlier thought only to be transcellular, but since an intercellular space has been demonstrated by electron microscopy, a penetration through the intercellular space has also been discussed as a possibility[39,40]. However, it has been suggested that the electron-microscopic finds may be artefacts, and if an intercellular space does really exist it has been assumed to be only of a submicroscopic order of magnitude[6].

Nothing is as yet known about the chemical composition of the intercellular material. In accordance with the histological view that no intercellular space exists[2–4], no such space has been postulated in the chemical and X-ray diffraction investigations of the stratum corneum (on lipids, water-soluble substances, etc.) and the results have been referred only to the horny cell itself.

The present investigation supports the hypothesis that the whole stratum corneum constitutes the barrier[6,38]. The barrier, however, is formed both of the cells and the intercellular space. The intercellular space contains a complex of structures. It is filled with a non-homogeneous substance which shows indications of a pattern with filaments. In this substance appear opaque components which are here assumed to constitute Unna's so-called keratin *A* (ref. 7). The non-homogeneous substance shows greater solubility than these components for the preparation technique. These observations may favour the suggestion that the penetration rate of chemicals and solvents may be due to the amount of various opaque components, that is, the membrane-like component with its incorporated bodies and the different granules embedded in the non-homogeneous substance. Variations of this amount could explain the differences in the penetration rate at different levels or in adjacent areas of the horny layer and in different sites of the body.

In a similar way the barrier function of the stratum intermedium may be in part regulated by the intercellular material in this layer.

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REPRESSION AND THE CONTROL OF CYCLIC ENZYME SYNTHESIS IN *Bacillus subtilis*

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THE synthesis of many enzymes is periodic in synchronous populations of *Bacillus subtilis*[1–4]. The synthesis of each enzyme occupies only a fraction of the cell cycle and the quantity of enzyme does not increase thereafter until the next cell cycle; thus the total amount of enzyme in a synchronous culture increases in a stepwise fashion. Since the synthetic periods of different enzymes are not simultaneous, a linear map can be constructed which represents the temperal order of synthesis of the different enzymes. This article reports experiments designed to discover what mechanisms determine the timing of these synthetic periods.

It has been suggested that the sequential duplication of the genome of *B. subtilis*[5] might in some way determine the order of enzyme synthesis. Indeed, Masters and Pardee have reported that the order of synthesis of four enzymes corresponds to the order of replication of their genes[4]. Various hypotheses have been advanced to explain the

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discontinuous enzyme syntheses and the way in which they appear to be related to the order of replication of the genes. These can be summarized as follows: (1) Enzymes are synthesized immediately after the replication of their structural genes[1] but not at other times. (2) Transcription is ordered in the same way as replication but does not necessarily occur simultaneously with replication. A corollary of this hypothesis would be that each gene may be transcribed at only one time during each cell cycle. (3) Periodic enzyme synthesis is the result of end-product repression. Enzyme and end-product form a system in which the concentration of one determines the rate of synthesis of the other. As a consequence of these interactions the intracellular concentrations of both enzyme and end-product oscillate[2,6,7]. According to this explanation, the order of enzyme synthesis may, but need not be, the same as that of gene replication.

Our evidence is consistent with the third explanation since we have shown that periodic enzyme synthesis continues in the absence of DNA synthesis and that the timing of synthetic periods can be altered by interference with repressor-levels.

The techniques for growing and synchronizing *Bacillus subtilis* (*W*23) and the methods used to assay enzyme activities and total DNA have been described previously[1,3]. The 5-fluorodeoxy uridine (FUdR) was a gift from Roche Products, Ltd.

Initiation of Enzyme Steps in the Absence of DNA Synthesis

In cultures of *B. subtilis* in which L-histidine is supplied as sole carbon source, DNA synthesis is periodic. As shown in Fig. 1, the DNA synthetic period occupies about 50 min of a cell cycle of 120 min.

Consequently there is no DNA synthesis during about 60 per cent of the cell cycle in histidine medium. However, the times of initiation of autogenous[2] steps in the synthesis of the enzymes histidase, aspartate transcarbamylase (ATCase), ornithine transcarbamylase (OTCase) and dehydroquinase (DHQase) are spaced over a period equal to the whole cell cycle[4]. It is clear that the initiation of some of these syntheses must occur during the period when no DNA is replicating. (Similar observations have been made on the yeast *Schizosaccharomyces pombe*[10].)

Direct evidence that periodic enzyme synthesis can continue in the absence of DNA replication was obtained by preventing DNA synthesis with FUdR[8,9]. Since strain *W*23 was not susceptible to this drug, a prototrophic revertant (23*TR*) of a thymineless strain (23*T*⁻) (ref. 11) was used. In this strain DNA synthesis was almost completely inhibited for at least 2 h following the addition of FUdR. During this period optical density and cell length continued to increase. Cell division, however, was inhibited and viability decreased exponentially.

A synchronous culture of 23*TR* was divided into two parts and FUdR added to one of them (Fig. 2). The subsequent synthesis of OTCase was followed in the two cultures. In both cases enzyme synthesis continued to be

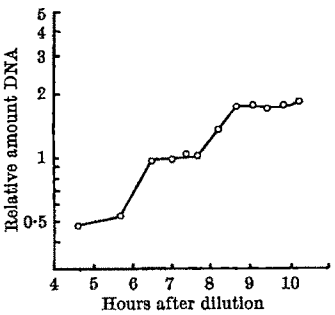


Fig. 1. DNA synthesis in a synchronous culture of *W*23 growing on minimal medium[12] with 0·2 per cent L-histidine as carbon source. Total DNA was estimated in the hot perchloric acid soluble fraction of cold trichloroacetic acid precipitates by the method of Burton[14]

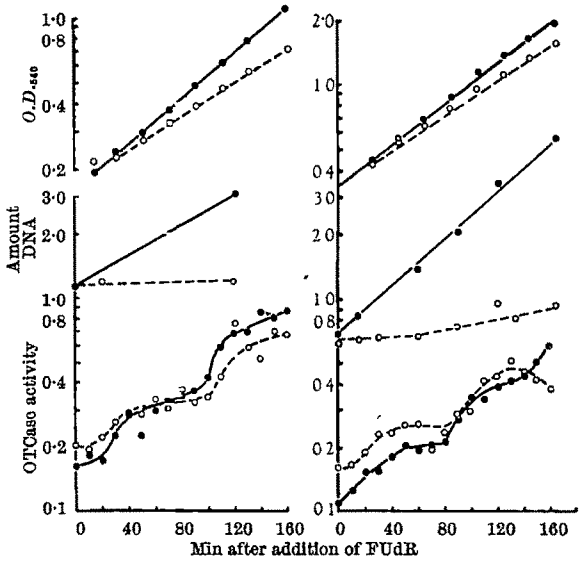


Fig. 2. DNA, $O.D._{540}$ and OTCase increase in two cultures of 23*TR* growing synchronously on minimal medium + 44 μg/ml. uracil with 0·2 per cent glycerol as carbon source. Upper curves, *O.D.*; middle curves, DNA (assayed as described in Fig. 1); lower curves, OTCase activity (assayed as previously described[3]). At 0 min 22 μg/ml. FUdR was added to half the culture. Open circles (dashed lines) + FUdR. Closed circles (solid lines) without FUdR

periodic and the synthetic periods in the two cultures remained in phase.

The fact that periodic enzyme synthesis can continue for at least two cycles after DNA synthesis has ceased demonstrates that the initiation of synthetic cycles is not dependent on continued gene replication.

Repression and the Timing of Enzyme Steps

It has been reported in previous papers[1,2,4] that genes are potentially active at all times during the bacterial cell cycle. This was inferred from the observation that the rate of specific enzyme synthesis in synchronous cultures can be greatly increased at any time by the addition of inducer or removal of repressor. Evidence that genes are in fact always active, albeit at a low level, is provided by the observation that enzyme synthesis is continuous when the level of repression is constant. For example, the basal synthesis of alkaline phosphatase (APase) in the presence of excess inorganic phosphate has been shown to be linear with doublings in rate once per cell cycle[2,3]. Similarly, the basal synthesis of the inducible enzyme sucrase (in the absence of sucrose) is continuous in slowly growing cultures (Fig. 3).

However, in cultures in which sucrose is provided as sole carbon source, the synthesis of this enzyme is periodic (Fig. 3). This is consistent with the hypothesis that the catalytic action of sucrase on sucrose results in repressor (catabolite) production and the consequent feedback on enzyme synthesis gives an oscillating system.

In the normal cell cycle, periods of enzyme synthesis occur once in every cycle[1,2,4]. If there is a fixed period during which a particular enzyme may be synthesized, steps in enzyme amounts should occur at intervals equal to one cell cycle. It should not be possible to have steps initiated at smaller intervals than this. However, if oscillations in repressor concentrations underlie the periodic enzyme synthesis, it should be possible to upset the timing of enzyme steps by experimental interference with repressor-levels.

This was done in the following manner. The synthesis of ATCase in a synchronous culture was repressed by the addition of uracil. Since ATCase activity is unstable[3] the amount of active enzyme decayed progressively in the presence of uracil (Fig. 4). Aliquots were derepressed by washing free of uracil at 0·5 and 1·0 cell cycles after repressor addition. (Growth was not affected by this treatment.) In both aliquots ATCase synthesis recommenced

immediately on removal of uracil and rose rapidly until it reached the normal level for cells growing in the absence of external uracil. Synthesis then stopped and did not recommence for a further 40 min; 30 min (0·5 cell cycles) also separated the times of initiation of the second autogenous step in the two cultures (Fig. 4). The timing of OTCase steps (in these cultures) was not affected by the treatment. The initiation of steps in synthesis of ATCase and OTCase normally occurs simultaneously[4]. This experiment shows that the addition and removal of uracil at different times can result in the displacement of ATCase steps relative to one another and to OTCase steps. Thus release of repression can result in a specific alteration in the timing of subsequent autogenous enzyme steps.

A similar effect has been observed during the derepression of alkaline phosphatase (APase) (Fig. 5). The synthesis of this enzyme is discontinuous after removal of inorganic phosphate from the medium. Probably the cessation of synthesis after removal of external repressor results from the release of inorganic phosphate from an internal source. That *subtilis* has such an internal supply of phosphate is suggested by the fact that growth continues for a period after removal of all external inorganic phosphate.

As in the experiments on derepression of ATCase, the second initiation of enzyme synthesis occurred at a fixed time after the removal of repressor from the medium. Thus there is no fixed time in the cell cycle during which steps in APase are necessarily initiated. Thus we suppose that synthesis of APase is autogenous[2] for a period after removal of external supplies of repressor.

These experiments show that the repressor level can determine not only whether or not periodic enzyme synthesis occurs but also the time in the cell cycle at which the enzyme steps are initiated.

The experiments described here show that periodic enzyme synthesis in *Bacillus subtilis* does not require continued gene duplication and that the timing of the synthetic periods can be specifically altered by the addition and removal of repressor. It was previously shown[1,2,4], and is confirmed here, that enzyme synthesis can be initiated at any time in the cell cycle by removing repressor and that enzyme synthesis is not periodic in the presence of constant repressor levels. Taken together, these experiments strongly suggest that the basis of periodic enzyme synthesis in this organism is cyclic variation in repressor concentrations. It is supposed that these oscillations are self-generating[7] and arise from the feedback of end-product concentration on enzyme synthesis.

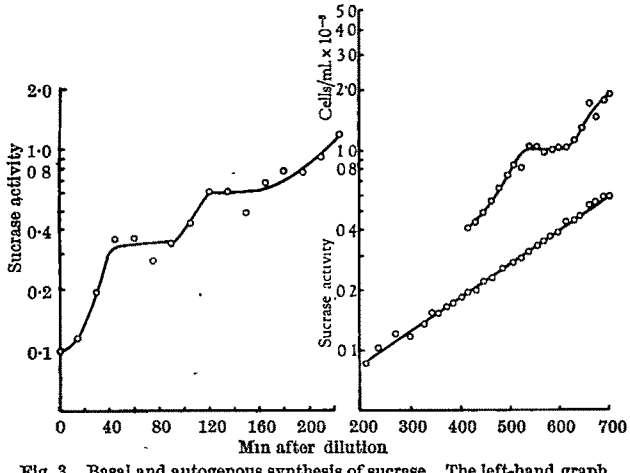


Fig. 3. Basal and autogenous synthesis of sucrase. The left-hand graph shows sucrase activity of *W*23 growing on minimal medium with 0·2 per cent sucrose as carbon source. The right-hand graph shows cell counts (upper curve) and sucrase (lower curve) of *W*23 growing on minimal medium with 0·2 per cent L-histidine as carbon source. The generation times were approximately 100 min on sucrose and 180 min on histidine. Sucrase was assayed as reported previously[1]. The units on the ordinates are arbitrary and are not directly comparable between the right and left graphs. Sucrase activity in the presence of sucrose is approximately 20-fold higher than in histidine cultures. The abscissa shows time in minutes from re-inoculation of the cultures from stationary phase

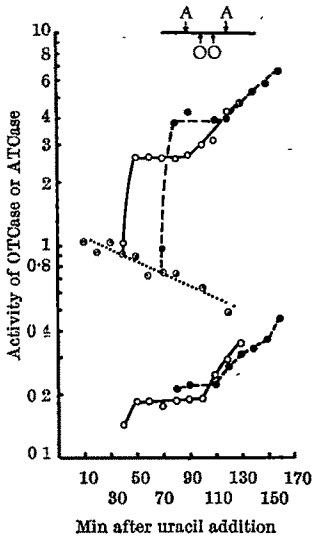


Fig. 4. Effect of uracil addition and withdrawal on the synthesis of ATCase (upper curves) and OTCase (lower curves) in a synchronous culture of *W*23. At 10 min, 2 × 10^{-4} M uracil was added to a synchronous culture of *W*23 growing on minimal medium + glycerol. Circles with central points represent ATCase in this repressed culture. At 40 min (open circles, solid lines) and 70 min (closed circles, dashed lines) 50 ml. portions were washed rapidly by filtration with minimal medium without carbon source at 37° C. The washed cells were resuspended in minimal medium + glycerol (without uracil) and sampling continued during subsequent growth. 5-ml. samples were taken into 50 μg/ml. chloramphenicol in ice, centrifuged and resuspended in 0·5 ml. of 80 μg/ml. cetyl trimethyl ammonium bromide

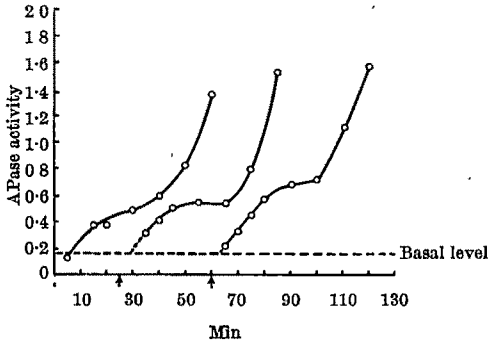


Fig. 5. Derepression of alkaline phosphatase. A stationary phase culture of 23*TR* was re-inoculated into low phosphate (2·5 × 10^{-5} M) minimal medium with 0·2 M *tris*, *p*H 7, + 0·2 per cent glycerol. The doubling time was 175 min. At the times indicated (arrows) 20 ml. portions were removed and washed free of phosphate by filtration with warm (37° C) minimal salts medium containing 0·2 M *tris* (*p*H 7). The cells were resuspended in this phosphate-free medium + glycerol. One-ml. samples were taken for enzyme assay, into 50 μg chloramphenicol in ice, during subsequent growth. The cells were lysed with lysozyme (0·1 mg/ml.) and 0·5 ml. aliquots incubated with 0·013 M *p*-nitrophenyl phosphate and 0·33 M ethylamine, *p*H 10·2, in a final volume of 1·5 ml. After incubation at 37° C, 0·5 ml. of 1 M potassium phosphate + 1 M sodium hydroxide was added to stop the reaction. Samples were centrifuged and the *O.D* at 405 mμ of the supernatants determined

These experiments suggest that periodic enzyme synthesis need not be restricted to organisms in which DNA synthesis is sequential and occupies the greater part of the cell cycle. Indeed, it is possible that similar cyclic enzyme synthesis will be found in any organism in which end-product repression occurs. Such cyclic synthesis has been predicted on theoretical grounds[7] and may have great importance in the development of differentiating organisms.

The apparent correspondence between order of enzyme syntheses and order of genetic replication requires explanation. In the model proposed by Pardee[6] periodic enzyme synthesis would continue for several cycles after gene replication had ceased. Goodwin[12] has devised a modification of this model in which 'free' periodic enzyme synthesis

is entrained to the period of gene replication by the synthesis of a small amount of messenger at the time of gene duplication. This model could explain the correspondence between genetic order and order of enzyme syntheses and, in principle, it can be tested.

The previous suggestion[3] that the instability of certain enzymes could result in stepwise increases in activity is now seen to be only part of the explanation. However, enzyme instability when it does occur will contribute to the discontinuous increase in enzyme amount, no matter what the cause of the initiation of the synthetic periods.

In summary, periodic enzyme synthesis in synchronous cultures of *Bacillus subtilis* continues in the absence of DNA synthesis and is therefore not directly dependent on periodic gene replication. The addition of repressor prevents periodic synthesis and its removal results in an immediate resumption of synthesis. The time of repressor removal and not the stage in the cell cycle determines the timing of the next period of enzyme synthesis.

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AN ENTRAINMENT MODEL FOR TIMED ENZYME SYNTHESES IN BACTERIA

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DETAILED analyses of enzyme dynamics in synchronously dividing cultures of micro-organisms have recently been reported[1–5]. These investigations have established the following points regarding the temporal ordering of events during the cell cycle in *E. coli* and *B. subtilis*. (1) Enzymes which are not continuously repressed double in activity suddenly at a characteristic time in the division cycle. (2) Repressed enzymes are synthesized continuously at low rates which double once every cycle. (3) Repressed enzymes can be induced at any time in the division cycle. (4) The normal doubling of an enzyme can be arrested by adding repressor, enzyme activity increasing when the repressor is removed. (5) The relative positions of steps for two different enzymes can be reversed by delaying the first enzyme with exogenous repressor and releasing it from repression after the second enzyme has doubled. (6) The potential for synthesis of a particular enzyme changes discontinuously at a characteristic time in the division cycle, which is not necessarily the same as the time of discontinuous enzyme synthesis. (7) The temporal sequence of doubling of potential for enzyme synthesis has the same linear order as the genetic map of the enzyme loci investigated (*E. coli*). (8) The temporal sequence of discontinuous enzyme bursts described in (1) is, on the average, the same as the sequence of enzyme loci in the genetic map (*B. subtilis*).

An obvious model relating the order of events during the cell cycle to the linear order of genetic loci postulates the transcription of genetic information temporally in the same fixed linear order as the spatial ordering of the genes. This model is attractive for its simplicity, but it is deficient in two respects. If genes can be transcribed only in a fixed sequence, then it should not be possible to (i) induce or derepress an enzyme at any point in the cell cycle, and (ii) reverse the order in which two enzyme steps occur during the cell cycle. It is thus necessary to seek alternative models which can explain all the observations so far, and also make some predictions which are subject to experimental test.

Observations (1)–(5) indicate that the enzyme steps which occur during the cell cycle are intimately connected with the operation of feedback repression circuits. One of the major dynamic consequences of negative feedback is the possibility of oscillations occurring in the variables involved in the control circuit. An extensive investigation of such oscillatory behaviour in the feedback repression circuits of resting or steady-state cells and the general consequences of such behaviour for the temporal organization of the cellular processes was made by Goodwin[6]. It will now be shown how timed enzyme syntheses in a population of synchronously dividing micro-organisms can be understood and analysed in terms of the same general dynamic principles, and how oscillatory behaviour and non-linear interaction can explain the phenomena already described. An explanation of discontinuous enzyme synthesis which introduces oscillations in feedback repression circuits has also been proposed by Kuempel *et al.*[2] and by Pardee[7].

Control Dynamics in Growing Cells

A set of relatively simple equations describing the operation of a closed feedback repression loop consisting of *m*RNA, enzyme, and enzyme product (repressor) can be derived making the following assumptions: (*A*) *m*RNA is the controlling factor for protein synthesis. (*B*) Enzyme is the controlling factor for production of enzyme product. (*C*) Repression of *m*RNA synthesis by enzyme product follows the same laws of surface adsorption as does enzyme inhibition.

Expressing *m*RNA concentration as X_i, enzyme as Y_i, and enzyme product as Z_i, the equations describing the dynamics of feedback repression can be written, on the above assumptions, in the form:

$$\frac{dX_i}{dt} = \frac{\lambda_i T_i}{\mu_i + K_i Z_i^n} - \nu_i X_i$$

$$\frac{dY_i}{dt} = \alpha_i X_i - \beta_i Y_i$$

$$\frac{dZ_i}{dt} = \rho_i Y_i - \gamma Z_i - \sigma_i$$

The Greek letters are all constants, γ being the constant of exponential growth of the cells, $\frac{dV}{dt} = \gamma V$. If the enzyme is stable then $\beta_i = \gamma$. σ_i is the rate of utilization of end-product Z_i per unit cell volume for growth. T_i is the concentration of the ith gene in the bacterial population, and n is the power of the repression (henceforth taken to be 1). Similar control equations have been derived previously[6,8,9].

These equations are complete (autonomous) except for the definition of how the quantity T_i varies in time. Keeping T_i constant, the equations define the dynamic behaviour of the repression circuit in cells which are growing but are not replicating their DNA, as occurs when 5-fluoro-deoxyuridine (FUdR) is added to a culture,

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for example. There are two possible general modes of stable dynamic behaviour which such a system can show. In the first case the variables settle down after a longer or shorter period of damped oscillations to their steady-state values and remain there. This mode does not involve continuous oscillations in the variables. The other stable mode of dynamic behaviour is oscillatory: the variables X_i, Y_i and Z_i undergo continuous oscillations, all of the same period but of differing amplitudes. about their steady-state values. The mode into which the control circuit goes depends on the relative values of the parameters. Although rhythmic variation in molecular populations have been widely demonstrated in protozoa and the cells of higher organisms[10–14], there has been little evidence to suggest that bacteria have endogenous rhythms (see, however, Dimmick[15]). However, the latest results of Masters and Donachie[5] provide strong evidence for autonomous periodicities in bacterial control processes. The assumption of oscillatory behaviour in bacterial feedback repression leads to a consistent intepretation of these results and provides a useful working model for cell division in general.

Consider first the behaviour of the variables X_i, Y_i and Z_i in cells which are synthesizing all molecular species (RNA, protein, metabolites, etc.) but without DNA replication. In the oscillatory mode and with the parameters scaled roughly to give relative biological quantities of the variables, non-linear oscillations such as those in Fig. 1 can be observed on an analogue computer[9]. All variables are then periodic functions of time so that, for example, $Y_i(t + T)$ is equal to $Y_i(t)$, T being the period of the oscillation which is the same for all three variables X_i, Y_i and Z_i. If the variable $N_{Y_i} = Y_i V$ is plotted on a semi-log plot, one obtains the graph shown in Fig. 2. This shows steps in the total number of enzyme molecules in the culture (compare Masters and Donachie[5], Fig. 2).

If there are several feedback repression circuits operating simultaneously in a cell growing without DNA replication, then we might expect to have a situation such as that shown in Fig. 3. Here only the enzymes, Y_i of the repression circuits are shown, and only three of these are considered. In a bacterial cell there may be as many as 100 or more feedback repression circuits, all of which could show oscillatory behaviour. No interactions are assumed to occur between these circuits, and the variables in Fig. 3 run freely with periods which are assumed to be approximately but not exactly equal. Since the periods of the individual variables are not equal, the enzymes do not maintain constant phase relationships with each other. Y_2 and Y_3, for example, reverse their phase relations in two to three cycles. It is a prediction of this model that cessation of DNA synthesis would result in phase reversals of this kind, with no fixed linear order of enzyme steps. When DNA replication is allowed to proceed in the cells, sequential enzyme syntheses should occur, and a simple model will now be proposed which has this consequence.

Oscillator Entrainment by Replicating Genes

Assume that when that part of the DNA coding for a particular enzyme is replicated there is a small burst of messenger synthesis. Hanawalt and Wax[16] have concluded

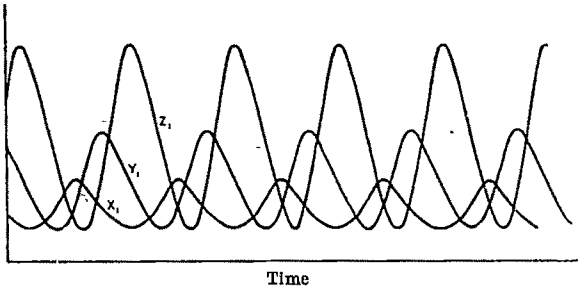


Fig. 1. Oscillations in the variables X_i, Y_i and Z_i

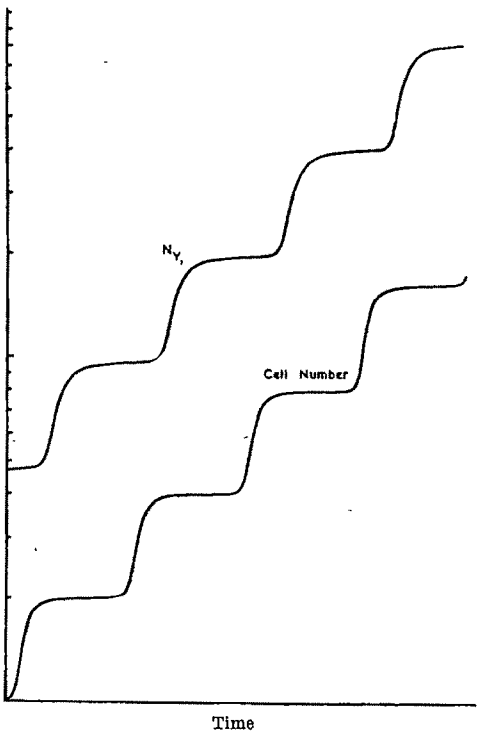


Fig. 2. [Steps in the variable $N_{Y_i} = Y_i V$ on a semi-log plot

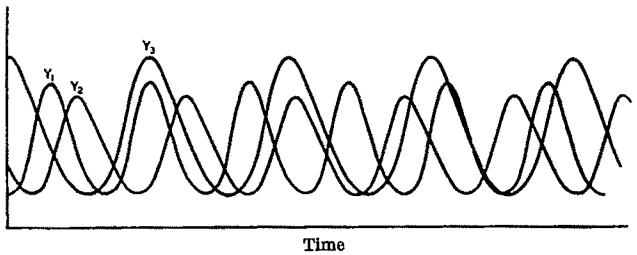


Fig. 3. Enzyme components of three feedback repression loops running freely in the absence of DNA replication

from their observations on the β-galactosidase system that such synthesis occurs at the moment of replication of the β-galactosidase gene in *E. coli*. If the doubling time of the culture is about 1 h and the DNA is replicated once every doubling time, then the frequency of these small bursts of *m*RNA synthesis at any locus will be also about one per hour. For any particular oscillating control circuit, such as that shown in Fig. 1, the effect of this burst is to add an additional small quantity of messenger X_i to the system as a pulse at regular intervals.

One of the commonest phenomena to occur in the interaction of non-linear oscillators is synchronous locking or entrainment of frequencies. In biology the best-known examples of this phenomenon are to be found in the literature on biological clocks, the most striking cases arising in the entrainment of circadian rhythms in organisms by light signals (Pittendrigh and Minis[17]). In a similar manner, the two periodic processes which we are considering here, DNA replication and oscillations in a closed feedback circuit, can lock together at the same frequency when they interact. In this event the interaction occurs because one of the variables is the same in both systems, namely, *m*RNA. The locking, or 'entrainment', will occur only if the frequency of the control circuit oscillations is sufficiently close to that of DNA replication, so that this must be assumed.

This entrainment phenomenon will occur for each control circuit which is operating and oscillating in the cell. However, the small bursts of *m*RNA synthesis which are assumed to occur when each gene locus is replicated

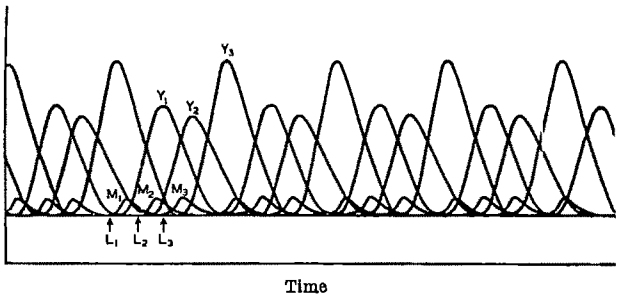


Fig. 4. Enzyme components of three feedback repression loops entrained by pulses of *m*RNA

will have a definite time order corresponding to the linear ordering of the genetic loci on the chromosome, since replication occurs linearly. The entrained control circuits will have the same temporal ordering, so the result is as shown in Fig. 4. This figure has been redrawn from results obtained on an analogue computer simulating the system. The small oscillations labelled M_1, M_2 and M_3 are the bursts of *m*RNA synthesis occurring just after the genetic loci replicate, three of which (L_1, L_2 and L_3) are represented as replicating at the times shown by the arrows. Only the enzyme components of the entrained control circuits have been shown in this figure. In comparison with Fig. 3, it is clear that the entrainment process imposes a definite temporal order on the enzyme oscillations and the linear relations of the genetic code are preserved in the phase relations of the oscillations. However, the non-linear interaction between the *m*RNA bursts and the control circuit operation resulting in entrainment is subject to disturbance by interference with the dynamics of the system, for example by altering repressor concentrations as described by Masters and Donachie[5]. There is also a finite probability that the control circuits will escape intermittently from the entrained condition so that spontaneous reversals of enzyme order can occasionally occur, as observed by Masters and Pardee[4] for the case of histidase and ATCase in *B. subtilis*. This entrainment model thus predicts a more labile dynamic behaviour than would occur in a deterministic tape-reading model.

It should be observed that there is an appreciable lag between the replication of any particular locus and the peak of the enzyme oscillation. If DNA replication occurs in a time period less than the cell cycle, then steps in enzyme synthesis occurring after DNA replication has ceased are to be expected, because of the dynamic time lags in the model. For example, if DNA synthesis takes only 46 min between L_1 and L_3, with L_3 the last locus to be replicated, then a step in Y_3 synthesis will be located at a point in the cell cycle some 20–30 min after DNA synthesis has ceased.

The model as it now stands can account for all the observations on the behaviour of enzymes and enzyme potentials which were summarized in the introductory paragraphs, and it makes some predictions. One is the drift in enzyme steps relative to one another when DNA replication is arrested in a culture, as shown in Fig. 3. Another consequence of this model distinguishes it from any model which postulates damping in the oscillations. The data so far available on enzyme dynamics in synchronously dividing populations of micro-organisms do not allow one to decide between these alternatives, since the number of steps observed is never more than three or four. Under chemostat conditions, however, the present hypothesis predicts that continuing periodicities should be observable in cell variables, and experiments are in progress to test this point.

A final prediction of this treatment of enzyme dynamics in bacterial cultures is that if continuing periodicities occur under chemostat conditions, then the synchrony maintained in the culture must be due to the transmission of some signal (or signals) between the cells. This is because a population of non-interacting oscillators, each subject to random variation, will quite rapidly drift out of phase with one another, even if they all have the same mean frequency. Some interaction between the control circuits of different cells would be required to keep the oscillations in phase, and a reasonable class of candidates for this interaction signal is the repressors themselves.

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EFFECT OF CARZINOPHILLIN ON BACTERIAL DEOXYRIBONUCLEIC ACID: FORMATION OF INTER-STRAND CROSS-LINKS IN DEOXYRIBONUCLEIC ACID AND THEIR DISAPPEARANCE DURING POST-TREATMENT INCUBATION

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PREVIOUS investigations with carzinophillin (CF), an antibiotic isolated from *Streptomyces sahachiroi* by Hata *et al.*[1], demonstrated its antibacterial activity as well as its marked anti-tumour effect in rodents[2]. In 1963, Endo *et al.*[3] reported that carzinophillin (CF) was able to induce λ-phage in *Escherichia coli*.

This article presents evidence that: (1) *E. coli* B_0, a strain sensitive to CF, shows a marked inhibition of DNA but not of RNA or of protein synthesis when treated with CF at doses that inhibit colony formation; (2) treatment of DNA of *E. coli* B_0 and of *Bacillus subtilis* results in formation of inter-strand cross-linking; (3) the CF-induced cross-linking effect disappears either in cells or *in vitro* during post-treatment incubation; (4) transforming activity of *B. subtilis* DNA, which is inactivated by this antibiotic, can be reactivated to some extent by post-treatment incubation *in vitro*.

Bacterial strains. *E. coli* B_0, a strain highly sensitive to mitomycin *C*[4] and CF but not to ultra-violet radiation and showing host cell reactivation, was used. *B. subtilis* strain 19 and strain 168 were a gift from Dr. D. Bobb of

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† U.S. Public Health Service Career Development Award.

the Palo Alto Medical Research Foundation. Samples of carzinophillin were kindly provided by the Kyowa Fermentation Industry Co., Ltd., Tokyo, Japan.

Determination of nucleic acids and protein. Exponentially growing cells of *E. coli* B_0 at a density of 1×10^8 cells/ml. were incubated with shaking at 37° C in minimal glucose-salts medium (M9) containing various concentrations of CF. 10 ml. of the sample were taken at intervals and analysed for nucleic acids and protein. RNA was assayed by the orcinol reaction[5], DNA by the diphenylamine reaction[6], and protein by the Folin reaction according to the method of Lowry *et al.*[7].

Transformation assays. These were carried out with an indole-requiring mutant of *B. subtilis*, strain 168, by the method of Nester and Lederberg[8].

The 'irreversibility' of thermal denaturation was demonstrated spectrophotometrically in 7·2 M sodium perchlorate according to the procedure of Geiduschek[9]. Denaturation experiments were performed in the following manner. DNA solutions (20–30 μg/ml.) were heated to a given temperature in a thermostatic cell compartment of a spectrophotometer and the absorbance (A) at 260 mμ was measured after equilibrium was attained. The heated cuvettes were then plunged into ice for 45 sec, transferred to water at 25° C for 20 sec, rapidly dried and transferred to a second spectrophotometer maintained at room temperature and the absorbance again measured. The heating and quenching cycle was repeated at successively higher temperature. Absorbance at 260 mμ, measured at increasing temperatures, yielded curves marked (*d*) in Figs. 2–4, while measurement at room temperature on quenched solutions yielded curves marked (*i*).

The DNAs used were prepared from exponentially growing cells of *E. coli* B_0 and *B. subtilis* 19, wild-type, by the method of Saito and Miura[10].

Fig. 1 illustrates the preferential inhibition of DNA synthesis by CF for *E. coli* B_0. When cells of *E. coli* B_0 were incubated in the presence of CF at doses that inhibited colony formation (the dose of CF that permitted 20 per cent of the colonies to form after 60 min incubation was 5 u./ml.), the amounts of DNA failed to increase during 60 min incubation; after 60 min it gradually decreased. However, the formation of RNA and protein was unaffected compared with that of untreated cultures.

During incubation with CF, the optical density of the surrounding medium at 260 mμ increased more than that observed with untreated cultures. The preferential inhibition of bacterial DNA synthesis by CF, accompanied by progressive and extensive breakdown of DNA as evidenced by the increase in optical density at 260 mμ, indicate that DNA is the primary target.

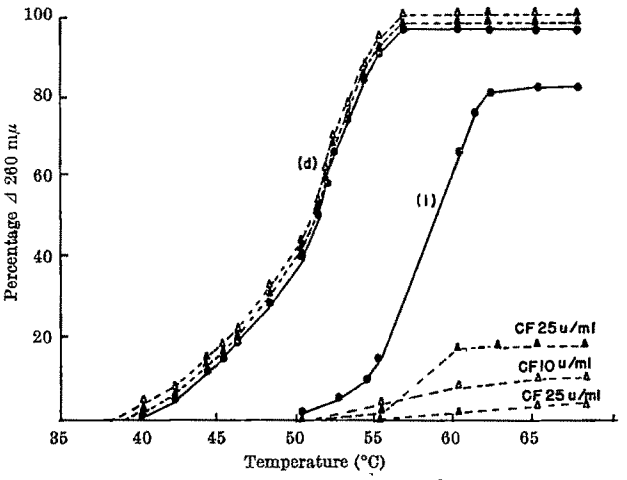


Fig. 2. Reversible thermal denaturation of CF-treated *E. coli* B_0 DNA:

$$\text{Percentage } \Delta 260\ m\mu = \frac{100\,(A(T) - A(25°))_i}{(A\ A\ \max)_d}$$

In vivo treatment of cells with CF: Cells cultured for 10 h in growing medium[17] were resuspended at a density of 2×10^8 cells/ml. in same medium containing 25 u./ml. of CF and incubated at 37° C for 30 min *In vitro* treatment of DNA with CF: 2·5 ml. of a solution of DNA (about 1 mg/ml) in 0·15 M sodium chloride plus 0·015 M sodium citrate was diluted 1 . 1 (v/v) with 0·14 M phosphate buffer (*p*H 7·0) containing CF. The mixture was incubated with gentle shaking at 37° C for 30 min. DNA was then precipitated by the addition of two volumes of cold 95 per cent ethanol, the precipitated DNA removed on a glass rod, drained, dissolved in 0·15 M sodium chloride plus 0·015 M sodium citrate, reprecipitated and redissolved. ——, Untreated DNA, – – – –, CF-treated DNA; △, *in vitro*; ▲, *in vivo*

It is known that other agents which specifically inhibit DNA synthesis, such as nitrogen mustard and mitomycin *C*, produce inter-strand cross-links in DNA as demonstrated by the reversibility of thermal denaturation[11,12]. To determine whether CF damaged the template DNA of *E. coli* B_0 in this manner, the reversibility of thermal denaturation of CF-treated DNA was investigated. Fig. 2 shows the results of denaturation experiments on CF-treated DNA.

Absorbance of DNA treated with CF both *in vivo* and *in vitro*, measured at the increasing temperatures ('standard' melting curves marked (*d*)), was very similar to that of untreated DNA, indicating no unstabilization of the DNA molecules such as follows deamination with nitrous acid[11]. With regard to the irreversible component (*i*), on the other hand, a change was observed. DNA treated with 25 u./ml. of CF *in vitro* was not irreversibly denatured at 65° C, at which temperature untreated DNA was almost completely denatured. Similarly, DNA extracted from CF-treated cells showed much less irreversibility than that of DNA extracted from untreated cells. These results are similar to those obtained with nitrous acid, nitrogen mustard[11] and mitomycin *C*-treated DNA[12], suggesting that, in CF-treated DNA, covalent cross-links between polynucleotide chains are formed.

It seemed reasonable that the presence of these cross-linkages in the DNA molecule was largely responsible for the biological effects

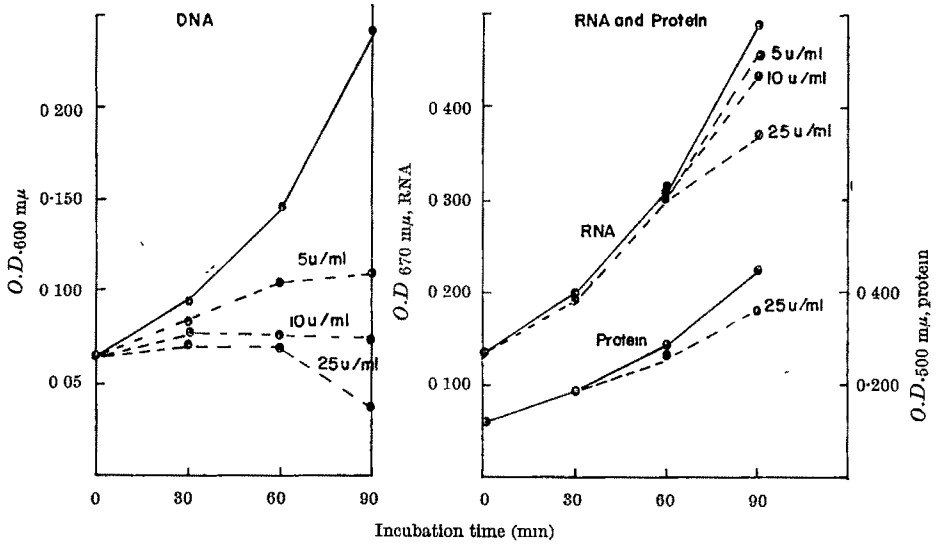


Fig. 1. Effect of CF on the synthesis of nucleic acids and protein ——, Untreated bacteria; – – – –, CF-treated bacteria

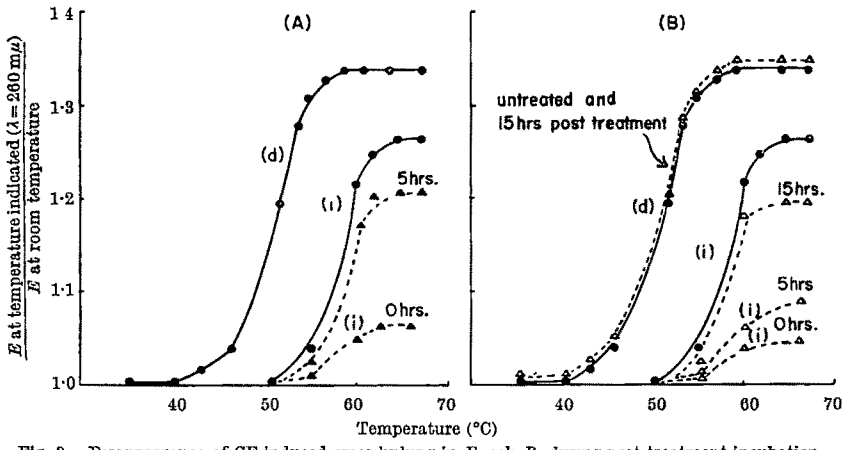


Fig. 3. Disappearance of CF-induced cross-linking in *E coli* B_0 during post-treatment incubation. *In vitro* post-treatment incubation was carried out as follows: after extraction of DNA from CF-treated cells, the solutions of DNA (200 μg/ml) in 0·15 M sodium chloride plus 0·015 M sodium citrate were incubated at 37° C for 5 or 15 h without shaking, and then subjected to thermal denaturation without reprecipitation. *In vivo* post-treatment incubation as described in text. ——, Untreated DNA, – – – –, CF-treated DNA; △, post-treatment *in vitro*; ▲, post-treatment *in vivo*

produced by CF. CF also has a filament-inducing action and the property of cross-resistance with mitomycin *C* and ultra-violet irradiation[13]. From these observations CF could be classified as a 'radiomimetic' agent along with poly-functional alkylating agents such as nitrogen mustard and mitomycin *C*. It has been shown that, during incubation, thymine dimers induced by ultra-violet irradiation were released into the acid-soluble fraction in several strains of *E. coli*[14,15]. Similarities in the biological effects between ultra-violet irradiation and radiomimetic substances imply that thymine dimers and the reaction products of radiomimetic substances and the DNA molecule might be handled in cells in a similar manner. For this reason the stability of CF-induced cross-linkages was investigated.

Cells of *E. coli* B_0 cultured for 10 h were treated with CF in M9 medium without glucose for 30 min at 37° C. The cells were then collected by centrifugation, washed and resuspended in the same medium, and incubated at 37° C for 5 h without shaking (*in vivo* post-treatment incubation).

DNA extracted from these cells, as shown in Fig. 3, showed partial restoration from the CF-induced effect to the normal state of DNA. During post-treatment also, incubation of extracted DNA (*in vitro* post-treatment incubation) for 5 h, a more limited disappearance of CF-induced cross-links than was seen with *in vivo* post-treatment incubation was observed. The same treatment for 15 h resulted in the disappearance of a large part of CF-induced cross-links.

It will be noted from the data in Fig. 3 that the absorption at 260 mμ of CF-treated DNA was unchanged after 15 h of *in vitro* post-treatment incubation. This observation could be interpreted to mean that the disappearance of CF-induced effect during post-treatment incubation *in vitro* is due to the splitting of cross-linkages in the DNA molecule *in situ*, because, if the disappearance of CF-induced cross-linkage occurs by excision of cross-linked nucleotides, a reduction in the rate of increase in the (*d*) curve should have been observed.

On the other hand, the rate of the disappearance of CF-induced cross-linking in intact cells was more rapid than that *in vitro*. This finding suggests the existence of some intracellular mechanism which is responsible for the recovery of DNA from CF-induced cross-linking effect. This interpretation is supported by the observation that, in several strains of *E. coli* showing host cell reactivation, mitomycin *C*-induced cross-linkages disappeared during *in vivo* post-treatment incubation, although the same treatment *in vitro* was ineffective[13].

It has been found recently that several sulphur mustard–DNA alkylation products are eliminated from DNA during post-treatment incubation *in vivo*[16]. In preliminary

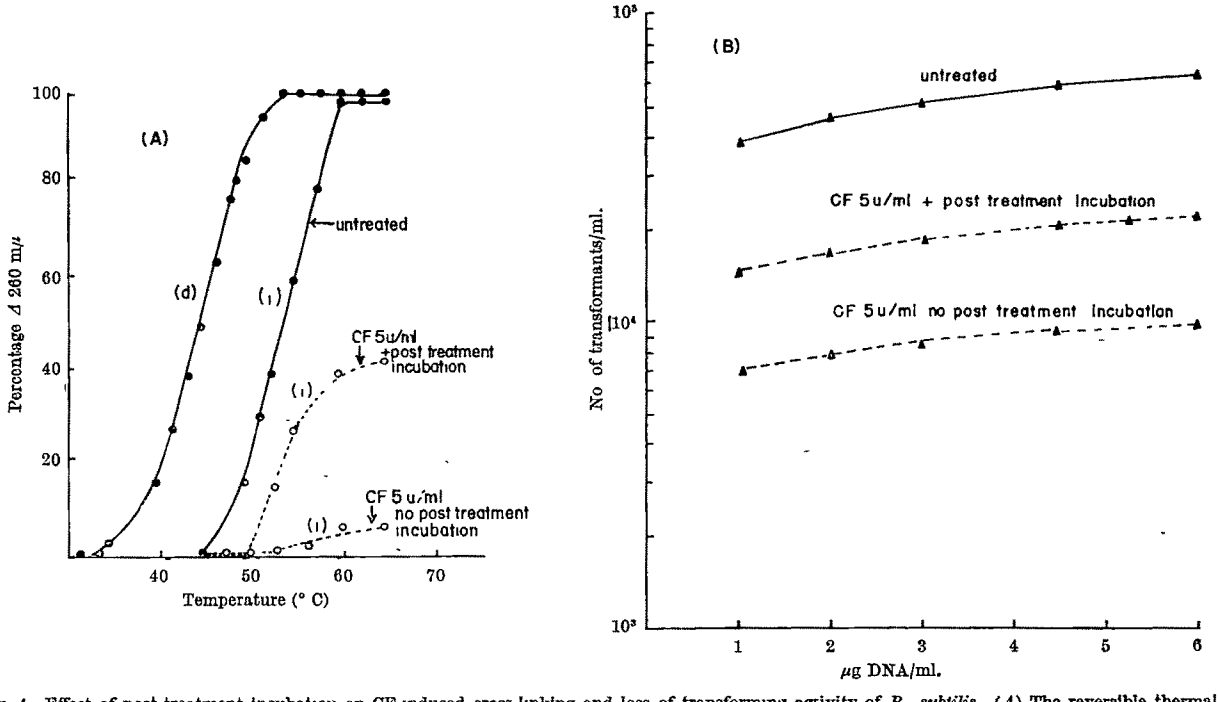


Fig. 4. Effect of post-treatment incubation on CF-induced cross-linking and loss of transforming activity of *B. subtilis*. (*A*) The reversible thermal denaturation of CF-treated *B subtilis* DNA and its reversal during post-treatment incubation. (*B*) The loss of transforming activity of *B. subtilis* induced by CF and its restoration during post-treatment incubation. In both experiments, same preparations of DNA were used. After incubation at 37° C the solutions of DNA were subjected to experiments without reprecipitation

experiments, we have also observed that, during post-treatment incubation of CF-treated cells, diphenylamine reacting material in the acid-insoluble fraction decreased and substances with an absorption maximum at 260 mμ in the acid-soluble fraction increased. Furthermore, the presence of glucose and casamino acid in the incubation medium enhanced the disappearance of the CF-induced effect. Such observations suggest that the elimination by excision of cross-linkages from CF-treated DNA occurs during post-treatment incubation *in vivo*.

We next investigated the possibility of the disappearance of the CF-induced DNA cross-linking effect during post-treatment incubation resulting in the reversal of the biological damage induced by CF. Fig. 4 (*A* and *B*) shows the result of experiments in which transforming DNA inactivated with 5 u./ml. of CF was subjected to *in vitro* post-treatment incubation. *B. subtilis* DNA reacted *in vitro* to CF under similar conditions and behaved in a manner analogous to *E. coli* B_0 DNA treated with this antibiotic with regard to the thermal denaturation. Transformation occurred at a frequency of 3·5 or 4·5 × 10^{-4} viable cells with the untreated transforming factor DNA (1 μg/ml.). Treatment of DNA *in vitro* with 5 u./ml. of CF for 60 min at 37° C reduced the transforming activity to about 15 per cent of the non-treated material. When the CF-treated DNA was incubated *in vitro* for 15 h, partial reversal of the DNA cross-linking effect and partial restoration of the decreased transforming activity of *B. subtilis* DNA were observed. This evidence implies a causal relationship between the cross-linking effect and the loss of transforming activity induced by CF.

We may, therefore, conclude that at least some of the biological effect of CF is mediated by the formation of inter-strand cross-linking in the DNA molecule. The *in vitro* reactivation system for damaged DNA is much simpler to investigate than that *in vivo*. Both inactivation and the subsequent reactivation occur in solution without any cell component.

Further investigations concerning the mechanism of the disappearance of cross-linkage *in vivo* are in progress to correlate our observations on the disappearance of cross-linking with DNA synthesis and mechanisms of resistance.

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CHANGES IN WALL TEICHOIC ACID RESULTING FROM MUTATIONS OF *Staphylococcus aureus*

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TEICHOIC acids are closely associated with the surface structures of many Gram-positive bacteria. Representatives containing either ribitol or glycerol are found in cell walls[1] and glycerol teichoic acids are associated with the wall-membrane interspace, or the membrane itself[2,3]. The metabolic function of these polyol phosphate polymers in the bacterial cell is, however, unknown. There is evidence that at least some teichoic acid is covalently bound to the structural mucopeptide of the cell wall of Gram-positive bacteria, but it is believed that they are not necessary for the maintenance of rigidity of the wall structure. Synthesis of both mucopeptide and ribitol teichoic acids by intact *Staphylococcus aureus* cells is inhibited by penicillin[4], and it has been suggested that the teichoic acids might act as a foundation structure on which the mucopeptide is polymerized[5]. On the other hand, the lability in alkali of the D-alanine ester linkages to the polyol phosphate suggests a function for the teichoic acids which would involve the potential flexibility of the molecular charge, that is, at alkaline *p*H the molecule would lose alanine, become more negatively charged, and repulse anions, with the reverse situation operating at lower *p*H when the phosphate groups would be less ionized and the alanine could become positively charged.

Experimental evidence for the suggested functions of teichoic acids is almost non-existent, but the location of the teichoic acids in cells has reasonably restricted proposals to processes intimately connected with surface function or with the biosynthesis of the cell envelope.

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Mutants of *S. aureus* NCTC 8511 were recently described with phenotypic characters that are suggestive of difference between the surface structures of the mutants and the parent strain. We initiated an examination of the walls of the parent and certain mutant strains to determine whether structural differences in the teichoic acids occurred. It was thought that the mutants could provide a valuable experimental tool for investigating teichoic acid function if such structural differences were found. This article shows that the teichoic acid of certain mutants differs markedly from that of the parent strain.

The parent strain and mutants were obtained from Dr. Ruth Korman of Cornell University, Ithaca, New York. She has isolated and described the characteristics of the mutants, and some of the major phenotypic differences are summarized in Table 1. The fact that large phenotypic changes are associated with simple mutational events has been rationalized by the hypothesis that the phenotypic changes are caused by a primary change in the surface structure which leads to secondary changes in, for

Table 1. Phenotypes of Strains Studied
(Information taken from ref. 7)

Parent strain 8511	Glucose (+), lactose (+), mannitol (+), sucrose (+), fructose (+), galactose (+), 'Novobiocin'-sensitive, coagulase (+), phage-sensitive
Mutant *HS*635	Glucose (+), lactose (−), mannitol (−), sucrose (−), fructose (−), galactose (−), phage-sensitive, 'Novobiocin'-sensitive
Mutant *HS*968	Lactose (+), mannitol (−), sucrose (+), fructose (+), galactose (+), 'Novobiocin'-sensitive, phage-resistant, coagulase (−)
Mutant *HS*1160	Mannitol (+), fructose (+), sucrose (−), maltose (−), galactose (−), phage-resistant, 'Novobiocin'-resistant, coagulase (−)

Table 2. Major Components identified in Acid Hydrolysates

Products were identified by paper chromatography in: (*A*) propan-1-ol-aq. ammonia (sp. gr. 0·88)–water (6 : 3 : 1, by vol.); (*B*) pyridine–ethyl acetate–water–acetic acid (5 : 5 : 1 : 1, by vol.), (*C*) butan-1-ol–ethanol–water–aq. ammonia (sp. gr. 0·88) (40 : 10 : 49 : 1, by vol.; organic phase). Whatman No. 1 paper was used for descending chromatography. Products were detected where appropriate by the periodate-Schiff reagents for glycols[8,9], ninhydrin reagent for amino acids and amino sugars, molybdate-perchloric acid for phosphates[10] and aniline phthalate for reducing sugars[11]. Polyols were identified with solvent *A* and *C*

Strain	Products
8511	Ribitol, alanine, glucosamine, anhydroribitol, ribitol 5-phosphate, ribitol 3-phosphate, ribitol diphosphates
*HS*635	Ribitol, alanine, glucosamine, anhydroribitol, ribitol 5-phosphate, ribitol 3-phosphate, ribitol diphosphates
*HS*968	Glycerol, alanine, glucose, glycerol phosphates, glycerol diphosphates
*HS*1160	Glycerol, alanine, glucose, galactosamine, glycerol phosphates, glycerol diphosphates

example, permeability to sugars, susceptibility to attack by phage and sensitivity to antibiotics. A mutant similar to *HS*635 is known to have lost its ability to ferment several sugars simultaneously because of impaired permeability to sugars[6]. Korman reported[7] that the mutant *HS*968 has serine in its walls, whereas this is absent from walls of the parent strain.

Our first approach to the question of whether the teichoic acids of the mutants differed significantly from that of the parent strain was to determine the phosphorus content of walls prepared from each. The percentage of phosphorus (shown in parenthesis) in the walls of the various strains was: 8511 (2·64), *HS*1160 (3·30), *HS*968 (2·72) and *HS*635 (1·92). Although these differences may be significant, it was likely that all strains contained a wall teichoic acid. A qualitative examination of material extracted from the walls with 2 N hydrochloric acid for 24 h at 20°–22° C was carried out in a study of characteristic components of teichoic acid. The procedure was more vigorous than that generally used for extraction of these polymers, but this was convenient for the purpose of screening for major differences in components. The procedure did not remove components of mucopeptides from intact cell walls. The extracts were hydrolysed in 2 N hydrochloric acid for 3 h at 100° C and subjected to paper chromatography after appropriate deionization. The major sugars, polyols and amino acids in the hydrolysates are shown in Table 2. No changes were observed in the nature of the polyols when hydrolysates were dephosphorylated with an alkali phosphatase.

These results show that a major change in the teichoic acid of the cell wall occurs when mutants *HS*968 and *HS*1160 are derived from the parent strain 8511. Unlike the parent, there is no indication of the presence of a ribitol teichoic acid in the mutants *HS*968 and *HS*1160, but the chromatographic evidence strongly suggests that a glycerol teichoic acid is present in their walls. Mutant *HS*1160 can also be distinguished from mutant *HS*968 and the parent strain 8511 on the basis of the presence of galactosamine in the hydrolysed extracts.

Further support for the conclusion that these changes in the composition of the hydrolysed extracts actually represent changes in teichoic acids was obtained by extraction of teichoic acids from walls with trichloroacetic acid at 4° C, precipitation with organic solvents and partial purification by reprecipitation[12]. The polymers obtained in this way from walls of the parent strain and the mutant *HS*968 gave essentially the same products on acid hydrolysis (2 N hydrochloric acid, 100° C, 3 h) as did the 2 N hydrochloric acid extracts. However, hydrolysates of the polymer from trichloroacetic acid extracts of the mutant *HS*1160 contained no galactosamine. Wall residues from *HS*1160 obtained after extraction with 10 per cent trichloroacetic acid at 4° C for 2–24 h were subjected to further extraction with 2 N hydrochloric acid at 20° C for 16 h. The extract was treated with 2 vol. of acetone and kept at 4° C for 24 h. The resulting precipitate was collected by centrifugation, dissolved in 2 ml. of cold 5 per cent trichloroacetic acid and treated with 2 vol. of acetone. After 72 h at 4° C the precipitate was recovered by centrifugation and washed with ethanol, acetone and ether. The washed precipitate was dried in air and dissolved in water; insoluble material was removed by centrifugation, and the water-soluble portion was freeze-dried. This material was hydrolysed with acid, and chromatography of the products showed that they were the same as those for 2 N hydrochloric acid extracts of the walls, including the presence of significant amounts of galactosamine.

The acid hydrolysates of the polymers obtained from the parent and the mutants *HS*968 and *HS*1160 by extraction with trichloroacetic acid contain the typical products of hydrolysis of teichoic acids. The polymer containing galactosamine in *HS*1160, however, is not readily extracted with cold trichloroacetic acid but is extracted with 2 N hydrochloric acid at 20° C. The galactosamine is mainly in a fraction that also gives on hydrolysis typical glycerol teichoic acid components. However, it is not possible to decide at present whether a glycerol teichoic acid containing galactosamine occurs in *HS*1160 or whether the galactosamine is associated with other polymeric material. The extraction of material containing galactosamine in the 2 N hydrochloric acid fraction but not in the trichloroacetic acid fractions suggests that at least two polymeric species may be present. Treatment of wall residues of 8511 or *HS*968 with 2 N hydrochloric acid after the usual extraction with trichloroacetic acid resulted in the extraction of more polymer containing phosphorus, the hydrolysis products of which seemed to be grossly similar to those obtained by direct extraction with trichloroacetic acid.

The teichoic acids of the parent strain and mutant *HS*635 thus appear to be grossly similar to the teichoic acids of the *S. aureus* group, whereas *HS*968 and *HS*1160 have teichoic acids similar to those of the *S. saprophyticus* group[13] (classification according to Shaw, Stitt and Cowan[14]). Baird-Parker's recent classification of staphylococci and micrococci[15] records that staphylococci in his *Staphylococcus* Sub-group I are the only staphylococci which contain ribitol teichoic acid in the wall. The *Staphylococcus* Sub-groups II–VI of Baird-Parker have glycerol teichoic acids in their walls. No glucose is found in the walls of Sub-group I, but glucose occurs in walls from members of all the Sub-groups which contain wall glycerol teichoic acid. Members of Sub-groups II, V and VI, however, are the only staphylococci reported to contain galactosamine in their walls. Organisms in Sub-groups I and VI are the only ones with the ability to produce acid from mannitol. *HS*1160, the mutant which has glucose and galactosamine in the teichoic acid fraction, also produces acid from mannitol. *HS*968 does not ferment mannitol and does not have galactosamine in its wall teichoic acid. On the basis of the limited tests that have been performed, it would seem that *HS*968 is related to the organisms in Sub-group III and *HS*1160 related to organisms in Sub-group VI. It would be of interest to know whether strains of Sub-group VI yield wall teichoic acid fractions containing both glucose and galactosamine. Glycerol teichoic acids containing either glucose or galactosamine, but not both, have already been shown to be present in *S. saprophyticus*[13].

There are several interesting questions raised by the finding of fundamental differences in the composition of teichoic acids in mutants in addition to the question of the relationships of the mutants to natural strains of staphylococci. These experiments have shown that changes occur in the teichoic acids of mutants with other phenotypic differences suggestive of surface structure changes; but whether the other phenotypic differences are directly related to the changes in the teichoic acid is not known. The nature of the mutational event or events which account for the changes in wall teichoic acid is also obscure. That the phenomenon is complicated is evidenced by the fact that a change in the mucopeptide amino acids also occurs in *HS*968 (ref. 7). Moreover, the change from a wall ribitol teichoic acid to a wall glycerol teichoic acid

might appear to be a gain mutation. It is not necessary, however, to postulate such an unusual phenomenon to explain this change. All the Gram-positive bacteria examined contain membrane glycerol teichoic acid (as it is now known that so-called 'intracellular' teichoic acid occurs between the wall and the protoplast membrane and is attached, at least in part, to the latter structure it is proposed to call this 'membrane teichoic acid'), whether or not they have ribitol, glycerol or no teichoic acid in the wall[16,17]. It is possible that an event which leads to a loss of ability to synthesize or insert ribitol teichoic acid into the cell wall could simultaneously permit the insertion of a membrane teichoic acid into the wall. This hypothesis may be complicated by the necessity to postulate a control process to regulate the production of membrane teichoic acid and to prevent its insertion into cell walls of those organisms which either do not require a wall teichoic acid or which normally make a ribitol teichoic acid for this purpose. The hypothesis, however, does not involve the postulation of new genetic capability in the mutants containing wall glycerol teichoic acid because the capability for synthesis of glycerol teichoic acid exists in the parent strain.

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ANATOMICAL RESPONSES OF PINE NEEDLES TO HERBICIDES

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SEVERAL herbicides have shown variable toxicity to young gymnosperms, with susceptibility of plants varying greatly with the chemicals and dosage used, species, and age of plants[1–7]. Among the more obvious deleterious responses of young pine seedlings to herbicides are twisting and curling of cotyledons, distortion of old needles, chlorosis and subsequent death of the plant.

Although less obvious than the effects already noted, the influences of herbicides on internal leaf anatomy are often profound. For example, Ashton *et al.*[8] investigated the influence of atrazine on leaf structure in *Phaseolus vulgaris* and found that the herbicide: (*a*) accelerated development of vacuoles and modified ontogeny of chloroplasts in developing leaves; (*b*) ultimately caused chloroplast destruction and reduced the airspace system in mature and primary leaves; (*c*) promoted cessation of cambial activity and caused thin walls to develop in sieve and tracheary elements in the stem.

In another investigation, Ashton *et al.*[9] noted that atrazine induced several progressive changes in fine structure of chloroplasts in *Phaseolus*, including: (*a*) spherical rather than discoid shape; (*b*) disappearance of starch from the lamellar system; (*c*) destruction of frets leading to disorganization of the lamellar arrangement; (*d*) swelling of compartments of the grana; (*e*) ultimate disintegration of the swollen compartments. Ashton *et al.*[9] concluded that these changes were effected by formation of a toxic substance or substances involving interactions of light and atrazine in the presence of chlorophyll.

Only very few investigations are available that deal with anatomical changes induced by herbicides in woody plants. Parascan and Vlase[10] found marked variation among species in resistance to 2,4-D. *Betula*, *Carpinus betulus*, *Salix* and *Populus* were very sensitive to the herbicide. *Fagus silvatica* and *Quercus* were less sensitive, while *Tilia*, *Acer campestre*, *Acer tataricum* and *Prunus cerifera* were classified as resistant. The overall responses of plants to the herbicide were grouped into two categories: (1) decreased growth without dieback; (2) dieback. The first group was characterized by leaf modifications such as reduction in size, lobing, fasciation and, in isolated cases, epinasty. These changes occurred in the species generally resistant to herbicides. The second group responded by forming swellings and cankers, bark splitting, and wilting and dieback of shoots. Work with 3-amino 1,2,4-triazole by Shiue and Hansen[11] showed that the herbicide caused chlorosis, needle distortion, mesophyll collapse, and change in shape of mesophyll cells of *Pinus resinosa* and *Picea glauca*. These experiments have now been extended to an investigation of the influence of several herbicides on leaf anatomy of young pine plants.

Experiments were performed on young red pine (*Pinus resinosa* Ait.) plants. Herbicides were incorporated in the soil or applied as soil surface sprays according to the following schedule:

Herbicides incorporated in soil and applied pre-emergence: atrazine, simazine, prometryne, propazine, ipazine, EPTC, DCPA, CDEC, and CDAA. Each herbicide was applied at 2 or 8 lb./acre (surface area basis).

Herbicides applied to soil surface when plants were six months old: atrazine, simazine, prometryne, propazine, ipazine, EPTC, DCPA, CDEC, CDAA, monuron and diuron. Each herbicide was applied at 16 lb./acre.

For the soil incorporation experiments, Plainfield sand was placed in greenhouse flats to a 2 in. depth and the appropriate herbicide sprayed on the soil surface at 2 or 8 lb./acre. The soil and herbicide were then mixed in a Patterson–Kelly twin-shell blender and distributed in 4-in. clay pots. One hundred *Pinus resinosa* seeds were planted in each of ten pots for each dosage of each herbicide. Pots planted with seed but without herbicide served as controls. Seeds germinated regularly, beginning about 10 days after planting. For the surface application experiments, each herbicide was sprayed to the surface of soil in pots containing 6-month-old seedlings.

Eight weeks after application of the herbicides, representative needles of all plants were collected and preserved in formalin–acetic acid mixture. Afterwards the needles were embedded in 'Tissuemat', cross-sectioned at 12μ and stained with safranin and fast green before the sections were mounted on slides.

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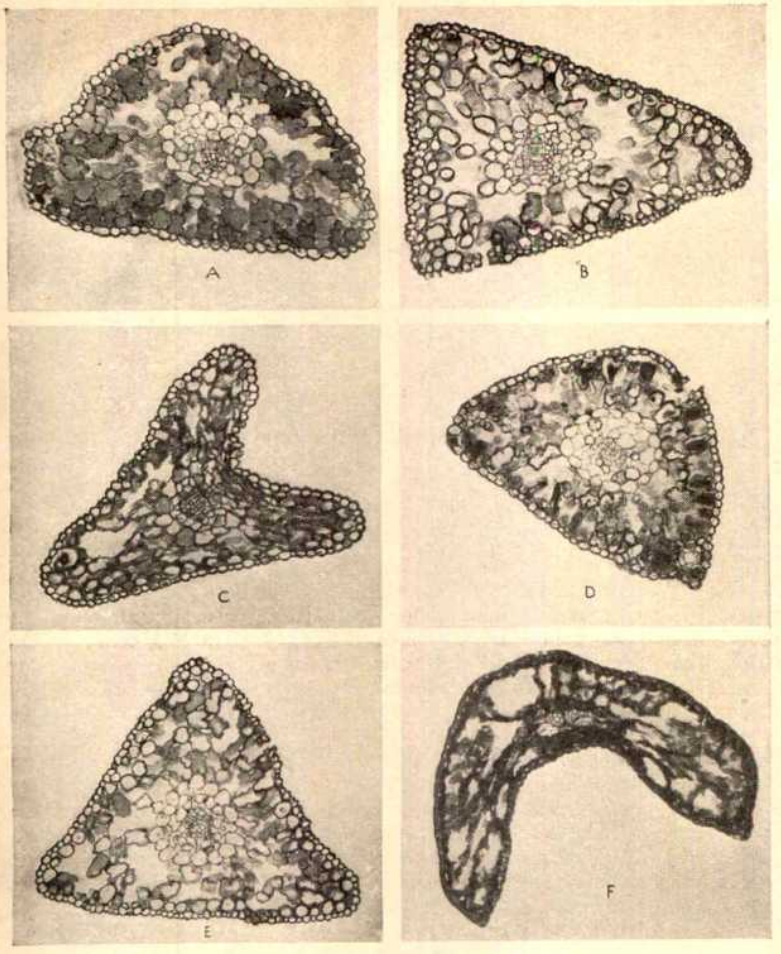


Fig. 1. Influence of various herbicides on young *Pinus resinosa* needles. *A*, Control (no herbicide). Needle semicircular in cross-section. Mesophyll cells are closely packed and infolded. *B*, CDAA incorporated in soil at 8 lb./acre (surface area basis), pre-emergence. Needle triangular in cross-section with granular, red-staining material in some mesophyll cells. Mesophyll cell contents often missing. Large gaps show among mesophyll cells. *C*, Prometryne incorporated in soil at 2 lb./acre (surface area basis), pre-emergence. Needle distorted and collapsed. Chloroplasts are not apparent and rounded mesophyll cells have large gaps among them. *D*, EPTC incorporated in soil at 8 lb./acre (surface area basis), pre-emergence. A normally shaped cross-section shows breakdown of chloroplasts. Red-staining granular material is present in mesophyll instead of solid red-staining chloroplasts. Mesophyll cells are frequently rounded and have spaces among them. *E*, CDEC incorporated in soil at 8 lb./acre (surface area basis). The triangular cross-section shows no red-stain in the mesophyll. Large gaps exist among rounded, frequently empty mesophyll cells. *F*, Diuron applied to soil surface at 16 lb./acre when seedlings were six months old. The typically brown needle was shrivelled. The mesophyll is totally collapsed and only the epidermis and some conducting tissues retained their normal form. (× 72)

Soil-incorporated herbicides caused far more injury than those applied to the soil surface. Within 10 days from the beginning of germination, toxicity symptoms were developing in plants growing in soil containing incorporated herbicides. In general, slight needle curling was followed by severe chlorosis, needle dieback, seedling distortion, depressed growth and death of many needles. Most injury was caused by atrazine, simazine, prometryne and propazine, whereas ipazine, EPTC, CDEC, CDAA and DCPA caused far less and sometimes only negligible injury.

The surface-applied herbicides fell into three groups. Atrazine, simazine, monuron and diuron were highly toxic. Prometryne, propazine, CDEC and CDAA caused intermediate damage, while EPTC, DCPA and ipazine caused slight to no obvious injury.

There was considerable variation among needles in the amount of injury sustained. When needles were severely damaged the internal anatomy was altered in a more or less similar way by the various herbicides. Such anatomical changes, however, covered a fairly broad spectrum within and among seedlings. Only generalized symptoms of herbicide effects on leaf anatomy are given at this stage, with further experiments contemplated to investigate details of variable injury by different herbicides.

Examples of sections of healthy and herbicide-damaged needles are shown in Fig. 1. Cross-sections of needles from control plants were approximately semicircular in outline. The mesophyll cells were closely packed and infolded and contained large, dark-red-staining chloroplasts. In contrast, the distorted needles of herbicide-treated plants showed considerable yellowing or browning, and were triangular (or nearly so) in cross-section. Many needles which had turned brown had shrivelled. The red stain of chloroplasts was very pale or absent and red-staining nuclei were thus unmasked. Mesophyll cells were predominantly rounded and had lost the characteristic infolding of normal mesophyll tissue. Mesophyll cells sometimes became vacuolated or nearly empty. Often conspicuous large gaps among mesophyll cells were apparent. In some needles red-staining granular material was present in the mesophyll. The cells of lignified epidermis and vascular bundles retained their outlines, but the mesophyll had disintegrated, permitting the needle to collapse. Sometimes only one of the three needle surfaces was concave as a result of mesophyll collapse.

The greater damage caused by soil incorporation of herbicides over surface application appears to be related to the amount of herbicide absorbed. Kozlowski and Kuntz[12] showed that very young pine seedlings readily absorbed soil-incorporated triazine herbicides and were killed, whereas surface-applied herbicides which were not easily absorbed by plant roots were less toxic. When various triazine herbicides were applied to the soil surface and leached, most of the herbicide remained in the surface inch of soil regardless of whether 2, 4 or 8 surface inches of water were used in leaching. Old seedlings escaped injury from surface-applied triazines largely because their absorbing roots were sufficiently deep to avoid taking up the surface-concentrated herbicide. Old seedlings were readily killed, however, when small amounts of triazine herbicides were incorporated in the zone of absorbing roots. Such observations are reinforced by those of Freeman[13], who noted that pine seedlings were killed by simazine within 5 weeks of germination. In contrast, oak seedlings were not damaged largely because of the rapid movement of the absorbing zone of their roots to levels below that of the herbicide concentration.

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EFFECTS OF ALCOHOL CONSUMPTION ON THE ACTIVITY OF LIVER ENZYMES IN *C57BL/Crgl* MICE

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INCREASED alcohol dehydrogenase (ADH) activity has been reported in mice, rats and men following the consumption of alcohol for prolonged periods[1]. These previous reports indicate that consumption of alcohol increases the activity of enzymes involved in its metabolism. In the present study the generality of the effect of alcohol on activity of alcohol dehydrogenase, xanthine oxidase (XO) and hexokinase (Hk) in liver has been investigated. Hk is not directly involved in alcohol metabolism and the importance of XO in the *in vivo* metabolism of alcohol has not been established. In addition, the influence of alcohol ingestion on liver protein content was investigated.

Male *C57BL/Crgl* mice, obtained from the Cancer Research Genetics Laboratory, University of California, Berkeley, were used for the experiments[2]. All animals were 60 ± 3 days old at the beginning of the experiments.

Fifty-eight mice were used in three experiments; twenty animals were used in the first experiment, twenty in the second and eighteen in the third. Animals were randomly assigned, and in equal numbers, to the experimental and control condition in each experiment. Experimental animals were given a 10 per cent solution of ethyl alcohol as their sole drinking source for 90–104 days; control animals were maintained on tap water for an equivalent period. All animals had free access to standard laboratory diet.

The mice were weighed to the nearest 0·1 g before being killed by decapitation. The liver was immediately removed and weighed to the nearest mg. In the second experiment, one lobe was dissected and separately weighed. This lobe was dried to constant weight (± 1 mg) *in vacuo* using a dry ice-isopropyl alcohol trap.

The fresh livers were homogenized with a 'Teflon'-glass homogenizer in distilled water at 0° and diluted to a final concentration of 50 mg/ml. A portion of the homogenate was centrifuged at 0° for 30 min at 3,000 r.p.m. (International model *PR*-1 centrifuge). The supernatant, excluding the lipid layer, was removed and used for alcohol dehydrogenase (ADH) assays and for some of the protein analyses. Uncentrifuged homogenates were used for XO and Hk assays and for some of the protein analyses. All enzyme assays were carried out in duplicate; duplicate assays agreed within 5 per cent.

ADH assays were performed as follows: 3·0 ml. of a glycine-semicarbazide buffer[3], 0·20 ml. of 0·015 M nicotinamide-adenine dinucleotide (NAD), and 0·050 ml. of a 0·35 M ethanol solution were mixed in 10 mm light path spectrophotometer cells. After pre-warming the incubation mixture at 37° for 10 min, 50 µl. of the supernatant from the liver homogenate were added and mixed. The rate of reduction of NAD was measured by the increase in absorption at 340 mµ. A Beckman spectrophotometer equipped with a Gilford model 210 automatic cuvette positioner, a model 220 optical density converter, a recorder and a 37° cell compartment were used. The absorbancies of four samples were sequentially recorded in automatic rotation at 30-sec intervals for 10–12 min. This procedure has already been described in greater detail[4]. In the absence of added ethanol, no change in absorbance was noted during a 10 min incubation.

Hk activity was determined by a procedure modified from that described for brain[5]. Three ml. of a solution containing 0·13 M glycylglycine, 0·02 M magnesium chloride, 0·001 M nicotinamide-adenine dinucleotide phosphate (NADP), 0·025 M glucose and 0·01 M adenosine triphosphate (ATP) (final *p*H adjusted to 8·0) and 0·06 ml. of a glucose-6-phosphate dehydrogenase solution (50 µg/ml.) were pre-warmed to 37°. The uncentrifuged liver homogenate (0·075 ml.) was then added and the rate of reduction of NADP was measured by the increase in absorption at 340 mµ using a Beckman *DU* spectrophotometer and auxiliary recording equipment.

XO activity was determined by the method described by Udenfriend[6]. 2-Amino-4-hydroxypteridine is readily oxidized by XO to isoxanthopteridine[7]. The activity of the enzyme may be determined spectrofluorometrically since the fluorescence of the substrate, 2-amino-4-hydroxypteridine, is quenched in phosphate buffer, while that of the product is not. To determine XO activity 10-ml. aliquots of 0·1 M sodium phosphate buffer containing 4×10^{-6} M 2-amino-4-hydroxypteridine were pipetted into 50 ml. Erlenmeyer flasks, which were then placed into a metabolic shaker and warmed to 30°. Afterwards, 0·25 ml. of the uncentrifuged homogenate was added and the mixture was incubated in the shaker-water bath for 60 min. A 2-ml. portion was withdrawn and analysed in an Aminco–Bowman spectrophotofluorometer. The activating wave-length was 250 mµ and the analysing wave-length was 420 mµ. The machine was set at 'zero' before each determination and the value of a blank (containing everything in the sample except the enzyme) read. Values of the blank did not change with time. After all determinations had been made 10 µl. of milk XO were added to each flask to oxidize completely the 2-amino-4-hydroxypteridine. The fluorescence of this sample was then determined after 30 min on a 2 ml. portion. These final readings did not differ from sample to sample by more than 2 per cent. Under these conditions of measurement, fluorescence was shown to be a linear function of the amount of substrate oxidized.

The protein content of the livers was determined in triplicate with the Folin phenol reagent[8]. The original liver homogenate was diluted with water to 2 mg/ml. Aliquots containing 1 mg of wet tissue were mixed with 5 ml. of alkaline copper sulphate reagent *C* in 12-ml. centrifuge tubes. After 10 min, 0·50 ml. of dilute Folin–Ciocalteau reagent was added and rapidly mixed. The optical density at 750 mµ was determined in a Beckman *DU* spectrophotometer after 60 min. Suitable aliquots of freshly diluted crystalline bovine albumin solutions (Armour) were run as standards. All results were referred to this standard (mg protein = mg nitrogen × 6·25). Protein results were expressed as a percentage of the wet

tissue weight. In the second experiment, protein determination was made both on the liver homogenates that had been centrifuged and on those that had not.

Liver-weight and body-weight were not significantly different in control and experimental animals. In one of the three experiments, the ratio of liver-weight to body-weight was significantly greater in the experimental animals ($P < 0.05$), but this finding was not replicated in the other experiments.

Dry-weight determination of a liver lobe of the animals used in the second experiment indicates that no differences in the ratio of dry weight/wet weight existed between experimental and control animals. The percentage dry-weight of the liver lobe was 33 per cent in both the experimental and the control animals (range, 32–34 per cent in both experimental and control groups).

Prolonged alcohol consumption significantly increased ADH activity in liver by about 20 per cent (Table 1). These differences were statistically significant and of the same order of magnitude regardless of whether ADH activity was expressed as μmoles/min/entire liver, μmoles/min/g liver or μmoles/min/g liver/g mouse. Since all methods of expressing the data gave approximately the same results, only those for μmoles/min/g liver are given in Table 1.

Table 1. PERCENTAGE OF PROTEIN AND ADH ACTIVITY IN EXPERIMENTAL AND CONTROL *C57BL/Crgl* MICE

Exp. No.	Condition	N	Protein in liver* (per cent) $\bar{X}$	S.D.	ADH activity (μmoles/min/g liver) $\bar{X}$	S.D.
(1)	Experimental (alcohol)	10	18·17	0·18	4·41	0·66
	Control (water)	10	16·55	1·13	3·62	0·67
	Percentage difference		12		18	
	P value		< 0·01		< 0·02	
(2)	Experimental (alcohol)	10	18·23	1·08	6·56	0·32
	Control (water)	10	16·13	1·66	5·25	0·39
	Percentage difference		12		20	
	P value		< 0·01		< 0·01	

* The percentage of liver protein is based on the analyses of the uncentrifuged homogenates.

Liver Hk activity was not significantly different in control and experimental animals (Table 2).

XO activity/g liver was significantly decreased in experimental animals, by 43 per cent in the second experiment and by 48 per cent in the third (Table 2).

Liver protein (expressed as a percentage of the liver wet weight) was significantly increased in experimental animals by 12 per cent in the first experiment and 12 per cent in the second. These differences were found whether the protein analyses were made on the centrifuged or uncentrifuged liver homogenates. For this reason only the results obtained with the uncentrifuged liver homogenate are summarized in Table 1.

The differences in ADH activity in liver between experimental and control mice, when enzyme activity is expressed per unit protein, were 6 per cent in the first experiment and 9 per cent in the second. These differences, although quite small in magnitude, are statistically significant ($P < 0.01$ and $P < 0.01$ for Experiments 1 and 2 respectively).

The finding that ADH activity in liver is increased following the consumption of alcohol is in good agreement with the results reported by several other investigators. McClearn, Bennett, Hebert, Kakihana and Schlesinger[1] have shown increased ADH activity in *C57BL/Crgl* and *DBA/2Crgl* mice maintained on 10 per cent alcohol for 2–4 weeks. Kakihana, Schlesinger and McClearn[1] were able to demonstrate increased ADH activity in 12-week-old *BALB/cCrgl* mice, but not in 6- or 9-week-old mice, which had been maintained on alcohol for a 2-week period; the younger *BALB/cCrgl* mice had high endogenous ADH activity. Figueoroa and Klotz[1] and Dejani, Danielski and Orten[1] also reported increased ADH activity in the rat following alcohol ingestion; Dejani *et al.* reported increased acetaldehyde dehydrogenase activity in rats following ingestion of alcohol.

Table 2. EFFECTS OF ALCOHOL CONSUMPTION ON XANTHINE OXIDASE AND HEXOKINASE ACTIVITY IN *C57BL/Crgl* MICE

Exp. No.	Condition	N	Xanthine oxidase activity (μmoles/h/g liver) $\bar{X}$	S.D.	Hexokinase activity (μmoles/min/g liver) $\bar{X}$	S.D.
(2)	Experimental (alcohol)	10	0·96	0·12	——	——
	Control (water)	10	1·42	0·19	——	——
	Percentage difference		43			
	P value		< 0·001			
(3)	Experimental (alcohol)	7	0·48	0·15	3·08	0·025
	Control (water)	7	0·93	0·48	3·22	0·14
	Percentage difference		48		4	
	P value		< 0·01		*N.S.*	

All these reports have focused on the activity of only one enzyme. The increased liver protein content observed in experimental animals was unexpected and complicates the interpretation of the observed increase in ADH activity. This difference in liver protein content could, for example, mean that the increased ADH activity is merely a secondary reflexion of the total increase in liver protein. If the argument is to be maintained that ingestion of alcohol increases activity of the enzymes involved in its oxidation, then it is necessary to demonstrate that enzymes not directly involved in alcohol metabolism are not affected by ingestion of the substrate.

Two other enzymes were measured in these experiments. Hk was not directly involved in alcohol metabolism and the importance of XO in the *in vivo* metabolism of alcohol was not established. The activity of XO decreased with ingestion of alcohol and the activity of Hk was not affected by this treatment, while the activity of ADH increased. These findings suggest that only those enzymes directly involved in the metabolism of alcohol are affected by ingestion of the substrate.

The fact that liver-weight and body-weight were not changed by the ingestion of alcohol was also surprising. Liver water content was also not changed. Because liver protein content was increased by ingestion of alcohol and because the liver weight and the water content of the liver remained unchanged, some liver constituent(s) must be decreased. What these constituents are remains to be determined.

The results of the ADH analyses in the two experiments are not in very good agreement in terms of absolute magnitudes of enzyme activity. Such day-to-day discrepancies are probably due to the fact that the various reagents are made up fresh for each experiment. Because comparisons are made between experimental and control animals within a given experiment, however, these discrepancies are not too serious. It should be noted that the relative differences between experimental and control mice are in good agreement for all experiments. The differences in absolute oxidation rate in the XO assays are attributed to difficulties in completely dissolving the substrate. Again, comparisons were made within experiments, and the relative differences between experimental and control animals were in good agreement for all experiments.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Improved Resolution X-ray Photographs of the Sun

A FOURTH successful firing of the new Sun-pointed *Skylark* research rocket[1] took place at 0520 G.M.T. on October 20, 1965, at Woomera, Australia. The flight was designated *SL* 306 and on board were instruments prepared by Culham Laboratory and University of Leicester Groups for study of the short-wave-length emission of the Sun. This note presents a number of new X-ray photographs of the Sun obtained by the Leicester Group, using a camera array similar to that successfully utilized in the three earlier *Skylark* flights[2–4]. Two significant changes in the present camera were the inclusion for the first time of a Fresnel zone plate[5] unit and the improvement of the geometrical resolution on one pin-hole camera unit to 1 arc min. The latter improvement was encouraged by the increased exposures available from the predicted trajectory of the new, more powerful, *Raven* 6*A* rocket motor. The zone plate was flown primarily to test its rocket-worthiness prior to use in 1966 in the first Mark II stabilized *Skylark*, when an attempt will be made to obtain photographs of the Sun in monochromatic radiation with resolution of 10–20 arc sec. In the present flight the zone plate camera was focused for the Lyman-α line of *C* VI, recently[6] found to be one of the strongest short-wave-length emission lines in the solar spectrum. Full details of the camera array are given in Table 1.

Table 1. DETAILS OF THE CAMERA UNITS (NOMINAL VALUES)

Unit	Filter (μ)	Wave-length band 10% (Å)	Focal length (in.)	Pin-hole or zone plate diameter (10^{-3} in.)	Resolution (arc min)
A	0·3 Aluminium	< 40	7·4	5	2
B	1·0 Polypropylene + 0·15 aluminium	< 32 and 44–48	7·4	5	2
C	0·3 Aluminium	< 40	3·4	1·4 (inner zone) 8·7 (outer zone)	0·1*
D	0·3 Aluminium	< 40	8·4	10	4
E	1·5 Polypropylene + 0·1 aluminium	< 32 and 44–48	8·4	2·5	1
F	1·5 Aluminium	< 24	8·4	10	2
G	1·5 Polypropylene + 0·1 aluminium	< 32 and 44–48	8·4	10	2

* This resolution would only be realized for monochromatic radiation of 33·7 Å and a finer grain emulsion than that used in this flight.

The flight performance of *SL* 306 was excellent, reaching an apogee of 213 km and providing effective exposure of the cameras during the controlled phase of almost 5 min. Further, analysis of the initial flight records has shown the pointing control was the best so far achieved. Throughout the whole pointed phase, roll drift and lateral pointing noise were too small to be measured on the low-speed telemetry records so far available. It seems likely that the lateral noise figure of 20 arc sec obtained on this control unit during tests at Elliott Automation may have been achieved in flight, owing to the use for the first time of a higher gain error-signal amplifier. The major pointing movement appears to have been a slow lateral drift of the instrument payload relative to the control bay, due to heating of the rocket during the launch phase. A slight elongation of the X-ray images reported here supports such a drift of about 2 arc min parallel to the north–south axis of the Sun.

The condition of the Sun[7] on October 20 was 'fairly quiet', only three small chromospheric plages being evident on the disk, at 20° N.–5° W., 20° N.–65° W. and 30° S.–30° E. Of these, the plage near the centre of the disk had brightened considerably on the day prior to the flight. The only other feature evident in the *H*-alpha photographs are a number of dark filaments in the north-west quadrant, being remnants of old active regions. The provisional sunspot number on the day was 17 and the 10·7 cm radio noise index was 73.

X-ray images *A*–*G* are reproduced in Fig. 1, and enlargements of *B*, *C* and *E* are shown in Fig. 2. The following observations may be made from initial examination of the negatives.

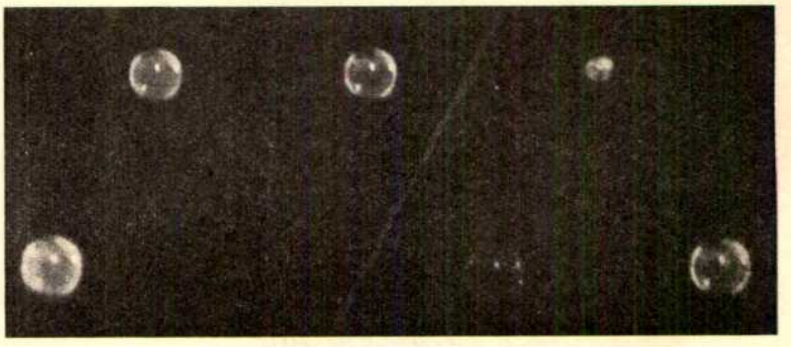
(*a*) X-ray enhancements are seen associated with all three chromospheric plages. Of particular note is the apparent size of the two low-latitude regions from image *E*. The measured size is 3 × 1 arc min and, after allowance for camera resolution of 1 arc min and the probable north–south thermal drift, it is clear that each X-ray source is even smaller than the 1 arc min estimated for earlier photographs[8].

(*b*) The very small motion of the pointing control parallel to the solar equator provides a true measure of the limb brightening which can be seen to have greater extent in an east–west direction than the plage-associated sources. Polar darkening is again clearly seen.

(*c*) A general increase in X-ray emission is found in the north-west quadrant of the disk, and this is particularly noticeable on the zone plate image. This emission probably indicates higher coronal densities in this area, possibly associated with the old activity seen as filaments in the chromosphere.

(*d*) Dark regions are clearly seen on the disk for the first time (compare images *B* and *G*) and it is notable that a dark region, probably therefore of lower density, is seen either side of the central plage, which was in process of rapid enhancement. A similar association of a dark patch with a newly forming bright X-ray region was seen on flight *SL* 301, but on that occasion the poorer image resolution made this association less distinct.

(*e*) Of particular interest is the higher disk emission on image *A* compared with image *B*, particularly as the transmission of filter *A* exceeds filter *B* only below 43 Å. It seems possible that this observation may be due to the greater sensitivity of camera *A* for the *O* VII lines at



A *B* *C*

D *E* *F* *G*

Fig. 1. Images *A*–*G*

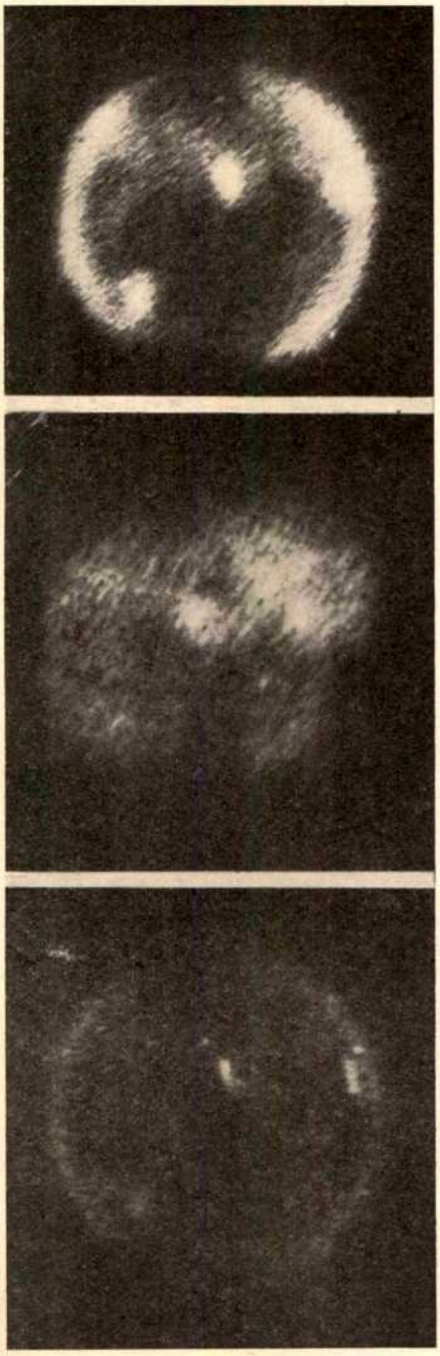
21·5 Å, reported earlier[9] to be very strong in the solar spectrum. The lower coronal temperature outside the active regions, found in analysis of the earlier photographs[8], would support the occurrence of abundant *O* VII, requiring the relatively low ionization energy of 170 eV, in the general corona.

(*f*) Image *C*, from the zone plate camera, is noticeably less distinct than the pin-hole camera images. This camera was set for prime focus at 33·7 Å, the Lyman-α line of *C* VI, but the thin aluminium filter also transmitted shorter wave-lengths. The two low-latitude plages appear relatively sharp, probably due to the abundance of *C* VI in the active corona, this being a relatively high-energy state, of ionization energy 380 eV. It seems likely that much of the out-of-focus emission from the disk, particularly evident in the north-west quadrant, may be again due to the 21·5 Å lines of *O* VII. Post-flight checks have shown that the zone plate was set in error for focus at 40 Å. This will have contributed to the blurring of the image but does not affect the conclusions drawn.

We thank the staff of the Royal Aircraft Establishment, Farnborough, and of Elliott Automation for their respective parts in successful stabilized *Skylark* vehicle, and the staff of the Weapons Research Establishment, Salisbury, for the launch. The technical assistance of colleagues in the Department of Physics in preparation of the cameras and, in particular, of John Larrad, who conducted the flight trial in Australia, are gratefully acknowledged. For the preparation of zone plates for this and future payloads and for laboratory study, we thank Prof. Möllenstedt of the University of Tübingen, Germany.

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B

C

E

Fig. 2. Images *B*, *C* and *E*

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COSMOLOGY

A Cosmological Model

(I) It is familiar that the Einstein equations for the density and pressure in a uniform universe:

$$8\pi G\rho = \frac{3}{R^2}(kc^2 + R'^2) - \Lambda \qquad (1)$$

$$8\pi G\frac{p}{c^2} = -2\frac{R''}{R} - \frac{R'^2}{R^2} - \frac{kc^2}{R^2} + \Lambda \qquad (2)$$

do not suffice to specify the model since there are two equations and three unknown functions, ρ, p and R. A very common assumption is $p = 0$, but models involving both positive and negative values of p have also been proposed. McCrea[1] pointed out that there is no objection in principle to negative pressure and that the steady-state universe can be interpreted in this way.

The model discussed here belongs to this negative pressure class but with a different 'equation of state'. Its particular interest is that it seems to provide a possibility of linking the cosmological parameters with familiar laboratory constants.

(II) Let us first make:

Hypothesis A :

$$p = -\frac{2}{3}\rho c^2 \qquad (3)$$

If this is inserted in the equation:

$$\frac{d}{dt}(\rho R^3) + \frac{p}{c^2}\frac{dR^3}{dt} = 0 \qquad (4)$$

which is obtained by eliminating Λ between (1) and (2), there results:

$$\frac{d}{dt}(\rho R) = 0$$

so that:

$$\rho R = L \text{ (a constant)} \qquad (5)$$

Following McVittie[2] we introduce the density parameter, σ_0, and the pressure parameter, ε_0, at the instant t_0, defined by:

$$\sigma_0 = \frac{4}{3}\pi G\rho_0 T_0^2,\ \varepsilon_0 = \frac{p_0}{\rho_0 c^2},\ T_0 = \frac{R_0}{R'_0} \qquad (6)$$

and also the acceleration factor $q_0 = -R''_0R_0/R'^2_0$. Thus equation (3) gives $\varepsilon_0 = -2/3$ and the Einstein equations can be written:

$$\Lambda = -3(q_0 + \sigma_0)T_0^{-2} \qquad (7)$$

$$k\left(\frac{cT_0}{R_0}\right)^2 = -(q_0 + 1 - \sigma_0) \qquad (8)$$

In these, q_0, σ_0 and T_0 can be regarded as having numerical values derived from observation. The equation corresponding to equation (7) in the zero-pressure model is:

$$\Lambda = -3(q_0 - \sigma_0)T_0^{-2} \qquad (7)'$$

Comparison of equations (7) and (7)′ brings out an important difference. In equation (7)′ it is possible for Λ to be zero or positive for positive values of q_0 and σ_0, and in fact it is widely assumed that $\Lambda = 0$. With equation (7) this is not possible. If q_0 and σ_0 are positive then Λ is certainly negative.

If, as seems very likely, $q_0 + 1 > \sigma_0$, the left-hand side of equation (8) must be negative so that $k = -1$. Thus:

$$\left(\frac{cT_0}{R_0}\right)^2 = q_0 + 1 - \sigma_0 \qquad (9)$$

and this, combined with equations (5) and (6), gives:

$$\frac{\sigma_0}{\sqrt{(q_0 + 1 - \sigma_0)}} = \frac{4}{3}\pi\frac{G}{c}LT_0 \qquad (10)$$

Reverting to equation (1), this can now be written:

$$R'^2 = c^2 + \frac{8\pi}{3}GLR - \frac{1}{3}(-\Lambda)R^2 \qquad (11)$$

which represents an oscillating hyperbolic universe.

If we introduce homogeneous variables $Y = R/R_0$, $X = t/T_0$, then equation (11) can be written:

$$\left(\frac{dY}{dX}\right)^2 = q_0 + 1 - \sigma_0 + 2\sigma_0 Y - (q_0 + \sigma_0)Y^2 \equiv \varphi(Y) \qquad (12)$$

and the age of this universe is AT_0, where:

$$A = \int_0^1 \{\varphi(Y)\}^{-\frac{1}{2}}\,dY = \frac{1}{\sqrt{(q_0 + \sigma_0)}}\left\{\sin^{-1}\frac{q_0}{\sqrt{(q_0^2 + q_0 + \sigma_0)}} + \sin^{-1}\frac{\sigma_0}{\sqrt{(q_0^2 + q_0 + \sigma_0)}}\right\} \qquad (13)$$

If, for example, $q_0 = 1{\cdot}85$ and σ_0 is small in comparison, A will be about 0·69.

(III) I am not competent to discuss the observational aspects, but I suspect that it might prove difficult to discriminate between the present model and a cognate zero-pressure one. The observational properties of the latter are closely bound up with the function:

$$I(z) = \int_0^z dz\ \{1 + 2(q_0 + 1)z + (q_0 + 1 + 3\sigma_0)z^2 + 2\sigma_0 z^3\}^{-\frac{1}{2}}$$

The corresponding function in the present theory is:

$$J(z) = \int_0^z dz\ \{1 + 2(q_0 + 1)z + (q_0 + 1 - \sigma_0)z^2\}^{-\frac{1}{2}}$$

which can, of course, be expressed in terms of elementary functions. If it is the case that q_0 is considerably larger than σ_0, it would not be easy to distinguish between $I(z)$ and $J(z)$ for the range of values of z (the red shift) which have so far been observed.

(IV) We now introduce a second assumption.

Hypothesis B. Equation (5) holds also for the electron, with the same constant L.

The negative pressure in this case would be a tension counteracting the electrostatic tendency of the electron to fly apart. The resulting energy is of the form $\frac{4}{3}\pi R^3.LR^{-1}c^2$ and so proportional to R^2. Such an energy, interpreted as being due to surface tension, has recently been proposed by Dirac[3] in his theory of the muon as an excited state of the electron.

Hypothesis B gives:

$$L = \left(m\Big/\frac{4}{3}\pi a^3\right)a = \frac{3}{4\pi}\frac{m}{a^2},$$

where m and a are the mass and radius of the electron. Thus, in equation (10):

$$\frac{\sigma_0}{\sqrt{(q_0 + 1 - \sigma_0)}} = \frac{Gm}{a^2c}T_0 \qquad (14)$$

The radius of the electron is a theoretical concept and it is not entirely clear what value should be attached to it. We adopt Dirac's expression, $3e^2/4mc^2$. Inserting numerical values, equation (14) becomes:

$$T_0 = 2 \times 10^{19}\frac{\sigma_0}{\sqrt{(q_0 + 1 - \sigma_0)}}\ \text{sec} \qquad (15)$$

In terms of Hubble's parameter H, expressed as usual in km/sec per megaparsec, this is:

$$H = 1{\cdot}54\ \frac{\sqrt{(q_0 + 1 - \sigma_0)}}{\sigma_0} \qquad (16)$$

and this appears to be consistent with observation, for example, $H = 75$, $q_0 = 1{\cdot}85$, gives $\sigma_0 = 0{\cdot}03$, which is in the permitted range[4].

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PHYSICS

Gravitational Deflexion of an Electromagnetic Wave

It is well known[1] that the velocity of propagation of an electromagnetic signal is governed by the boundary conditions imposed by the guiding structure, the type of modulation and the characteristics of the detector. It is possible that the velocity associated with an unmodulated transverse electromagnetic wave in free space may not be unique.

The deflexion of a light beam as it passes the Sun is calculated on Newtonian theory as:

$$\theta = \frac{2M}{av^2}$$

in astronomical units, where v is the velocity of the light corpuscles at infinity, M is the mass of the Sun, and a is the radial distance. For $v = c = 1$ this expression gives a deflexion equal to half that given by general relativity.

I have previously shown[2–4] that the constant velocity of light relative to any frame of reference may, in the steady state, be considered as a boundary condition imposed by the material boundary of the frame of reference. Physically, any electromagnetic energy arriving from a moving source may be considered to be guided in at c by the energy stored in the near fields associated with the current induced in the receiving aerial. Consider now the power density, P_r, of a wave transmitted into space by a dipole aerial. The distant field may be expressed in the usual form:

$$P_r = \frac{K}{r^2}(1 - \sin 2\omega t)$$

where K is a constant. The net transmitted power flow is associated with the first term inside the bracket and the velocity of propagation of this energy is c. If, however, this transmitted power is inherently guided by the stored energy component associated with the second term in the bracket, then it is the Sun's gravitational interaction with this stored energy term which is relevant to determining the deflexion of a light beam. The root-mean-square velocity, along the radius vector, associated with this stored energy term is simply $c/\sqrt{2}$ and the deflexion of a light beam is then:

$$\theta = \frac{4M}{a}$$

the same result as is predicted by general relativity.

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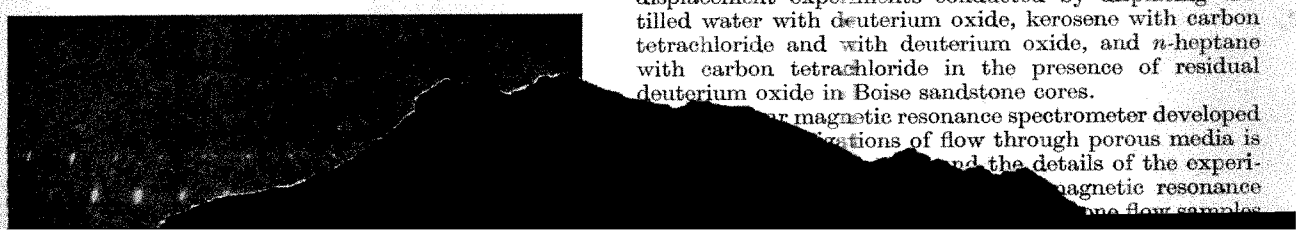
Epitaxial Deposition of Cadmium Selenide

In the past few years there has been considerable interest in the use of evaporated films of cadmium selenide (CdSe) for thin-film transistors and photoconductive devices[1]. As normally prepared, such films consist of polycrystalline material which has a rather poor electron mobility.

We have found that it is possible to produce large-area single-crystal films of cadmium selenide in a conventional evaporator by using mica as an orientating substrate.

Photoconductive grade cadmium selenide is evaporated from an alumina boat on to freshly cleaved mica in an ambient pressure of 10^{-5}–10^{-4} torr. The mica substrate is mounted in an almost enclosed oven to give a uniform substrate temperature, and the cadmium selenide film thickness is monitored by optical reflexion through the substrate. Initial nucleation is performed at a temperature of 200° C, and evaporation is continued until the reflectivity has increased by about 20 per cent. The evaporation is then interrupted, the substrate temperature raised to 400° C, and the evaporation completed. Deposition rates of 100–150 Å/min are used at this latter stage, and the films built up to a total thickness of 750–3000 Å. Thicker films than this tend to flake off the substrate.

To illustrate the single-crystal nature of the films, Fig. 1 shows a transmission electron diffraction pattern of a 750 Å film. The whole area of the film produced (1·8 cm diam.) is single-crystal.



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Fluid Displacement Phenomena in Porous Media observed by a Wide-line Nuclear Magnetic Resonance Method

Laboratory investigations of fluids moving through porous materials have been handicapped by the lack of direct methods of measuring fluid content without disturbing flow. Investigations of flow in porous media have been dependent on the properties of the particular tracer used to follow the flow. Experiments using labelled fluid have been criticized on the grounds that the movement of the tracer may not completely reflect the movement of the fluid.

The problem of measuring fluid content in porous materials has been studied extensively, however, and several methods have been developed through the use of various techniques, including electrical resistivity, X-ray absorption, radioactive tracers, neutron diffraction, magnetic susceptibility, and γ-ray absorption.

A new experimental method, based on nuclear magnetic resonance principles, which is free from the inherent limitations of the foregoing methods, was developed for examination of flow of fluids through porous media[1,2]. The sample to be investigated is placed within a coil and the amount of hydrogen-containing liquid is obtained directly by measuring the number of hydrogen atoms present inside this coil. No additional tracer is needed, since each hydrogen nucleus acts as its own tracer.

This method depends on the observation that if a sample containing nuclei with spin quantum number other than zero is placed in a direct-current magnetic field and is simultaneously irradiated by a small radio-frequency field rotating in a plane perpendicular to the steady magnetic field, the nuclei absorb radio-frequency energy at unique resonant frequencies proportional to the magnetic field strength[3]. The amount of energy absorbed by a liquid sample containing hydrogen atoms in its chemical constitution (such as water or hydrocarbon) is a measure of the number of hydrogen atoms in the observed volume.

In situ determination of fluid saturations in a miscible displacement experiment was performed for the first time without addition of a tracer through use of the nuclear magnetic resonance apparatus[1,2] and has already been reported[4]. This communication presents the results of detailed investigations of several miscible and immiscible displacement experiments conducted by displacing distilled water with deuterium oxide, kerosene with carbon tetrachloride and with deuterium oxide, and n-heptane with carbon tetrachloride in the presence of residual deuterium oxide in Boise sandstone cores.

...r magnetic resonance spectrometer developed ...tions of flow through porous media is ...nd the details of the experi- ...agnetic resonance ...ne flow samples

1 in. diameter) of the nuclear magnetic resonance apparatus. The nuclear resonance absorption spectrum was first observed under steady-state conditions and the derivative of the nuclear magnetic resonance absorption curve was obtained[2]. Then, the hydrogen-containing liquid was displaced vertically, from the bottom up, by a hydrogen-free liquid with a frontal velocity of the order of 10^{-3} cm/sec. The derivatives of the nuclear magnetic resonance absorption curves were obtained as the front between the hydrogen-containing and the hydrogen-free liquids was passing through the section at which the sample coil was located.

Since the nuclear magnetic resonance absorption signal amplitude is directly proportional to the number of hydrogen atoms present in the observed volume, provided the line width is the same, these experimentally obtained derivative curves were numerically integrated to obtain the nuclear magnetic resonance absorption signal amplitudes. The initial signal amplitude corresponds to 100 per cent saturation of the hydrogen-containing liquid. The saturation at subsequent intervals of injected pore volume were calculated by comparing the corresponding signal amplitudes with the initial signal amplitude.

In these experiments the line widths of nuclear magnetic resonance absorption curves were essentially the same, hence the signal amplitudes were used for comparison. In cases where the line width might change due to the wettability characteristics of the porous medium or changes in the viscosity of hydrogen-containing fluid, the area underneath the nuclear magnetic resonance absorption curve should be taken to be directly proportional to the number of hydrogen atoms present in the observed volume.

Using the procedure described, miscible displacement of distilled water with deuterium oxide (a hydrogen-free liquid) was observed. Although both the viscosity and the density ratios between distilled water and deuterium oxide, 0·80 and 0·90, respectively, were favourable, the concentration profile obtained was slightly asymmetric (Fig. 1*a*). This tailing of the breakthrough curve cannot be described by the one parameter diffusion model[4], which predicts a perfect S-shaped concentration profile having the 50 per cent concentration at one pore volume. Use of either two- or three-parameter dispersion models[5] to describe this concentration profile may indicate the presence of dead-end pores[6] or stagnant volume[5,7] in the Boise sandstone.

The effect of unfavourable viscosity ratio, 2·27, on the miscible displacement profile was observed by displacing kerosene with carbon tetrachloride. Viscous fingering of carbon tetrachloride is indicated by the early breakthrough and the greatly extended shape of the concentration profile (Fig. 1*b*).

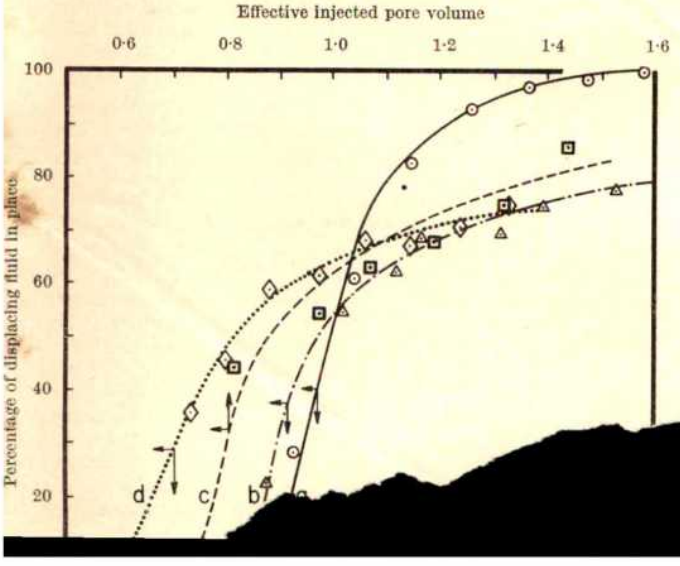
In order to investigate a multi-phase fluid flow in a porous medium, a sandstone sample was saturated with deuterium oxide which was in turn displaced with *n*-heptane until a residual amount was left (this is indicated when the nuclear magnetic resonance absorption signal amplitudes stop increasing). The residual deuterium oxide saturation, 21 per cent, was measured by obtaining a nuclear magnetic resonance absorption signal from this flow sample and comparing its amplitude with that obtained from a similar sample with 100 per cent heptane saturation. Then, *n*-heptane was displaced with carbon tetrachloride and a highly irregular concentration profile was observed (Fig. 1*c*). Since this curve is plotted on an effective pore volume basis (79 per cent of the total pore volume), it is apparent from Fig. 1*c* that the presence of deuterium oxide has more effect than merely changing the porosity available to flow. The irregular shape of the concentration profile rather indicates a change in the pore size distribution caused by the presence of deuterium oxide. Since deuterium oxide is present in the core as the wetting phase, the non-wetting phase, *n*-heptane, can occupy only the larger pores, thereby resulting in a dendritic, non-wetting phase fluid distribution in which the large volumes of *n*-heptane are trapped in non-communicating blocks[6].

For a study of immiscible displacement, kerosene was displaced with deuterium oxide. A very early breakthrough of deuterium oxide did occur and its saturation increased steadily as more and more viscous fingers (viscosity ratio of 1·75) reached the sample coil (Fig. 1*d*).

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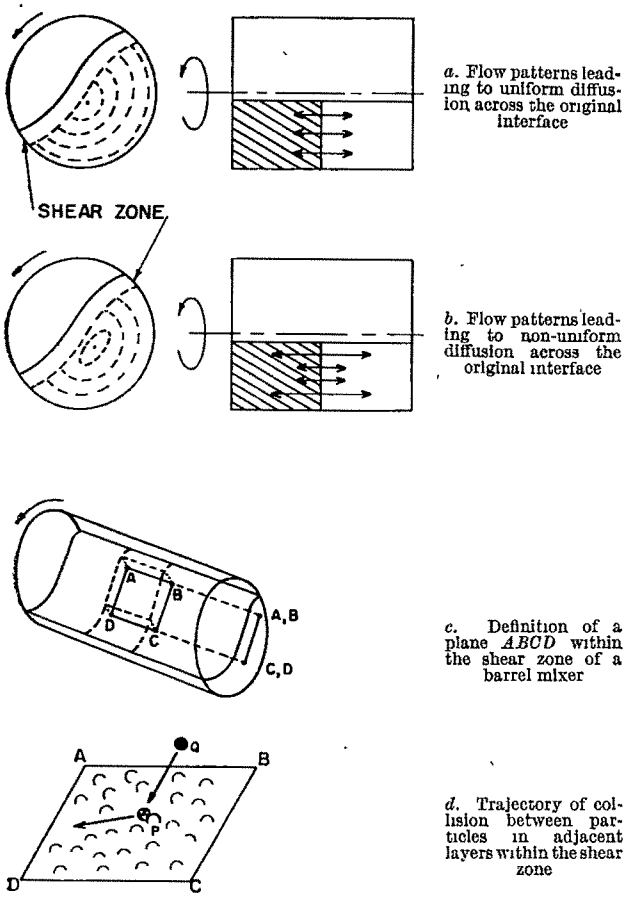
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[illegible]anism of Solid–Solid Mixing

[illegible] materials it is often stated [illegible] the process is the



a. Flow patterns leading to uniform diffusion across the original interface

b. Flow patterns leading to non-uniform diffusion across the original interface

c. Definition of a plane *ABCD* within the shear zone of a barrel mixer

d. Trajectory of collision between particles in adjacent layers within the shear zone

Fig. 1. Schematic representation of the diffusional mechanism of solid–solid mixing

process, regions of high concentration of one component remain, then such an aggregate may be too small to be segmented by the action of the mixer in a finite time, and diffusion out of the aggregate must take place before final mixing can occur.

In a barrel mixer, loaded axially as shown in Fig. 1*a*, the observed diffusion is a result of the particles acquiring a component of motion along the axis of the mixer. Figs. 1*a* and 1*b* illustrate the shear zone and two possible motions of the diffusion 'front' during mixing. (The diffusion 'front' is any hypothetical plane of constant composition perpendicular to the axis of rotation which signifies the progress of mixing at a given time.) If regions exist in the mixer in which a finite number of particles do not move into the shear zone, the diffusion 'front' will not be uniform. Thus, it must be established whether or not there are any systematic variations in concentration on any plane perpendicular to the axis of the mixer.

An idealized system of white and yellow beads, 200μ in diameter, identical in size, shape and densit[redacted] diff[redacted] in refractive index by 0·03 units, was ob[redacted] investigation. This permitted ready analy[redacted] taken from the mixer bed. These beads[redacted] axially in a simple barrel mixer, 4-in. diamet[redacted] length, constructed of 'Lucite'. Samples[redacted]

Table 1. Illustration of 'Plane Front' Diffusion in an Ideal System of Particles loaded axially in a Barrel Mixer

No. of revolutions	$\sigma^2_{NS}-\sigma^2_{RS}$
2,000	0·05
4,000	1·52
7,000	1·48
10,000	0·62
20,000	0·82
30,000	−1·33
50,000	−1·99
100,000	2·02

the observed and random variances are shown in Table 1. Thus, no systematic variation in concentration in a plane perpendicular to the mixer axis was observed in this system. It now remains to describe the particle motion in the mixer that leads to the observed diffusion as a plane front.

The principal mixing zones within a barrel mixer are shown schematically in Fig. 1*a*. Consider a plane *ABCD*, as shown in Fig. 1*c*, located within the shear zone of the mixture. Visual observations of motion in the mixer reveal that the great majority of particles enter this shear zone during each revolution of the mixer. The photographic investigation of mixing reported by Carley-Macauly and Donald (ref. 3, Fig. 12) illustrates this motion into the shear zone. Distributed at random across the plane *ABCD* are particles which may be considered to be half-spheres. For simplicity, consider that the centres of the particles all lie in the plane *ABCD*. Due to the gradient in the shear zone, particles passing over the plane *ABCD* will collide with those in the plane and are likely to be deflected. The deflexions result in a displacement of the particles parallel to the mixer axis. This is shown schematically in Fig. 1*d*, in which particle *Q* after collision with particle *P* moves in a direction along the mixer axis and into a 'hole' in the random assembly of particles.

The randomness of position within the assembly of moving particles and the action of a shear gradient are together the necessary and sufficient conditions to generate random motion of a particle in a mixer. Since one component of motion is parallel to the mixer axis, net diffusion will take place. As almost every particle enters the shear zone during each revolution, on the average, the diffusion 'front' is at all times a plane parallel to the original interface between the components of the mixture in an ideal system. Note that early investigations[4] revealed that density differences between components of the mix lead to collisions in the shear zone in which the resultant motion of the particles is predominantly toward or away from the centre of mass of the rotating charge.

Recent experimental analysis by Kaye and Sparrow[5] utilized a system of balls flowing down a stationary inclined plane in an attempt to simulate motion within a rotating mixer. They interpreted their results in terms of diffusion in the limited surface region, rather than in terms of diffusion within the more extensive shear zone, as shown in Fig. 1*d*. The results shown in Table 1 show that diffusion in solid–solid mixing cannot, as Kaye and Sparrow[5] suggest, be limited to a depth of eight or nine [redacted]meters from the free surface, but must occur [redacted] whole shear zone into which the great [redacted]les enter during each revolution. In the [redacted]d materials, limitation of diffusive [redacted]egion is unrealistic, since the shear [redacted] particle diameters in from the

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GEOPHYSICS

Munro Jets and the Origin of Tektites

The theory of Spencer[1] that tektites splashed out of terrestrial craters met with support from Cohen[2], Gentner and Zähringer[3] and others, but also with objections, summarized by O'Keefe[4]. The main objection was that the air resistance of bodies as small as tektites on their upward flight is too large to allow them to escape from the crater at the velocities (2–8 km/sec) required for their observed ranges. Vand[5,6] proposed a detailed mechanism of a tektite jet formation through an implosion of a conical cavity lined with molten glass which is formed immediately after impact, and showed that a jet of tektites can survive the upward atmospheric journey if of sufficient total mass; that is, if it originated in a crater more than some 4 km in diameter. The escape is further facilitated by the low density of the hot train left after the passage of the meteorite. The high-velocity jet overtakes the general debris and keeps tektites from crystalline contamination. If moldavites are from the 24 km diameter Ries Kessel crater in Germany, the tektite jet from an initial conical cavity 4 km deep and 1 km in diameter would be a cylinder about 5 km long and 40 m in diameter, moving initially at 2·36 km/sec and carrying some 10^{10} kg glass with it. The original of the required cone wall implosion velocity of about 0·15 km/sec was not well understood, as such a phenomenon had not then been observed in laboratory cratering experiments, and gravity collapse could only account for part of the velocity.

However, the work of Watson and Gibson[7] on jets formed from imploding bubbles seems adequately to elucidate the operating mechanism. Photographs are given of beautiful Munro jets formed when hemispherical cavities on the surface of a liquid are collapsed by a shock-wave travelling from below. Shock strength of some 10 kbar produces 1·9 km/sec Munro jets from cavities in water.

After a meteoritic impact, a travelling sh[illegible] be reflected upwards from underlying d[illegible] Reflexion and refraction of shock-wav[illegible] phenomena. For acoustic waves[illegible] travelling from medium 1 to m[illegible]

Typical boundaries which may be encountered under terrestrial conditions at smaller depths are those of granite (d = 2·65, c = 5·5 km/sec) over diorite (d = 2·9, c = 6·5 km/sec) or at depths of some 40 km (Mohorovičić discontinuity) of diorite over dunite (d = 3·4, c = 7·75 km/sec). We find for P_r/P_i for the above two cases 0·12 and 0·16 respectively.

Assuming that Ries Kessel was formed by an iron meteorite 1 km in diameter travelling at 15 km/sec and having kinetic energy equivalent to 10^5 megaton TNT, we find by analogy with nuclear explosions that a 100 kbar shock would be found at 2·5 km from the blast centre. According to Shoemaker[8], the blast centre occurs at 4 to 5 meteorite lengths after ground penetration. Assuming that the meteorite arrived at an angle 30° from horizontal, as required by the range of moldavites, the centre of explosion would be at 2 km depth and therefore a downward travelling 100 kbar shock would be found at 4·5 km depth. If at this depth there happened to be a discontinuity reflecting about 15 per cent of pressure, an upward travelling 15 kbar wave would form, which would attenuate to 10 kbar when reaching the cavity.

The implosion velocity which occurs on the arrival of the reflected wave is a spalling effect due to its reflexion at the free cavity interface. The implosion velocity at the free interface is then twice the instantaneous particle velocity, which is given by $v = P_s/c\,d$, where c and d are the velocity of propagation of the longitudinal wave and density at shock pressure P_s. Substituting for glass d = 2·5, c = 5 km/sec, P_s = 10 kbar, we obtain v = 75 m/sec or the implosion velocity $2v$ = 0·15 km/sec, which is just the required value for the cavity collapse to generate a backsplash jet of the required velocity.

We may further speculate that the general inward motion of other crater materials caused by the passage of waves reflected from below will also be responsible for the formation of a central peak in some craters, if underlain by denser strata at favourable depth. In fact, the central peak of Steinheim basin may have been formed through reflexion at the underlying limestone-granite boundary, and the peaks of certain other large craters through reflexion from Mohorovičić discontinuity. Similar interpretation of central peaks of lunar craters might help in mapping such discontinuities under the lunar surface.

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GEOLOGY

of Silcretes of Central Australia

[illegible], Stephens[1] has suggested that the wide-[illegible]tes of Central Australia can be explained by a [illegible]ree events: (1) mobilization of silica by

Although Stephens's concept of linking the lateritic leaching processes in one great region with the deposition of silica in the adjacent one is appealing in its simplicity, severe doubts come to mind if one attempts to visualize the process of fairly regular distribution of the silica over a surface, measuring hundreds of miles across, by means of streams and rivers. No planation surface is so featureless as to allow complete, or major, inundations by rivers, and even during great floods, rivers would, therefore, tend to follow particular linear trends along which the silica supplied and eventually precipitated would have to be confined. Thus, if rivers flowing across the region actually did contribute a major amount of allochthonous silica to the formation of the silcrete sheet, this could only have happened by feeding the dissolved silica into the groundwater and by its further slow distribution and rise with this latter agent away from the river courses. It is more likely, however, that river contributions of silica were subordinate or negligible, and that the formation of the siliceous duricrust followed, predominantly or entirely, the pattern generally accepted for the genesis of such surficial encrustations, whether ferruginous, aluminous, manganiferous, calcareous, or siliceous—mobilization of the substances concerned from the bedrock (or parent material) *in situ* by chemical weathering, and precipitation after movement over very short, or at best moderate, distances in the groundwater, usually by means of evaporation[2]. It would be necessary, therefore, to make allowance, in the silcrete areas, for a period during which the climatic conditions were just right for both mobilization and precipitation of silica to occur more or less *in situ*. A climate drier than the equatorial one with its lateritic weathering processes would presumably have to be envisaged.

My own experience with duricrusts was essentially gained in West Africa, and although I was predominantly concerned there with ferruginous and aluminous types[3,4], I have made some observations on siliceous precipitates which support the generally accepted view outlined here. These investigations will be published separately.

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I take Prof. Brückner's remarks to mean that he almost exclusively favours the hypothesis of relative accumulation of silica within the profile in which it has been released by weathering. Examples of this are widespread in Australia for both laterite and silcrete, but in the case of the Central Australian silcretes the general and detailed evidence is markedly in favour of absolute accumulation following lateral movement in drainage waters. In addition to the general evidence set out in my letter the following details are significant. (1) Water-worn quartz gravels are frequently found in the matrix of silcrete. (2) Water-worn silcrete from some higher residuals is often included in the silcrete of lower surfaces, thus pointing to a complex erosional and depositional history rather than simple widespread planation. (3) Some of the silcrete overlies gypseous, calcareous and alunitic lacustrine deposits from which it is most unlikely to have been derived. (4) The present ephemeral stream pattern and the associated soils and rock exposure taken together with the higher situated water-worn quartz gravels point to topographic inversion of a landscape in which the originally lower silicified surfaces are now preserved as the higher topographic elements.

It is my intention to complete, during 1966, a monograph giving the results of an intermittent, but very prolonged, investigation of Australian laterites and silcretes and their relationships.

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Cromer Forest Bed Series

During the past seven years much new evidence has been obtained concerning the stratigraphy of the Cromer Forest Bed Series of the Norfolk and Suffolk coasts, and this requires the sequence put forward by Clement Reid[1,2] to be revised. These new investigations have not yet been completed and it will be some time before the full stratigraphical and palaeoecological results will be available. But because of the importance of the Cromer Forest Bed Series for the European Middle Pleistocene succession it will be of general interest to give a simplified description of our provisional stratigraphical results.

Reid's classic Cromer Forest Bed Series succession is shown in Table 1.

Table 1. Clement Reid's Sequence of the Cromer Forest Bed Series and Related Deposits

Cromer Till (First Till)	
Arctic Freshwater Bed	
Leda-myalis Bed	
Upper Freshwater Bed	Cromer Forest Bed Series
Forest Bed (estuarine)	
Lower Freshwater Bed	
Weybourne Crag	

The only emendation that this sequence has received since the nineteenth century is Solomon's[3] suggestion that the Arctic Freshwater Bed precedes the *Leda-myalis* Bed.

The new stratigraphical evidence is summarized in the sketch-sections of Figs. 1 and 2 of the coastal exposures at West Runton and Mundesley. A lower temperate series, principally estuarine sediments (*a*), is overlain by sands and gravels (*b*), often ferruginous near their surface and forming iron pan. These gravels are often associated with clay-conglomerates derived by re-working of the lower estuarine beds. Arctic freshwater beds (*c*) are associated with these gravels at West Runton, and ice-wedge casts and involutions occur within them. Freshwater late-glacial marls and silts (*d*) lie on these beds and then come the temperate organic mud beds (*e*) forming the so-called 'Upper Freshwater Beds' at West Runton. These temperate freshwater beds are the type sediments of the European Cromerian Stage[4]. The soil (*f*) of this stage extends north and south of the main exposure at Goss' Gap, West Runton. Along this stretch of the coast the soil and the peat are covered by estuarine silts, gravels and sands (*g*) of a marine transgression which occurred in the latter half of the Cromerian. The well-known bed of *Mya truncata in situ* at West Runton, noted by Trimmer[5],

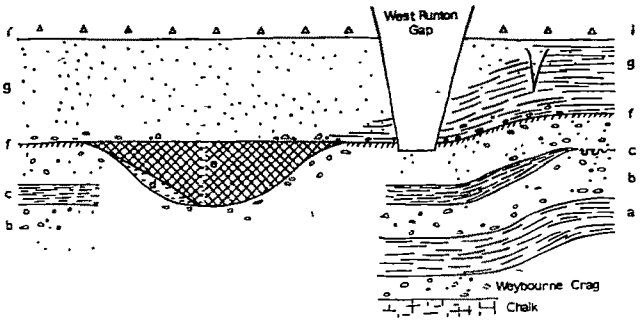


Fig. 1. Sketch section at West Runton, about 400 m length and 7 m depth. Key to letters in Table 2

appears to be the soil of the Cromerian land surface bored by *Mya* in the first stages of the transgression. At Mundesley the transgression silts and beach gravels of the same transgression cover thin temperate peats of the Cromerian freshwater episode, which lie in hollows of the ferruginous gravels and sands of horizon (*b*). Similar transgression silts occur at Corton in Suffolk overlying the freshwater 'Rootlet Bed', which again lies on ferruginous gravel.

At Mundesley, freshwater sediments (*h*) again occur over the transgression deposits. They are arctic in character and are associated with ice-wedge casts and involutions. Similar ice-wedge casts penetrate the transgression deposits at Corton, and at West Runton. The succession is capped by the lowest till (*i*) of the coast sections, representing the first ice advance over East Anglia.

Regarding the correlation of these temperate and cold horizons, the lower temperate series may be equivalent in age to the topmost temperate horizon (*L*5) of the Ludham borehole[6]. This temperate stage is provisionally named 'Pastonian' as the sediments occur on the foreshore and in boreholes opposite Paston Cliffs, near Mundesley.

Table 2

Stage	Deposit (letters refer to Figs. 1 and 2)	Environment	Identified by C. Reid as:
Lowestoftian	*i* Till	Glacial	Cromer Till
	h Mossy silts, sands	Cold freshwater	Arctic Freshwater Bed
	Ice-wedge casts and involutions	Permafrost	
Cromerian	*g* Silts, sands	Temperate estuarine, marine	*Leda-myalis* Bed
			Forest Bed (estuarine)
	f Soil	Temperate	
	e Muds, peats	Temperate freshwater	Upper Freshwater Bed
Beestonian	*d* Silty marls	Late-glacial freshwater	
	c Silts	Cold freshwater	Arctic Freshwater Bed
	b Gravels, sands, clay-conglomerates	Cold	Forest Bed (estuarine)
	Ice-wedge casts and involutions	Permafrost	
Pastonian	*a* Silts and sands	Temperate estuarine, marine	Weybourne Crag

The middle cold horizon appears to be a hitherto unrecorded cold stage, here named the 'Beestonian' because the characteristic arctic beds of the stage are well exposed in the Beeston Cliffs, near Sheringham. The upper temperate horizon, including both freshwater and estuarine facies, must be included in the Cromerian Stage by definition. The stage names and their boundaries shown in Table 2 are given according to the system described by West[7].

Reid's divisions are not everywhere comparable with those of the sequence described here. Table 2 shows the general relation of the two sequences as far as possible: we have not included in this the Lower Freshwater Bed, as the identity of this is not yet certain. It will be best to abandon the names suggested by Reid, as neither the sequence nor the divisions can be maintained.

This work has been supported by a Nature Conservancy grant. We thank the Paramoudra Club of Norwich and Mr. R. A. Markham of Ipswich Museum for their help in excavations made at West Runton.

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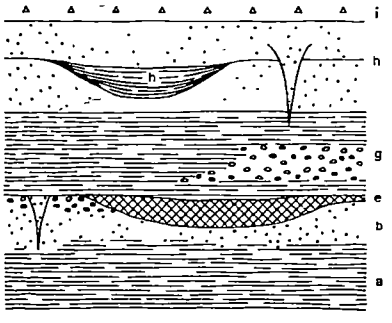


Fig 2. Composite section at Mundesley, about 6 m depth. Key to letters in Table 2

[1] Reid, C., Geology of Cromer, *Mem. Geol. Surv. England and Wales* (1882).
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CRYSTALLOGRAPHY

Nature of the Twinning in the Lower Strontium Soaps

Morley and Vand[1] have proposed the space group $P2_1/n$ for strontium laurate. During the investigation of the structure of strontium caprylate I found[2] that the crystals were all twinned and upper-layer Weissenberg and precession photographs showed conclusively that the crystals were triclinic with the remarkable feature of $\beta^* = 90°$.

Crystals of strontium caprate and strontium laurate, prepared by metathesis, have now been grown by slow cooling of aqueous solutions. The crystals so formed are rather less perfect than those of the caprylate but are quite good enough to confirm that all three soaps are of the same form; all are triclinic and in all cases $\beta^* = 90°$.

The twinning shows up clearly in the [100] projections of the soaps, where the two members of the twin have a common c^* axis. In strontium laurate the angle between the c^* and b^* axes (γ^*) is such that the reflexions from the two members of the twin with k even coincide exactly. A diagram of the composite reciprocal lattice plane is shown in Fig. 1. In the caprate and caprylate, although the twinning reveals itself in the same way, the coincidence of the reflexions on the rows when k is even is no longer exact.

The structures exhibited some unusual symmetries. The [100] projection shows that the Fourier transform of the unit cell contents contains a mirror plane but, since the reciprocal net is non-orthogonal, the weighted reciprocal lattice itself does not exhibit the mirror plane.

The probable mode of twinning is across the (001) planes at the ends of the hydrocarbon chains. In this plane, remote from the ionic groups, there will be little difference in energy between the two orientations. Fig.

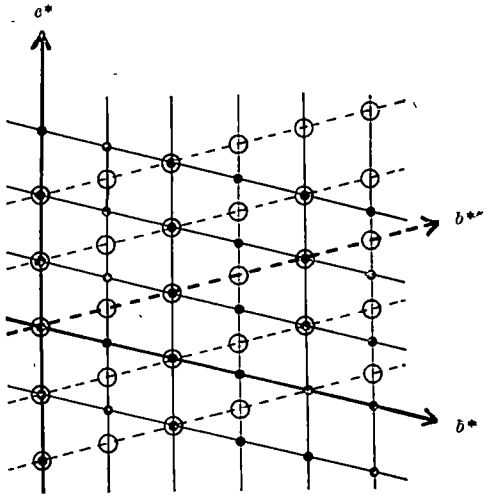


Fig. 1. The composite reciprocal lattice of the [100] projection

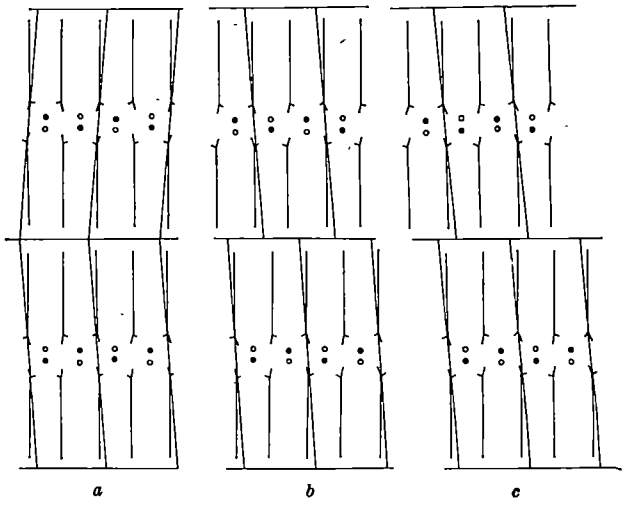


Fig. 2 *a*, The twinning mode; *b*, stacking fault with a slip of *b*/2; *c*, stacking fault with a slip of *b*/4

2*a* shows a sketch of the unit cell and the twinning mode. Since the angle β*, that is, the angle between the (100) and (001) planes, is exactly 90° the two lattices of the members of the twin coincide exactly in the [010] projection. It is interesting to note that the mode of twinning preserves the continuity of the lines of apparent symmetry across the twin plane.

In addition to the twinning in all three soaps there is evidence of a stacking fault. This reveals itself in the streaking of some sets of reflexions in the *c** direction. This fault probably originates in the same plane. A shift of *b*/2, as shown in Fig. 2*b*, would bring the ends of the hydrocarbon chains into a similar environment, and the influence of the ionic groups, 12 Å or more away, is likely to be very small. Such a stacking mistake would restrict the streaking to the reflexions when *k* is odd. Alternatively a shift of *b*/4, illustrated in Fig. 2*c*, would result in the continuity of the apparent line of symmetry across the fault plane and only reflexions with $k = 4n$ would be sharp. The first proposed mode agrees better with the observations. There is no streaking at all on the (00*l*) reflexions and the streaking of the (0*kl*) reflexions is greater when *k* is odd, but there is some streaking of the reflexions with *k* even. There is no observable difference in the sharpness of reflexions for which $k = 4n$ and $k = 2(2n + 1)$.

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CHEMISTRY

A C23-type Modification of $ThAs_2$

$ThAs_2$ was reported to crystallize in the tetragonal Cu_2Sb or *C*38 type of structure[1]. In an attempt to synthesize this phase by reacting arsenic with thorium powder at temperatures between 600° and 900° C, a new modification was obtained. This low-temperature form α-$ThAs_2$ is orthorhombic and of the *C*23 type. Its lattice constants are:

$$a = 7{\cdot}287 \pm 3 \text{ Å}$$
$$b = 9{\cdot}784 \pm 4 \text{ Å}$$
$$c = 4{\cdot}002 \pm 2 \text{ Å}$$

The same structure type is also found in $ZrAs_2$ (ref. 2), $HfAs_2$ (ref. 3), TiP_2, ZrP_2 and HfP_2 (ref. 4). The transition to the tetragonal high-temperature modification β-$ThAs_2$ appears to occur at approximately 1,100° C. A sample held at 1,150° C for one day was in parts converted into the *C*38 modification.

Our α-$ThAs_2$ samples showed *p*-type thermoelectric power of 10–20 μV/°C at room temperature and a diamagnetic susceptibility $\chi_M = -47{\cdot}10^{-6}$ cgs units/mole at 80° and 300° K. Both modifications were found to be metallic. This result is not trivial in the case of α-$ThAs_2$. In the ordinary *C*23 type the co-ordination is:

$$M\text{—}5X_I + 4X_{II},\ X_I\text{—}5M,\ X_{II}\text{—}4M$$

Six of the neighbouring anions form a trigonal prism around the cation while the remaining three anions are located outside the square prism faces coplanar with the cation. For large cations, such as Pb^{2+} in lead chloride, this co-ordination leads to a rather dense packing so that one might attribute its occurrence to geometry only. However, three of the nine Cl neighbours of each Pb are considerably closer ($2Cl_I$ at 2·67 Å, $1Cl_{II}$ at 2·89 Å) than the rest (at 3·06, 3·09, 3·14 and 3·30 Å), indicating that the electron configuration of the cation is also of importance: Pb^{2+} readily forms *p* bonds. Hybrid wave functions fitting the actual 9-co-ordination of the cation can be constructed by admixing an *s* and all five *d* orbitals to the *p* functions. If, therefore, in addition to geometry the electronic configuration of the cations is also important, then one would expect the *C*23 structure to occur in connexion with cations which have all five *d* orbitals available for bond formation. To our knowledge, there are no cations which contain localized *d* electrons and show a 9-co-ordination.

Among the non-transition elements, above all Ba^{2+}, but also Pb^{2+}, fit both criteria and it is not surprising, therefore, that their halides are the main representatives of the *C*23 structure. A tetravalent cation is needed in order that the bonds in *C*23-type chalcogenides be saturated, and this is indeed the case in the non-metallic phases ThS_2 and $ThSe_2$. A pentavalent cation such as Pa^{5+} may form PaPS, PaAsS, etc., which we think may be isostructural with Pb(OH)Cl (*C*23-type with X_I and X_{II} being different anions). Extrapolating further one might expect hexavalent cations to be associated with the *C*23-type phosphides and arsenides. This is obviously not the case in the $ZrAs_2$ family of compounds, and their adherence to the *C*23 structure seems rather surprising at first glance. However, the *C*23 structure of the $ZrAs_2$ family differs from the ordinary $PbCl_2$ structure in that the corresponding atomic parameters give rise to bonded zig-zag chains involving half the anions. Ideally, bond saturation may therefore again occur with tetravalent cations, as can be seen from the representative ionic formula $Zr^{4+}As^{3-}As^{-}$. Although the anion–anion distances within the chains in $ZrAs_2$ and $HfAs_2$ are too long to correspond to single bonds, the 'strive' towards bond saturation is nevertheless obvious in these compounds. Due to the larger size of the cation the chain formation will be less pronounced in α-$ThAs_2$. I do not know the exact As–As distance in α-$ThAs_2$ since I did not determine the atomic parameters, but it is probably around 2·7 Å. The metallic behaviour of my samples gives further evidence that the As–As distance within the chains is longer than the single-bond diameter of arsenic (2·42 Å, ref. 5). Since phases, the bonding of which nearly satisfies the conditions for semiconductivity, are often superconductors, α-$ThAs_2$ was also tested for superconductivity properties. However, down to 1·2° K, no transition to the superconducting state was found[6].

As would be expected, the metallic character of the high-temperature modification is more pronounced. Indeed, no anion–anion bonds occur in the *C*38 structure of β-$ThAs_2$ so that saturation of the bonds is impossible. In this connexion it should be noted that in the corresponding isostructural uranium compounds, UP_2, UAs_2

and USb_2, the uranium atoms do not contribute all their six valence electrons necessary to fill up the anion valence shells, and as a consequence these compounds are also metallic.

Attempts to prepare the diphosphide ThP_2 always failed. Instead of ThP_2 almost pure Th_3P_4 of the cubic $D7_3$ type formed, which is a semiconductor like the isomorphous Th_3As_4. It would be expected, however, that ThP_2 exists and that it is isomorphous with α-$ThAs_2$. Six faint low-angle lines which I observed on my X-ray photographs, and which do not belong to the Th_3P_4 pattern, can indeed be attributed to a $C23$ type ThP_2 with the following lattice constants:

$$a = 6{\cdot}95 \text{ Å}$$
$$b = 9{\cdot}42 \text{ Å}$$
$$c = 3{\cdot}90 \text{ Å}$$

I thank Miss E. Viefhaus and Mr. H. U. Boelsterli for assistance in the X-ray work and sample preparation, and Dr. E. Mooser for advice.

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New T_3B_7 Compounds

In the course of a study on transition-element compounds of composition TB_2 (T = transition element, B = phosphorus-group element) two compounds with a body-centred cubic structure were found, a semiconducting one in the system Re–As and a metallic one in the system Mo–Sb. Both compounds formed on reacting, at 600°–900° C, the powdered elements in various ratios. A crude analysis of $ReAs_x$ by thermal dissociation at 1,000° C gave $x \approx 2{\cdot}4$. Our arsenide, therefore, must be identical to the phase $ReAs_{2.3}$ reported by Wiechmann *et al.*[1]. The non-metallic behaviour as well as the diamagnetism of rhenium arsenide confirmed the suspected stoichiometry Re_3As_7 which corresponds to the normal valencies of Re and As. From density measurements, the unit cell [$a = 8{\cdot}722 \pm 2$ Å] was found to contain four formula units of Re_3As_7. Since in the X-ray diagram only the general extinctions for body-centred cubic symmetry are observed, seven space groups are possible. However, the striking resemblance between the diagram of Re_3As_7 and that of Ir_3Ge_7 led me to postulate a $D8_f$ structure for Re_3As_7. Best agreement between calculated and observed intensities was obtained for the following parameter values:

$$x_{Re} = 0{\cdot}338, \quad x_{As} = 0{\cdot}159$$

Assuming the $D8_f$ type to be correct* for Re_3As_7, the coordination is as follows:

Re —4As_{II} in 2·50 Å	As_I — 4Re in 2·60 Å
4As_I in 2·60 Å	
1Re in 2·83 Å	As_{II} — 3Re in 2·50 Å

This proximity is, in fact, reasonable since it makes allowance for saturated covalent bonds as can readily be seen by invoking a charge transfer $Re_3^+As_3^-As_4$ and by allowing half-bonds to form between Re and As_I. The non-metallic character of Re_3As_7 was established by resistivity measurements on sintered samples at temperatures above 300° C. Assuming $\rho \approx \exp(\Delta E/2kT)$, an energy gap $\Delta E \approx 0{\cdot}7$ eV was derived. At room temperature the samples were n-type with a thermoelectric power of about 100 μV/°C. The magnetic susceptibility χ_M was found to be -210 and -250×10^{-6} cgs units/mole at liquid-nitrogen and room temperature, respectively.

As expected, the corresponding technetium arsenide occurs in the same structure with $\alpha = 8{\cdot}702 \pm 2$ Å.

Attempts to prepare the corresponding rhenium antimonide failed. Rhenium did not react with antimony up to 1,300° C. The phosphide Re_3P_7 has a different structure and is isomorphous with $Re_3As_4P_3$ and $Re_3As_3P_4$.

The second compound, Mo_3Sb_7, is metallic and has a practically temperature-independent paramagnetism (+570 and $+580 \times 10^{-6}$ cgs units/mole at 80° and 300°, respectively). The crystallographic data of Mo_3Sb_7 are†:

$a = 9{\cdot}569 \pm 2$ Å, $D8_f$ type with $x_{Mo} = 0{\cdot}341$, $x_{Sb} = 0{\cdot}161$

A survey[2] of all previously known $D8_f$-type compounds (Table 1) shows that they are metallic and that in them the total number of valency electrons per formula unit (counting all outer d electrons of the transition element) lies between 51 and 55. The occurrence of the $D8_f$ structure in Mo_3Sb_7 therefore is less surprising than in the non-metallic arsenides Tc_3As_7 and Re_3As_7, the valency electron number of which is 56.

I have tried to supplement the list of $D8_f$ compounds by preparing a series of binary and ternary phases of adequate composition (see Table 1). These attempts were successful only in a few cases:

$Ir_3Ga_4Ge_3$	: $a = 8{\cdot}702 \pm 3$ Å
$Ir_3In_4Sn_3$	: $a = 9{\cdot}371 \pm 2$ Å
$Ir_3Ga_3Ge_4$	: $a = 8{\cdot}694 \pm 3$ Å
$Ir_3Ga_3Sn_4$	: $a = 9{\cdot}113 \pm 2$ Å
$Ir_3In_3Ge_4$	: $a = 8{\cdot}980 \pm 2$ Å
$Ir_3In_3Sn_4$	: $a = 9{\cdot}369 \pm 2$ Å
$OsIr_2Sn_7$	: $a = 9{\cdot}367 \pm 3$ Å

All my samples were prepared either by sintering the components in the form of pressed powder pellets or by melting, quenching and subsequent annealing at temperatures between 450° and 900° C. Since all $D8_f$ compounds form peritectically, it is possible that these preparation techniques are inadequate in many of the systems investigated and that the failure which I incurred in synthesizing the compounds in parentheses in Table 1 does not necessarily exclude their existence. Fortunately, in the case of Ru_3Sn_7, Ir_3Ge_7, Ir_3Sn_7, $Ir_3Ga_3Sn_4$, $Ir_3In_3Sn_4$ and $Ir_3In_4Sn_3$, the peritectical transformation occurs at a high temperature (between 700° and 900° C). On cooling, the solidified reguli suddenly start to glow, indicating a very rapid exothermal peritectical reaction. In the case of Ni_3Ga_7, on the other hand, the transformation occurs at 250° C (ref. 3) and is accordingly slow.

Table 1. Occurrence of the $D8_f$-type Phases in Relation to the Number of Valency Electrons

Existing phases are underlined; the new ones are marked with an asterisk

45: (Ru_3In_7).
46: (Mo_3Sn_7).
48. (Ir_3Ga_7), (Ir_3In_7).
49: (Re_3Sn_7), ($Ru_3Ga_3Ge_4$), ($Ru_3In_3Sn_4$).
50: (Nb_3Sb_7), (Ta_3Sb_7), ($Mo_3Sn_3Sb_4$).
51: $Ir_3Ga_4Ge_3$*, $Ir_3In_4Sn_3$*, Ni_3Ga_7, Ni_3In_7 Pd_3Ga_7, Pd_3In_7, Pt_3Ga_7, Pt_3In_7, (Ta_3MoSb_7), (Pd_3Al_7), ($Pd_3In_4Tl_3$), (Pd_3Tl_7), ($Au_3Cd_3In_4$).
52: $Co_3Al_3Si_4$, Ru_3Sn_7, $Ir_3Ga_3Ge_4$*, $Ir_3Ga_3Sn_4$*, $Ir_3In_3Ge_4$*, $Ir_3In_3Sn_4$*, ($NbMo_3Sb_7$), (Ru_3Ge_7), (Ru_3Pb_7), (Os_3Ge_7), (Os_3Sn_7), ($Ir_3Al_3Ge_4$), (Pt_3AuIn_7).
53: Mo_3Sb_7*, ($Ta_3Sb_4Te_3$), (Mo_3As_7), (Mo_3Bi_7), (W_3As_7), (W_3Sb_7).
54: $OsIr_2Sn_7$*, ($Pt_3In_4Sn_3$), (Au_3Ga_7), (Au_3In_7).
55: Ir_3Ge_7, Ir_3Sn_7, ($Ru_3Sn_4Sb_3$), (Rh_3Ge_7), (Rh_3Sn_7), (Ir_3Si_7), (Ir_3Pb_7), ($Pd_3Ga_3Ge_4$).
56: Tc_3As_7*, Re_3As_7*, ($Mo_3Sb_4Te_3$), (Re_3Sb_7), ($Ir_3In_3Sb_4$), ($PtIr_3Ge_7$).
57: (Pt_3IrGe_7), ($Au_3Ga_4Ge_3$).
58: ($Ir_3Sn_4Sb_3$), (Pd_3Ge_7), (Pd_3Sn_7), (Pt_3Ge_7), (Pt_3Sn_7).

Note. I was unable to synthesize the phases indicated in parentheses.

I thank Miss E. Viefhaus, Mr. R. Béchade and Mr. H. U. Boelsterli for their help in the X-ray work, the

magnetic measurements and the preparation of the samples, respectively.

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* This structure type has since been confirmed by S. Rundqvist, Uppsala (personal communication)

† After this letter was submitted, a publication of Brown appeared in which he also reported on Mo_3Sb_7, giving $x_{Mo} = 0.338$ and $x_{Sb} = 0.163$ (Brown, A., *Nature*, **206**, 502; 1965).

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Thermoelectric Power and Chemisorbed Hydrogen

Chemisorption and catalysis on palladium have been attributed to holes in the *d* band of the metal[1]. Earlier work has shown that ambient gases change the thermoelectric power of silver[2] and copper[3]. The present communication demonstrates how simultaneous measurements of thermoelectric power and resistance show how chemisorbed hydrogen affects the Fermi-level of palladium at the metal–gas interface.

A 7-mg film of palladium of geometrical area 175 cm² was formed by evaporation of a wire in high-vacuum on to the glass walls of a reaction vessel. After annealing, the film shows a thermoelectric power of $-7{\cdot}5$ μV deg.$^{-1}$ compared with $-9{\cdot}5$ for bulk palladium[4]. Hydrogen gas was admitted to the film at 20° C, and the hydrogen uptake, resistance and the thermopower of the film all measured for various uptakes. Fig. 1 shows that the resistance of the film is raised by chemisorbed hydrogen, as found by Suhrmann[5], and the thermopower changes in the positive direction. Point *M* denotes monolayer coverage estimated for atomic adsorption on the geometric area.

Like Surhmann[5], we assume a surface dipole $Pd^{\delta+}-H^{\delta-}$, on the basis of surface dipole measurements for other transition metals[6]. Such chemisorption will tend to lower the Fermi-level and, assuming the usual density of states picture for palladium, nickel and platinum as in Fig. 2 (ref. 7), this means an increase in $N_d(E)$ since the Fermi-level lies above the maximum. The following two equations for thermopower S and the conductivity σ ($\propto (1/R)$) are relevant[8]:

$$S = -\frac{\pi^2 k^2 T}{3|e|}\left\{\frac{\partial \ln \sigma(E)}{\partial E}\right\}_{E=\zeta} \qquad (1)$$

$$\sigma = \text{const}\ lA = \text{const}\ 'A/N_d(\zeta) \qquad (2)$$

$\sigma(E)$ denotes the conductivity calculated as if the Fermi-level was at energy E, and the differential coefficient is taken at the true Fermi-level $E = \zeta$. The electronic charge is $-|e|$. In equation (2), A denotes the area of the Fermi surface, and l the mean free path of the electrons. A is a measure of the number of electrons at the Fermi surface, which is quite distinct from the total number of electrons discussed in earlier resistance work[5].

According to Mott[7], the current in transition metals is carried by *s* electrons, which are trapped by holes in the *d* band, so that $l \propto 1/N_d(\zeta)$, where $N_d(\zeta)$ is the density of states in the *d* band at the Fermi surface (see also ref. 8, p. 377). Substituting for $\sigma(E)$ in equation (1) gives:

$$S = -\frac{\pi^2 k^2 T}{3|e|}\left\{\frac{\partial \ln A}{\partial E} - \frac{\partial \ln N_d(E)}{\partial E}\right\}_{E=\zeta} \qquad (3)$$

The relatively high resistance of transition metals has been attributed[7] to the $N_d(\zeta)$ factor in equation (2). If we assume this is dominant in changes in S, $\Delta_c S$ due to chemisorption effecting changes in the Fermi-level from $\zeta(M)$ to $\zeta(M^+-H^-)$ in Fig. 2 is given by:

$$\Delta_c S = \frac{\pi^2 k^2 T}{3|e|}\,\Delta_c\left\{\frac{\partial \ln N_d(E)}{\partial E}\right\}_{E=\zeta} \qquad (4)$$

If hydrogen chemisorption raises $N_d(E)$, then according to equation (4) we expect a positive variation of S, and according to equation (2) a decrease in σ (increase in R), both of which are found. We conclude, in contrast to copper[3], that for palladium the main effect of adsorbed gas on electron transport properties is exerted through the scattering or 'mean free path' term. This was really to be expected, if for transition metals the conduction electrons are *s* electrons, since we do not expect much change in A_s (as distinct from A_d), the area of the Fermi surface in the *s* band, with energy E, so the first differential in equation (3) is small. We would point out that, for a transition metal where the Fermi-level lies below the maximum of $N_d(E)$, electron abstraction chemisorption will cause a negative variation of S and a decrease in R.

From the practical point of view the changes in S are some fifty times greater than in R, hence S is a much more sensitive indicator of electronic changes in Fermi-level due to chemisorption. After about one-quarter of the palladium surface is covered by hydrogen, S starts to decrease again. This effect may be due to a change-over from one lattice plane to another. An alternative possibility is a change-over from chemisorbed H^- to H_2^+, as first noted from surface potentials[9]. S may also indicate chemisorption on defects and dislocations if present in sufficient concentration. We have so far made measure-

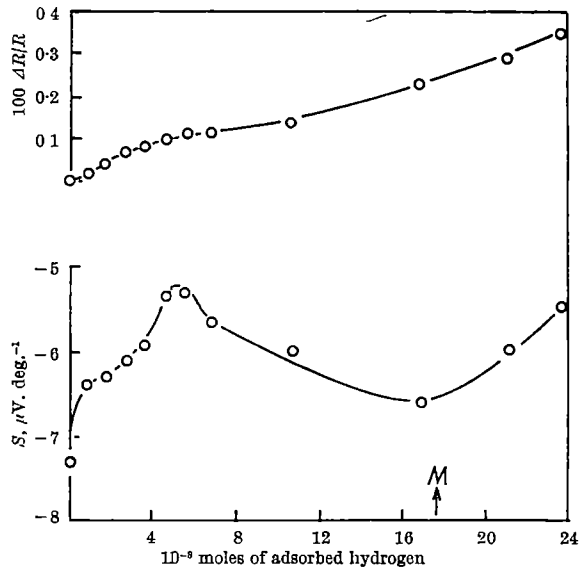


Fig. 1. Percentage change in film resistance $100\Delta R/R$ ($R = 45\Omega$) and changes in thermoelectric power S with 10^{-8} moles of adsorbed hydrogen. *M* denotes the monolayer

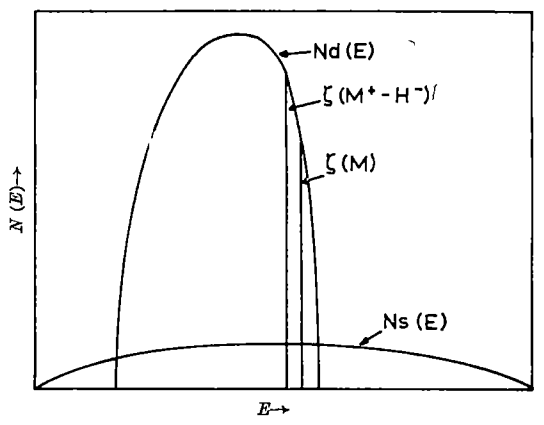


Fig. 2. The density of states diagram for palladium (also nickel, platinum) showing the way the Fermi-level changes from $\zeta(M)$ to $\zeta(M^+-H^-)$ when electrons are transferred from the metal to the chemisorbed hydrogen

ments on palladium, nickel, rhodium and platinum, and we intend to extend our measurements to other metals, and to investigate how the Fermi-level changes during an actual catalytic reaction. The foregoing theoretical treatment with further development should enable us to correlate resistance and magnetic measurements during chemisorption, which has not been satisfactorily achieved to date.

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Study of some of the Parameters involved in Split-stream Chromatographic Injection

The two essential requirements placed on a split-stream injector are that: (1) the injected sample be uniformly distributed in the split stream as the splitting point is reached; (2) the width of the splitted portion entering the column be appreciably smaller than the eventual width of all the eluted components.

It will be assumed in the investigation that follows that the injected sample, if liquid, is not vaporized sluggishly, and that the wall of the mixing tube connecting the injected point to the splitting point does not retain the sample. The first of these effects would interfere with good sample distribution in the split stream, and the second would cause tailing of the portion injected in the column.

Let L, S, D and F designate the length and cross-section of a round mixing tube, the diffusion constant of some component of interest and the volumetric flow of the split stream. The time required for substantial diffusion of a molecule packet across the mixing tube is of the order of S/D and the time $a\,S/D$ will be allotted to this event, a being a 'safety factor' larger than unity intended to allow for nearly completely uniform diffusion of the component in the mixing tube. The velocity of the carrier gas being F/S, the length of an adequately long mixing tube should be at least the product of that velocity times the allotted diffusion time, that is:

$$L \geqq a \frac{S}{D} \cdot \frac{F}{S} = a \frac{F}{D} \qquad (1)$$

This length is notably independent of tube size.

The height equivalent to a theoretical plate (H) of the mixing tube is given by the expression:

$$H = 2\frac{DS}{} + \frac{F}{24\pi D}$$

and since fairly large splitting ratios will be aimed at, for which the velocity will be appreciably higher than that required for minimizing the expression above, H will be given with sufficient accuracy by:

$$H \cong \frac{F}{24\pi D}$$

The space variance of any component distribution after travelling along the mixing tube of length L will be LH and the volumetric variance will be:

$$W = S^2 L H = S^2 a \frac{F}{D} \frac{F}{24\pi D} = \frac{a}{24\pi} \frac{S^2 F^2}{D^2}$$

Let N designate the splitting ratio. The volumetric variance w of the component entering the column will be:

$$w = W = \frac{a}{24\pi} \frac{S^2 F^2}{N^2 D^2}$$

and its space variance will be:

$$\frac{w}{s^2} = \frac{a}{24\pi} \frac{S^2 F^2}{N^2 D^2 s^2}$$

where s designates the open tubular column cross-section. This variance should be appreciably smaller than the variance of the inert component eluting from the column, which is itself smaller than the variance of the retarded components. This variance is, at optimum velocity:

$$l \sqrt{\frac{s}{3\pi}} = \frac{1}{3\pi} n s$$

where l designates the column length, and n its number of plates.

At optimum velocity for any component, the column flow is a fraction $1/c$ of the optimum flow for the inert component, c being usually between 2 and 3:

$$f = \frac{1}{c} D \sqrt{48\pi s}$$

hence:

$$F = Nf = \frac{N D}{c} \sqrt{48\pi s} \qquad (2)$$

and we should have:

$$\frac{a}{24\pi} \frac{S^2 F^2}{N^2 D^2 s^2} = \frac{a S^2 48\pi N^2 D^2 s}{24\pi c^2 N^2 D^2 s^2} \leqq \frac{1}{b} \frac{ns}{3\pi}$$

or:

$$S \leqq c s \sqrt{\frac{n}{6\pi a b}} \qquad (3)$$

where b designates a second 'safety factor', larger than unity, designed to ensure that the main contribution to any band variance is that due to the band passage in the column.

Substituting the value of F from equation (2) in equation (1) we obtain:

$$L \geqq a N \sqrt{48\pi s} \qquad (4)$$

Equations (3) and (4) constitute two essential constraints on the mixing tube, giving as they do an upper bound on its cross-section and a lower bound on its length.

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Stereospecific Catalytic Labelling of Amino-acids and Mandelic Acid with Isotopic Hydrogen

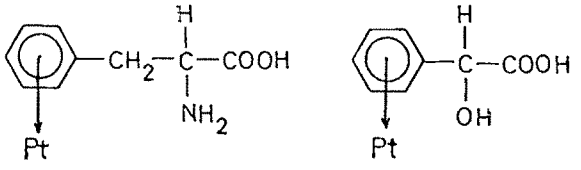
A comparison has been made between the catalytic labelling[1] of organic compounds with deuterium and tritium and radiation-induced techniques[2]. Of fundamental importance in deciding the choice of method, particularly for tracer applications, is the possible change in configuration which may occur during substitution by isotopic hydrogen. In radiation-induced procedures, a preference for retention of configuration has been observed in crystalline systems such as the octyl phthalates[3] and inositols[4], whereas a predominance of inversion occurs in the liquid octanols and inositol ethers[5] and in simple amino-acids[6]. In the catalytic technique, results from the only heavy-water system studied show that, in optically active lactic acid, deuteration of the α-position is generally accompanied by inversion[7].

For biological purposes, the labelling of amino-acids, particularly phenylalanine, in one step with retention of configuration, could be valuable. During charge-transfer mechanism studies of catalytic exchange with isotopic water[6], it has been observed that appreciable isotope incorporation only occurs if a compound contains a double bond or an aromatic ring. In simple amino-acids, such as alanine, it would be expected that catalytic exchange with D_2O would be low, as the system is essentially aliphatic in nature. However, by analogy with lactic acid, if the electron lone-pair on the nitrogen assists the absorption process and thus increases the exchange, isotope incorporation may occur predominantly by inversion since the asymmetric centre would be in close proximity to the catalyst surface. In phenylalanine, it is possible for the molecule to be π-complexed to the catalyst surface through the aromatic ring[8] and thus be highly deuterated without inversion of configuration. In addition to the biological implications, such a result would be further evidence to support the π-complex theory of catalysis[9].

For the present investigations, alanine, phenylalanine and mandelic acid have been exchanged with D_2O in the presence of hydrogen pre-reduced platinum, since platinum is the most active of the Group VIII transition metals for exchange[10]. In a typical experiment, L(−)-phenylalanine (0·4 g) in D_2O (5·5 g) was heated to 130° C with pre-reduced platinic dioxide (0·02 g) for 48 h. After purification of the deuterated compound, deuterium analyses were performed on a Metropolitan–Vickers *MS–2G* mass spectrometer and a Varian *A*-60 nuclear magnetic resonance spectrometer.

mechanism of exchange probably involves two competing dissociative mechanisms as previously discussed for the alkyl benzenes[8,11]. The relatively high isotope incorporation in the ring suggests that aromatic exchange again occurs predominantly by the dissociative π-complex substitution mechanism[12].

Lack of deuteration and inversion at the tertiary carbon atom of phenylalanine during the low-temperature exchange indicates that the molecule is tilted during adsorption so that the proton at this position never touches the catalyst surface (as in (I)). This effect has already been discussed during the catalytic exchange of the alkyl benzenes[8]. As the temperature of exchange is increased from 30° to 130° C, it is obvious that the effect of the second competing dissociative exchange reaction increases.



(I) (II)

The results with mandelic acid (adsorbed as in (II)) are consistent with the phenylalanine data. The only hydrogen in the α-position in mandelic acid is adjacent to the aromatic ring and attached to a tertiary carbon atom. Thus, it is not surprising to find that some inversion

Table 1. DEUTERATION OF ALANINE, PHENYLALANINE AND MANDELIC ACID

Compound	Weight (g)	D_2O or H_2O (g)	Catalyst (g)	Temp. of exchange (°c)	*d*-		*l*-		Statistical equilibrium *D* § (%)	Approach to statistical equilibrium (%)
					α*	*D* § (%)	α*	*D* § (%)		
L(+)-Alanine					+14·0 ± 0·3°	—				
,,	1·00	2·36 (D_2O)	0·050	30†	+13·7°	1·6			42·9	3·8
,,	1·00	2·36 (D_2O)	0·050	130	+2·7°	21·0			42·9	52·5
L(−)-Phenylalanine							−34°			
,,	0·70	14·0 (H_2O)	0·035	30†			−34°	—		
,,	1·00	22·0 (D_2O)	0·050	30†			−34°	17·7	70·6	25·1
,,	0·40	5·0 (H_2O)	0·02	130			−17°	—		
,,	0·40	5·5 (D_2O)	0·02	130			−17°	23·0	69·3	33·2
Mandelic acid ‡					+150°	—	−158°	—		
,,	1·52	3·4 (D_2O)	0·100	30†	+143°	27·6	−149°	24·7	60·4	45·7 ¶ 40·7 ‖
,,	1·52	3·1 (H_2O)	—	130	+24°	—	−40°	—		
,,	1·52	3·1 (H_2O)	0·100	130	+55°	—	−54°	—		
,,	1·52	3·4 (D_2O)	0·100	130	+19°	36·0	−30°	33·0	60·4	59·6 ¶ 52·8 ‖

* Rotations for alanine measured with 1·5 per cent compound in 1·0 N hydrochloric acid. Rotations for phenylalanine and mandelic acid performed with 1·0 per cent compound in water.

† These runs shaken for 3 days at 30° C; the remainder stood for 2 days at 130° C.

‡ Aldrich quality resolved grade.

§ In terms of non-labile hydrogens only.

¶ and ‖ Percentage approach to statistical equilibrium for *d*- and *l*-isomers of mandelic acid respectively.

From the results given in Table 1, alanine exchanges very slightly at room temperature but deuterates extensively with appreciable inversion of configuration at 130° C, thus indicating that the lone-pair on the nitrogen may assist the adsorption process. This is confirmed by a study of the nuclear magnetic resonance of the distribution of isotope in the alanine deuterated at 130° C, since 47 per cent of the hydrogen at the asymmetric carbon atom is replaced by deuterium while only 30 per cent deuteration of the methyl hydrogen occurs.

The significant feature of the present results is that L(−)-phenylalanine readily exchanges at 130° C with a predominance of inversion of configuration whereas, after shaking for three days at 30° C, deuteration is still relatively efficient with retention of configuration. Distribution of the isotope in the labelled phenylalanine from the latter reaction shows that deuteration has occurred essentially in the phenyl group with no observed isotope at the asymmetric carbon atom. This would indicate that the occurs with deuteration, even at 30° C. At the higher temperature, however, interpretation is difficult because of a thermal-induced inversion of configuration which occurs without catalyst.

For biological reactions involving labelled phenylalanine, the present result is significant since, with D_2O and moderate activity-levels of T_2O, the catalytic procedure can be used, at room temperature, to tag optically phenylalanine with retention of configuration. However, care should be exercised in extrapolation of the technique to high levels of T_2O (c./ml.) since an additional radiation-induced activation of the reaction would be expected[13], with possible consequential changes in stereochemical configuration[6,14]. The present results are important since they should act as a general guide to the type of results to be expected during the catalytic tritiation and deuteration of all amino-acids.

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Stereospecific Addition of Hydrogen to Fumaric Acid in Irradiated Single Crystals of Succinic Acid

Pooley and Whiffen[1] reported an interesting proton–deuteron exchange reaction of the primary radical $HOOCCH_\beta H_{\beta'}CH^{\alpha}COOH$ (or $DOOCCH_\beta H_{\beta'}CH_\alpha COOD$)[2] in an irradiated crystal of succinic acid. By means of an electron spin resonance technique, they detected $DOOCCD_\beta H_{\beta'}CHCOOD$ in addition to the primary species, but they did not detect $DOOCCH_\beta D_{\beta'}CHCOOD$, nor $DOOCCH_2CDCOOD$ (hyperfine splitting constant for H_β is larger than that for $H_{\beta'}$[2]). It was shown later[3,4], however, that formation of this $DOOCCD_\beta H_{\beta'}CHCOOD$ species is associated with an unidentified impurity in the crystal; the impurity is too low in concentration to be detected with ordinary experimental techniques. During the present investigation it was found that fumaric acid behaved quite similarly to the impurity: in a crystal of succinic acid which contained 0·001 mole fraction of fumaric acid, the ratio

$$[DOOCD_\beta H_{\beta'}CHCOOD]/[DOOCCH_2CHCOOD]$$

was found to be 0·2, that is, the same as the ratio found in the impure crystal[3].

The crystals used in this investigation were grown by slowly cooling aqueous solutions of mixtures of succinic acid and fumaric acid. Heavy water was used for the deuterium-substituted crystals. Concentrations of fumaric acid in the crystals were estimated from those of their mother liquors. The relation between the concentration of fumaric acid in the crystal and that in its mother liquor was determined by means of radioactive fumaric acid HOOCC*H = C*HCOOH. Irradiation of the crystals was accomplished by an X-ray source (30 kV, 15 m.amp) at room temperature, and the concentration of the radicals was about 7×10^{-4} radical/molecule. The electron spin resonance absorption was measured at 9 Gc/s at room temperature.

In any crystals which were grown from the aqueous solutions, only the primary species was detected. In the deuterium substituted crystal, which contained more than a 0·0001 mole fraction of fumaric acid, the $DOOCCD_\beta H_{\beta'}$-CHCOOD species was detected, in addition to the primary species. When 2,3-deuterofumaric acid was used, the absorption from $DOOCCD_2CDCOOD$ replaced this absorption. Thus, it was concluded that the radical $DOOCCD_\beta H_{\beta'}CHCOOD$ was formed by addition of hydrogen (deuterium) to fumaric acid.

Concentration of $DOOCCD_\beta H_{\beta'}CHCOOD$ increased with increasing concentration of fumaric acid in the crystal, until the latter concentration became about 0·01 mole fraction. For the crystal in which the concentration of fumaric acid was higher than 0·01, ratio

$$[DOOCCD_\beta H_{\beta'}CHCOOD]/[DOOCCH_2CHCOOD]$$

was found to be about 0·65.

From the signal-to-noise ratio of the absorption pattern of the crystal, the ratio

$$[DOOCCH_\beta D_{\beta'}CHCOOD]/[DOOCCD_\beta H_{\beta'}CHCOOD]$$

was found to be less than 1/300. In an isolated molecule of fumaric acid, these two species should have been formed with an equal reaction rate. Therefore, there was no uncertainty that this large difference in the addition rates arose from the crystal field which surrounded the molecule of fumaric acid. Such an effect of the crystal field on a chemical reaction had already been reported in the case of proton–deuteron exchange reaction of the radical in irradiated crystals of D,L-aspartic acid[4,5].

So far as we know, a high stereospecific efficiency, such as that observed at present, has been found only in the case of enzymatic reactions[6]. Moreover, it is well known that an enzyme is highly selective in its reactions. Such high selectivity was also found in the present case. So far as our examination was concerned, fumaric acid was the only compound from which a radical was formed with the use of an irradiated crystal of succinic acid. The other compounds examined (inactive compounds), which are more or less similar to fumaric acid in structure, were as follows: maleic acid, oxalic acid, crotonic acid, acrylic acid, tartaric acid, and D,L-aspartic acid. It was not known, however, whether a crystal, grown from a solution containing one of the inactive compounds in addition to succinic acid, contained a significant concentration of molecules of this compound.

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Anomalies in Contact Angle Behaviour for Ethyl Alcohol–Water Mixtures

Measurements of static contact angles for alcohol/water mixtures on pretreated copper surfaces are presented in Fig. 1. The results indicate that, for ethyl alcohol concentrations up to 8 per cent by weight, a constant contact angle of 72° is attained. Analogous behaviour has been observed by Franke[1] when measuring mutual diffusivities where a minimum value was reported at 10 per cent concentration. Hoffman *et al.*[2] have shown a maximum value of the temperature coefficient of the surface tension of ethyl alcohol/water mixtures at 8 per cent weight.

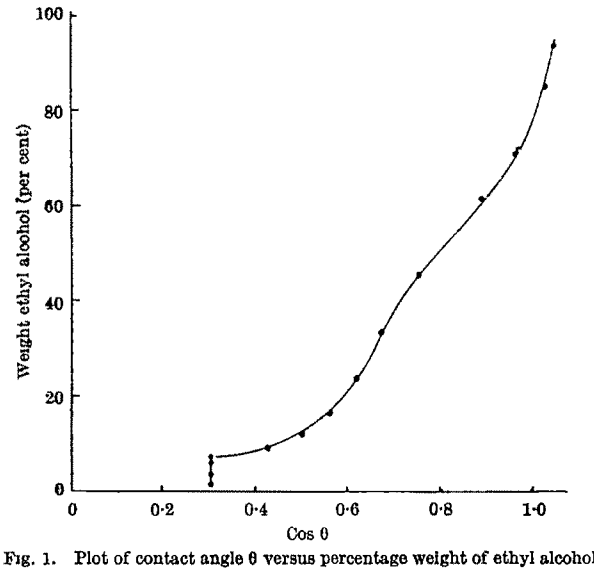


Fig. 1. Plot of contact angle θ versus percentage weight of ethyl alcohol

This behaviour again demonstrates the anomalous bulk and surface properties of dilute alcohol/water mixtures.

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BIOCHEMISTRY

Impaired Ability of Ribosomes to react with Polyuridylic Acid after Hypophysectomy of the Rat: Removal of the Disability by a Cell Sap Factor

The liver ribosomes of hypophysectomized rats are less able to incorporate amino-acids into protein *in vitro* than those of normal rat liver[1]. Ribosomal preparations from hypophysectomized rats contain less polysomes than those from normal rats[2], and the rate of synthesis of messenger ribonucleic acid (*m*RNA) and other kinds of RNA is depressed[2,3] after hypophysectomy. The action of growth hormone in promoting protein synthesis and growth can be explained in terms of its action in stimulating the synthesis of *m*RNA[2,4], but this hypothesis does not explain all the facts. It has been noted[4], for example, that the percentage stimulation of phenylalanine incorporation obtained by addition of 25 μg of polyuridylic acid (poly U) to the cell-free system with ribosomes from liver of hypophysectomized rats was as great as, or greater than, that obtained when ribosomes from liver of normal rats were used; but the absolute level of incorporation with ribosomes from hypophysectomized rats was still less than that obtained with normal rat liver ribosomes. The amount of poly U used was arbitrary and was probably not optimal for the system; thus it was decided to reinvestigate this point under conditions which were optimal for incorporation of phenylalanine into polypeptide in the presence of poly U.

The total ribonucleoprotein particle population of the sodium deoxycholate-treated, 10,000*g* supernatant material of liver homogenate was prepared by centrifugation at 100,000*g* for 4 h over 1 M-sucrose containing medium *M* (0·02 M *tris* buffer, *p*H 7·5, 100 mM potassium chloride, 40 mM sodium chloride, 10 mM magnesium acetate and 6 mM mercaptoethanol) as previously described[2,5]. The optimum conditions for incorporation of amino-acids had already been elucidated[5]. Two, which are not widely observed, are to treat the cell sap with 'Sephadex' to remove inhibitors and to use at least 20 times as much sap protein as ribosome protein to ensure that sap factors are not limiting[5]. Observing these conditions, the amount of poly U which was necessary to saturate the system was determined. It was found that incorporation of phenylalanine rose rapidly with increasing amounts of poly U, came to a plateau when the amount of poly U added was about ten times the concentration of ribosomal RNA, but thereafter continued to rise very slowly. In subsequent experiments, ribosomes containing 10 μg RNA were incubated with 250 μg poly U in medium *M* with 5 mM ATP, 0·6 mM guanosine triphosphate and 0·3 μc. D,L (3-^{14}C) phenylalanine (34 mc./mmole) and various amounts of 'Sephadex'-treated cell sap. The radioactive protein was prepared and counted in a manner previously described[5].

Table 1 shows typical results. It will be seen (lines 1 and 2) that even when saturating amounts of both cell sap and poly U are present, the incorporation of phenylalanine into polypeptide with ribosomes of hypophysectomized rat liver is much less than that obtained with ribosomes from normal rat liver. This result, which has been repeatedly obtained, must mean that hypophysectomy of the rat impaired the ability of liver ribosomes to react with poly U to form an active polysome. It gives experimental support to the argument, previously expressed[4,6,7], that stimulation of protein synthesis by growth hormone cannot be explained simply and entirely in terms of altered rates of *m*RNA synthesis; other factors are involved. Similar results have been obtained with heart ribosomes of the rat[8] and with muscle ribosomes of alloxan-diabetic and insulin-treated rats[9].

Table 1. Stimulation of the Incorporation of Phenylalanine by Poly U with Liver Ribosomes from Normal and Hypophysectomized Rats

Amount of cell sap protein per unit of ribosomal RNA	C.p.m./μg ribosomal RNA: Normal rat ribosomes		Hypophysectomized rat ribosomes	
	No poly U	Poly U (250 μg)	No poly U	Poly U (250 μg)
20	960	10,300	560	4,800
50	1,700	13,800	750	7,300
100	1,200	21,500	860	12,800
200	1,270	33,000	970	26,500
400	1,910	26,500	1,310	25,800
800	2,300	19,000	1,570	18,600
1,000	2,560	12,000	1,850	11,500

The amount of cell sap required to saturate the ribosomes had been calculated from experiments carried out in the absence of poly U[5]. It seemed possible that more sap might be required for saturation when large amounts of poly U are present. Ribosomes were incubated, with or without poly U, in the presence of large quantities of cell sap. Saturation was achieved with a twenty-fold excess of sap to ribosomes[5], but in the experiments summarized in Table 1 up to a thousand-fold excess was studied. Three interesting observations emerged. (1) In the absence or presence of poly U, increasing the sap concentration above the 'saturating' level resulted in increased incorporation. This was not caused by ribosomal contamination of the cell sap, for incubation of it without ribosomes resulted in little incorporation and no particles could be detected when cell sap was studied in a Spinco model *E* analytical ultracentrifuge. (2) In the presence of poly U, but not in its absence, concentrations of sap greater than about 200 times that of the ribosomes had an inhibitory effect. (3) In the presence of poly U when the cell sap concentration reached 200–400 times that of ribosomes, the difference in incorporation of phenylalanine with ribosomes from normal and hypophysectomized rats disappeared. Cell sap contains a factor other than the amino-acid activating enzymes and *s*RNA necessary for maximum incorporation of phenylalanine in the presence of poly U; this factor is required to a greater extent by ribosomes from liver of hypo-

physectomized rats than by ribosomes from normal rat liver.

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Bacterial Degradation of Glycoprotein Sugars in Human Saliva

Previous reports[1,2] have indicated that sialic acid in human, wax-stimulated saliva is rapidly released and metabolized by the oral flora. Heat (100°, 5 min), or antibiotic, prevents both these reactions from taking place. Further investigations have now shown that the other sugars associated with the salivary glycoproteins (3 hexoses, 2 hexosamines and fucose) are all spontaneously lost from saliva by bacterial action, and this loss may also be prevented by either heat or antibiotics. Wax-stimulated saliva contains, with the exception of fucose, all these sugars in two distinct forms: those in the salivary glycoproteins and those in the many and varied bacteria (10^8/ml.) also present in the saliva. Many of the bacteria in the saliva contain rhamnose, a 6-deoxyhexose of the same group of sugars as fucose, but it is possible to distinguish between these two sugars by either paper or thin-layer chromatography[3]. By this method it was shown that the 6-deoxyhexoses in freshly collected, wax-stimulated saliva consisted of rhamnose and fucose. It was also shown that, on incubation, the saliva rapidly lost all its fucose and sialic acid, while the rhamnose remained. The hexoses and hexosamines, however, occurred in the salivary glycoproteins and the bacteria, and it was not possible to distinguish between these sources by direct analysis or by chromatography. On incubating wax-stimulated saliva, 50–70 per cent of the hexoses and hexosamines were lost, but it was not possible in this instance to distinguish whether the remaining sugar was derived from the salivary glycoproteins or from the bacteria. If the saliva was collected directly from the salivary ducts (parotid, submaxillary and sublingual) and pooled, there were now present so few bacteria that all the sugars from the glycoproteins remained intact on incubation and the saliva remained clear and viscous. The addition of bacteria, in the form of dental plaque or salivary sediment, to the viscous duct saliva caused a rapid loss of sugars, a drop in viscosity and the formation of a precipitate. After incubation no fucose or sialic acid was present and the amount of residual sugars was approximately equal to that from the added bacteria. In none of the experiments described here has it been possible to detect any but traces of free sugars in the saliva when bacteria have been present, and these sugars were also rapidly metabolized when added in a free state to the saliva. It is thus apparent that the carbohydrate components of the glycoproteins in saliva are labile in the oral environment and are rapidly released and metabolized by bacterial action. The loss of these sugars, particularly sialic acid, provides an explanation for the observed drop in viscosity and formation of a precipitate that occurs spontaneously in wax-stimulated saliva[1,2]. Previous suggestions that the drop in viscosity is due to a mucinolytic depolymerization of the salivary glycoproteins[4] are valid so far as the viscosity-drop is concerned, but this mechanism should produce a product of increased solubility, and, in fact, a heavy precipitate always appears concomitantly with the drop in viscosity. It is tempting to postulate that this is the mechanism whereby the material from the saliva forms a matrix holding together the many bacteria adjacent to the teeth, which is commonly referred to as dental plaque. Support for this is found in the observation that dental plaque is practically devoid of both sialic acid and fucose ($<0{\cdot}002$ per cent), the two essential sugar constituents of salivary glycoproteins that can be identified as belonging exclusively to the glycoproteins and not to the oral bacteria.

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High Content of Hyaluronic Acid in Normal Human Heart Valves

During the course of investigations of the effect of age on acid mucopolysaccharide content of the human heart valves, we found hyaluronic acid to be a major component. In all it represented about 60 per cent of the acid mucopolysaccharide present in the tissue. This observation is in striking contrast with reports from other laboratories[1,2] which have shown hyaluronic acid to be a minor component of both bovine and pig heart valves. This communication reports the distribution of the acid mucopolysaccharide fractions of the normal human heart valves.

For total acid mucopolysaccharide determination, individual heart valves (approximately 50 mg) were dried, de-fatted and subjected to papain digestion and the protein separated by precipitation with trichloroacetic acid. The acid mucopolysaccharide is precipitated by cetylpyridinium chloride, dissolved in sodium chloride and reprecipitated with ethanol. For fractionation, extracted acid mucopolysaccharide of about ten valves of the same age-group was pooled. The acid mucopolysaccharide was fractionated to hyaluronic acid, chondroitin sulphuric acids and heparin according to Schiller *et al.*[3]. Applying this method, hyaluronic acid, chondroitin sulphates, and heparin were obtained at 0·4 M, 1·2 M, 2·1 M sodium chloride solution, respectively. Chondroitin sulphate *B* was further separated from chondroitin sulphate *A* or *C* by hyaluronidase (testicular) digestion. The total acid mucopolysaccharide is expressed as uronic acid and determined by the carbazole reaction[4]. The results of such a fractionation procedure are shown in Table 1. The total acid mucopolysaccharide present in the mitral heart valve is 0·93 mg uronic acid/100 mg dry weight and is significantly higher than in the tricuspid valve ($P < 0{\cdot}01$ analyses were carried out on fourteen individual valves). In both valves there is no change in the distribution of various fractions of acid mucopolysaccharide and it can be seen that hyaluronic acid represents 58 per cent

Table 1. Total Acid Mucopolysaccharide (AMPS) Content and Individual AMPS Fractions from Human Heart Valves

Heart valves*	Total AMPS†	Fraction (per cent) HA	CSA—*A*/*C*	CSA—*B*	Heparin
Mitral	0·93	58·8	27·3	13·6	Trace
Tricuspid	0·74	57·7	28 0	14·3	Trace

HA, hyaluronic acid; CSA—*A*/*C*; chondroitin sulphate *A* or *C*; CSA—*B* chondroitin sulphate *B*.
* These heart valves were obtained from subjects of 20–30 years of age.
† Mg of uronic acid/100 mg dry de-fatted valve.

of the total acid mucopolysaccharide present. Chondroitin sulphate *A* or *C* is about 28 per cent while chondroitin sulphate *B* is 14 per cent. The actual amount of chondroitin sulphate *B* is a little higher than this value because of the low carbazole value of iduronic acid in chondroitin sulphate *B*. Trace amounts of acid mucopolysaccharide were found in the 2·1 M sodium chloride fraction (heparin) but the attempt to identify this fraction as heparin was not successful. Paper chromatography[5] of acid hydrolysed fraction showed spots corresponding to glucosamine in the 0·4 M sodium chloride fraction and galactosamine in the 1·2 M sodium chloride fraction. This confirms the presence of hyaluronic acid only in the 0·4 M sodium chloride fraction and rules out any contamination of chondroitin sulphates into this fraction and also the presence of chondroitin (chondroitin and chondroitin sulphates contain galactosamine). Similarly the 1·2 M sodium chloride fraction had chondroitin sulphates and not heparitin monosulphate (heparitin monosulphate contains glucosamine). The significance of the difference in hyaluronic acid content of human and animal heart valves is not clear. Further investigations are in progress which are aimed at elucidating the relationship of this high content of hyaluronic acid in human heart valve with function.

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K88, an Episome-determined Protein Antigen of *Escherichia coli*

Recently, I. Ørskov, F. Ørskov, W. J. Sojka and J. M. Leach[1] described a new genetical donor strain of *Escherichia coli*. This strain, *G7* (O8 : K87(B) : K88ab(L) : H19), was at first isolated from a case of pig enteritis. Investigations in different countries have shown that this serotype is frequently found in enteric diseases in pigs. A characteristic feature was the simultaneous existence of two K antigens in the same strain. The K87(B) antigen is a polysaccharide as are all hitherto examined B (and A) antigens[2]. The K88 antigen is thermolabile, that is, its ability to agglutinate and to bind antibody and its capacity to induce antibody formation in rabbits are destroyed by heating to 100° C for 1 h and thus it must be grouped as an L antigen according to established definitions[3]. Since the first detection of the K88 antigen it has been found in many *E. coli* strains, mainly from young pigs with intestinal diseases, and in different serological combinations[4–6]. Furthermore, it has been shown that K88 exists in at least two varieties, one of which is symbolized as K88ab, and the other K88ac (ref. 4). Strain *G7* is a genetical donor and can, in conjugation experiments, transfer chromosomal markers to suitable recipients. In addition the genetic determinant of the K88 antigen can be passed to other *E. coli* serotypes by episomal transfer[7].

When *E. coli* cultures, collected from beef broth agar plates, are suspended in saline, considerable amounts of the surface antigens are released into solution. By gentle heating the amounts of released antigens can be increased considerably.

In order to isolate the K88 from such an extract containing as few other contaminating antigens as possible, the K88 factor was transferred to *D*282 (O8 : K27(A)$^-$: H$^-$, lac$^+$), a strain that carried no K and no H antigen. An overnight broth culture of *D*282 (0·003 ml.) and 0·3 ml. of a broth culture of donor strain *D*432 (O8 : K87(B) : K88(L) : H19, lac$^-$) were inoculated together in 50 ml. broth and incubated overnight at 37° C. The mixed culture was spread on bromothymol blue lactose plates and after incubation lactose-positive colonies (*D*282) were tested for K88 by slide agglutination. About 15 per cent of the lactose-positive colonies had acquired the K88 antigen. One such clone (*D*520) was purified and used for the production of K88 antigenic material. Apart from the acquisition of K88, *D*520 had all fermentation and antigenic markers of the *D*282 parent.

For production of K88 antigenic material, *D*520 was heavily inoculated on to thick broth agar plates (14 cm diameter) which were incubated for about 20 h at 37° C. The confluent surface growth was scraped off into *p*H 7–8, 0·1 molar phosphate buffer, 3–5 ml. buffer per plate, and the suspension was then heated for 20 min at 60°–65° C in a water-bath and finally centrifuged at 10,000 r.p.m. for 10 min. The supernatant was stored for at least 3 days at 4° C. During this time some impurities separated out and centrifugation was then repeated and followed by filtration through a 0·65μ 'Millipore' filter. Sterility was ensured by addition of 0·01 per cent 'Merthiolate'. Since it was observed that the K88 antigenic material was soluble in buffers of *p*H < 3·5 (partial denaturation) or > 6·5, but completely insoluble between *p*H 4·5 and 5·5, dilute acetic acid was added to bring the *p*H to the isoelectric region. The precipitated material was collected by centrifugation and washed twice with a *p*H 5, 0·005 molar McIlvaine buffer. For purification, the product was repeatedly redissolved in *p*H 7, 0·01 molar phosphate buffer by gentle stirring in the cold, filtered as already described, and precipitated again by addition of approximately the same volume of *p*H 4·8–5·0, 0·15 molar McIlvaine buffer. As determined by the biuret reaction, approximately 2 mg of K88 antigen was thus obtained from one plate.

Extraction and purification of the K88 antigen were controlled by a filter paper disk modification of the Ouchterlony gel-precipitation technique. Filter paper disks 6 mm in diameter, as used for sensitivity tests, were saturated with either bacterial agar plate culture (by placing them directly on the confluent growth), with bacterial extracts or with different K88 preparations and then placed on a 2 mm agar layer in a Petri dish. Antisera (mostly undiluted) were applied in the same way. K88, purified by repeated isoelectric precipitation, showed but one precipitation line against *D*520 rabbit serum (Fig. 1). The material sedimented uniformly in the analytical ultracentrifuge—a small contamination by some heavier component could be removed by short (15 min) centrifugation at 40,000 r.p.m. The K88 agglutinins could be selectively removed from the homologous OK serum by absorption with the purified K88 antigen.

As shown by the disappearance of the K88 line in the Ouchterlony test, the antigen is denatured by heating to above 70° C or by treatment with mineral acid, sodium

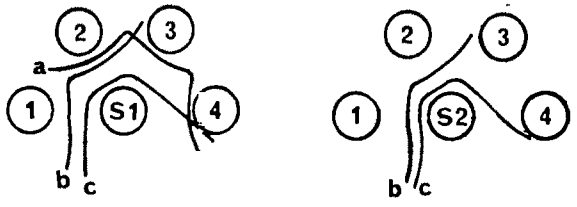


Fig. 1. Gel precipitation using purified K88 antigen, bacterial extracts of strains with and without K88 antigen and sera against such strains. Bacterial extracts: (1) *D*282 (O8 · K27$^-$: H$^-$); (2) *D*520 (O8 : K27$^-$: K88 : H$^-$); (3) *D*214 (O1(1) · K(B) : K88 : H2); (4) Purified K88 antigen. Sera: S1, OK antiserum *D*520; S2, OK antiserum *D*282. Precipitation lines: *a*, K88 line; *b*, lipopolysaccharide line (O antigen); *c*, non-specific, common antigen

hydroxide solution or formamide in the cold. The K88 precipitation line is stained by amido black but not by lipoprotein stains such as oil red or Sudan black[8]. The effect of proteinases on the material was investigated in the following manner: K88 antigen (six parts) in *p*H 7, 0·1 molar phosphate buffer was digested with crystalline trypsin (1 part)[9] at 28°–30° C. After various reaction times, the trypsin was inactivated by addition of soy-bean inhibitor (two parts)[9] and the mixture compared by agar-gel diffusion with undigested K88, using OK antiserum *D*520. Under similar conditions α-chymotrypsin, *B. subtilis* proteinases, papain and *Streptomyces griseus* proteinase (pronase)[9,10] were applied. Extensive splitting and weakening of the K88 precipitation line indicated that the antigen was affected by *B. subtilis* proteinases, papain, pronase and, to a lesser degree, by α-chymotrypsin. Trypsin had no visible effect.

Solutions of repeatedly precipitated K88 antigen were dialysed exhaustively against distilled water and freeze-dried. The biuret reaction gave nearly 100 per cent protein using bovine serum albumin as a standard and the ninhydrin method for proteins[11] yielded an extinction of 0·015/μg nitrogen/2·6 ml. in a 1 cm cell. The orcinol and indol reactions[11] gave 1·5–3 per cent carbohydrates with glucose as a standard, and the elemental analysis yielded 14 per cent nitrogen and 0·1 per cent phosphorus.

The ultra-violet absorption curve of K88 antigen in 0·1 N sodium hydroxide showed the broad absorption band at about 280 mμ, which is characteristic for proteins, and no shoulder at 260 mμ (nucleic acids).

Bacteria carrying the K88 antigen were also directly treated with proteinases and tested for loss of the antigen by slide agglutination. Whereas digestion with papain soon caused autoagglutinability of the organisms, pronase did not have this effect, but removed the antigen as expected. Treatment with 50 per cent aqueous urea had the same effect.

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Solubility in Phenol of Aortic Polysaccharide–Protein Complexes

Buddecke[1] has reported a successful separation of glycoprotein from glycosaminoglycuronoglycan in papain digests of aortic tissue by extraction with 90–95 per cent w/w phenol[2]. This result prompted us to attempt a fractionation, by this method, of the glycoproteins from the glycosaminoglycuronoglycan protein complexes present in material obtained from aqueous extracts of aortic intima.

An extract was prepared by homogenizing finely minced aortic intima in distilled water, stirring slowly for several days and dialysing. Insoluble material was removed by centrifuging and a crude mixture of the polysaccharide–protein complexes was precipitated with ethanol in the presence of potassium acetate. The precipitate was dissolved in water, dialysed, centrifuged and reprecipitated as before. The sediment was washed with ethanol, diethyl ether and air dried.

This material was then extracted with phenol[2]. Determinations of sialic acid (as *N*-acetyl neuraminic acid)[3], uronic acid (as glucuronic acid)[4] and hexosamine (as glucosamine)[5] were used to define the distribution of material. Samples of chondroitin 4-sulphate and chondroitin 4-sulphate protein complex from nasal septum and a crude glycoprotein mixture from pig aorta[6] were included. The results are shown in Table 1.

In view of the observed insolubility in phenol of chondroitin 4-sulphate, chondroitin 4-sulphate protein complex (Table 1) and aorta chondroitin 6-sulphate[1,7], and the reported solubility of aorta glycoprotein in this solvent, a successful separation of these two groups of components should be possible. The results show an almost equal distribution of material between the phenol-soluble and phenol-insoluble fractions. When similar preparations were fractionated with ammonium sulphate[6], the product obtained at full saturation contained mostly phenol-soluble material (Table 1, row 4).

Table 1. Distribution of Sialic Acid, Uronic Acid and Hexosamine between Phenol-soluble and Phenol-insoluble Fractions

Sample		Sialic acid	Uronic acid	Hexosamine
(1) Chondroitin 4-sulphate	Phenol-soluble	—	<1	<1
	Phenol-insoluble		>99	>99
(2) Chondroitin 4-sulphate protein complex	Phenol-soluble	—	<1	<1
	Phenol-insoluble	—	>99	>99
(3) Aqueous aorta extract	Phenol-soluble	38	51	54
	Phenol-insoluble	62	49	46
(4) Crude glycoprotein mixture (ref. 6)	Phenol-soluble	94	87	92
	Phenol-insoluble	6	13	8

Results are expressed as percentages of the total amount in both fractions. Analytical values (expressed as g/100 g material) for samples (1)–(4) were respectively: sialic acid, 0·1, 0 8, 2, 4; uronic acid, 33, 28, 6, 4; hexosamine, 28, 28, 7, 9.

As the glycoprotein components of aorta are usually characterized by the presence of significant amounts of sialic acid and the absence of uronic acid[6] and the glycosaminoglycuronoglycan protein complexes are characterized by relatively large amounts of uronic acid with little or no sialic acid[1,7,8], we conclude that no significant separation of glycoprotein from glycosaminoglycuronoglycan protein complex has been obtained by the use of phenol, and that both types of polysaccharide–protein complex contain phenol-soluble and phenol-insoluble components. These findings are at variance with those of Buddecke[1], who found no phenol-insoluble glycoprotein and no phenol-soluble glycosaminoglycuronoglycan. These differences may be explained in terms of the relative complexity of the aqueous extract where the components are present in forms which may approximate to their native state and where interactions between the various molecular species may occur, thus modifying the properties of the individual molecules. Enzymatic digestion[1], while simplifying the subsequent experimental procedures, is known to modify the polysaccharide–protein structures and this might lead to the observed differences in solubility properties.

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Stimulation of Protein Synthesis of Frog Embryo Cells by Larval and Adult Frog Liver Ribonucleic Acid Preparations

RECENT work from our laboratory has characterized certain quantitative properties of DNA and chromatin from developing frog embryos in terms of RNA-polymerase-mediated RNA synthesis[1,2]. Apart from knowing how much RNA can be synthesized using chromatin or DNA as a primer, it would be of interest to know some of the qualitative properties of the RNA synthesized. The present investigation examines two questions: Can added RNA stimulate protein synthesis of intact frog embryo cells? If it can, is this stimulation due to species of tissue-specific RNA molecules directing the synthesis of the appropriate proteins? Liver RNA preparations can stimulate haemin synthesis of minced frog embryos[3] or mouse ascites tumour cells[4], and Niu *et al.*[5] have claimed that liver RNA induces the synthesis of liver specific proteins in ascites tumour cells.

Groups of 25 or 30 early gastrulae of *Rana pipiens*, stage 10 of Shumway[6], were removed surgically from their jelly and membranes, and each was cut into four or eight equal-sized fragments in sterile Niu–Twitty saline medium[7]. The fragments from each group of gastrulae were cultured for periods of 24–50 h at 18° C in 1·5 ml. of Niu–Twitty saline, which contained an RNase inhibitor, polyvinylsulphate, at a concentration of 10 μg/ml. Carbon-14 labelled amino-acids of an algal hydrolysate or ^{14}C-phenylalanine were added to the cultures at levels of 0·5 or 1·0 μc./ml. RNA was prepared by the cold phenol method of Brown and Littna[8] and in some cases the interphases of these extractions were further extracted with phenol at 60° C (ref. 9). In one experiment, RNA was generated from frog liver DNA with *Micrococcus lysodeikticus* RNA polymerase in a cell-free system[1]. The RNA preparations were dialysed against Niu–Twitty medium and the RNA concentration estimated by the orcinol reaction[10]. RNA was added to the cultures at a concentration of 1 mg/ml. At the termination of the period of culture the explants were washed several times with 0·7 per cent sodium chloride and homogenized in this medium. The homogenates were then precipitated with cold 5 per cent trichloroacetic acid (TCA) and washed with 1–4 alcohol–ether. They were then treated with 5 per cent TCA at 90° C for 20 min, and washed with 5 per cent TCA and ether. These final centrifugal residues were dissolved in formic acid, and the pigment granules removed by centrifugation at 10,000*g*. The supernatants were plated and counted with a thin-window proportional counter. All homogenizations, dialyses and centrifugations were performed at 4° C.

In order to obtain anti-haemoglobin sera, blood was collected from the hearts of adult frogs. The red blood cells were washed by centrifugation with 0·7 per cent sodium chloride and haemoglobin was prepared by the method of Drabkin[11]. Anti-haemoglobin sera were obtained by injecting two rabbits subcutaneously three times a week during three alternate weeks with a mixture of equal parts of a haemoglobin preparation (2 per cent protein) and Freund's adjuvant. The rabbits were bled 1 week after the final injection. The reaction of the antiserum with a preparation of haemoglobin, frog serum, and centrifugal supernatants (10,000*g*) of adult frog livers, kidneys or brains revealed only one antigen–antibody precipitate line in agar-diffusion tests[12]. The one reacting antigen in centrifugal supernatants from the organs is haemoglobin from the blood cells in the organs, as the precipitate lines showed reactions of identity with the precipitation line of the haemoglobin preparation. The antiserum appeared to be specific for haemoglobin.

In the experiment designed to test for the synthesis of the globin portion of haemoglobin, centrifugal supernatants (10,000*g*) were obtained from the minced gastrulae cultured with ^{14}C-amino-acids, with or without added RNA. After washing the cultures four times with 0·9 per cent sodium chloride, the tissues were homogenized with 1 ml. of 0·9 per cent sodium chloride. One ml. of anti-haemoglobin serum was added to 1 ml. of centrifugal supernatant from each culture in 2 ml. plastic centrifuge tubes. These were mixed, placed at 37° C for 1 h and at 6° C for another hour, and centrifuged at 10,000*g*. The centrifugal residues were washed four times with 0·9 per cent sodium chloride by centrifuging at 10,000*g*. These residues were then dissolved in formic acid and counted.

The results of a number of experiments in which RNA prepared from different sources was added to the cultured gastrula fragments is represented in Table 1. Comparisons of incorporation of labelled amino-acids into the protein fraction should be made only for the controls and experimental cultures of each individual experiment since the kind and amount of isotopic compound used and duration of culture varied. Examination of these results shows that RNA isolated from the hot phenol (60° C) extracts of the interphase of cold phenol extracts of frog liver RNA stimulated protein synthesis of the embryonic cells. RNA generated *in vitro* from frog liver DNA and yeast-soluble RNA gave lower levels of stimulation. There was no significant increase of incorporation of labelled amino-acids into the protein fraction when RNA of the 105,000*g* centrifugal residues of stage 18 frog embryos, or calf thymus RNA or ammonium polyuridylate was utilized. The absence of stimulation of ^{14}C-phenylalanine incorporation into the TCA precipitates obtained from gastrula cells cultured with ammonium polyuridylate (1 mg/ml.) runs contrary to the idea of specific stimulation, as polyuridylic acid is known to code for polyphenylalanine synthesis[13].

Table 1. EFFECT OF VARIOUS RNA PREPARATIONS ON PROTEIN SYNTHESIS OF CULTURED FRAGMENTS OF FROG GASTRULAE

Exp. No.	Culture conditions	RNA preparation (1 mg/ml.)	Average c.p.m /mg protein	% stimulation
(1)	24 h; 1 μc./ml. ^{14}C algal hydrolysate	None	3,407	
		Frog liver; hot phenol (60° C) extract of interphase of cold phenol extract	4,960	46
(2)	40 h; 0·5 μc./ml. ^{14}C algal hydrolysate	None	7,170	
		RNA generated from liver DNA–RNA polymerase system	8,452	18
(3)	42 h; 0·5 μc./ml. ^{14}C algal hydrolysate	None	7,602	
		Stage 18 tailbud embryo 105,000*g* centrifugal residue (hot phenol, 60° C, *p*H 8·5 extract)	7,229	—
(4)	50 h; 0·5 μc./ml. ^{14}C algal hydrolysate	None	4,873	
		Calf thymus RNA (Nutritional Biochem. Co.)	4,996	
		Yeast soluble RNA (Cal. Bio. Chem.)	5,547	14
(5)	40 h; 0·5 μc./ml. ^{14}C-phenylalanine	None	7,585	
		Ammonium polyuridate	7,955	

The use of a specific anti-haemoglobin serum to precipitate haemoglobin, as well as molecules with similar combining groups, revealed that RNA isolated from various centrifugal residues of adult frog livers and the livers and kidneys of 50 mm frog larvae can stimulate the incorporation of labelled amino-acids into the proteins precipitated by this antiserum (Table 2). Stimulation of protein synthesis also occurred in the supernatant fractions

Table 2. EFFECT OF ADULT FROG LIVER AND LARVAL LIVER–KIDNEY RNA ON HAEMOGLOBIN SYNTHESIS OF FRAGMENTS OF FROG GASTRULAE CULTURED FOR 50 H

RNA preparations added	Average c.p.m. anti-haemoglobin precipitates	% stimulation	Average c.p.m. anti-haemoglobin supernatant	% stimulation
None	902		5,687	
Frog liver centrifugal residues				
600*g*	2,130	135	8,132	43
10,000*g*	2,086	125	10,736	88
105,000*g*	4,464	394	18,283	221
Larval liver–kidney centrifugal residues				
600*g*	3,171	251	11,268	98
10,000*g*	2,099	132	8,707	63
105,000*g*	4,795	431	14,046	146

obtained after the antigen–antibody precipitates were removed by centrifugation. The larval livers and kidneys are known to synthesize haemoglobin[14], but the livers of the adult frog are inactive in this respect and would not be expected to possess 'messenger RNA' for haemoglobin synthesis.

The results of this investigation demonstrated that certain RNA preparations can stimulate protein synthesis of frog embryo cells, but suggest that this is a non-specific effect not related to the action of tissue-specific messenger RNA-molecules.

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Metabolism of Glutamic and Aspartic Acids in Whole Rumen Contents

Amino-acids occur at low concentrations in the liquid phase of rumen contents[1,2] as intermediates in the breakdown of dietary proteins and in the production of microbial protein. The metabolism of these amino-acids has been investigated, mainly in washed suspensions of mixed rumen micro-organisms and pure cultures. There is abundant evidence of interaction in the metabolism of the amino-acids by the rumen microflora[3,4]. To obtain meaningful rates of utilization, physiological concentrations of all the amino-acids must be used. We report experiments in which glutamic and aspartic acids uniformly labelled with carbon-14 were added to whole rumen contents in true tracer amounts under conditions closely approaching those in the living animal.

For these experiments a sheep was fed a diet of pelleted dried grass meal continuously from a conveyor belt. The pellets from the machine arrived on average at intervals of 1–2 min, the food being eaten on presentation. Under this régime concentrations of metabolites in the rumen remained constant: in other words, a steady state was maintained. It was thus possible to compare entry rates obtained by the isotope dilution technique with the feeding rate.

Representative samples of uniform size were obtained using the continuous externalized circulation of the rumen content described previously[5] and transferred immediately for incubation at 40° C under nitrogen + carbon dioxide (50 : 50). Experiments were begun by injection of the isotopic amino-acid, and terminated by injection of 0·05 vol. of 10 N sulphuric acid, followed by chilling to 0° C. Samples were centrifuged at 1,500 r.p.m. for 2·5 min and the cloudy supernatant fluid centrifuged at 20,000 r.p.m. for 10 min.

The residue from high-speed centrifugation consisted almost entirely of bacteria and was washed twice with saline containing carrier inert amino-acid. There was little variation in the composition of the rumen content from day to day and the procedure of isolation of this fraction of the bacteria was rigidly standardized so that a constant proportion was collected. This proportion was found in separate experiments in which carbohydrates labelled with carbon-14 were metabolized, the ratio of radioactivity in amino-acids isolated from a hydrolysate of the bacterial fraction and in a hydrolysate of unfractionated contents giving this proportion.

The supernatant fluid from high-speed centrifugation was analysed for radioactivity remaining in the amino-acid and for radioactivity in short-chain fatty acids. Carbon dioxide was collected in parallel experiments and counted as barium carbonate. Amino-acids were separated and estimated as their 2 : 4-dinitrophenyl derivatives[6,7] and the short-chain fatty acids by steam distillation and gas chromatography[8]. Both glutamic acid-U-^{14}C and aspartic acid-U-^{14}C were metabolized rapidly with the incorporation of a great part of their radioactivity into short-chain fatty acids and carbon dioxide. The first phase of the decay curves corresponded to half-lives of 72 and 54 sec and entry rates for the whole rumen of 92 and 98 mmoles per day for glutamic and aspartic acids respectively.

Feeding rates of glutamic and aspartic acid calculated from analysis of hydrolysates of the diet were 80 and 88 mmoles per day. The shape of the decay curves indicates some re-entry of the amino-acid from secondary pools

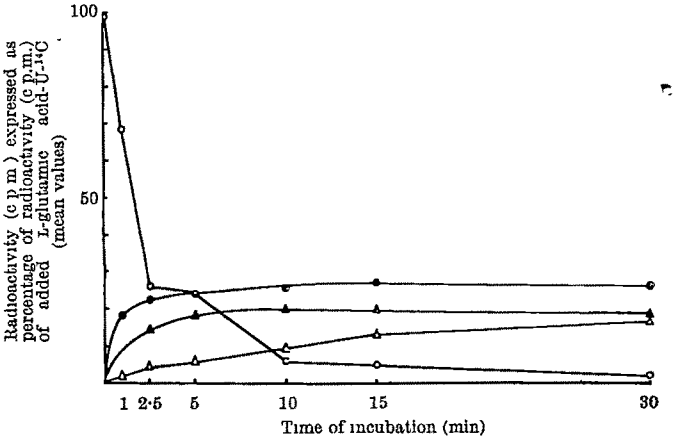


Fig. 1. Time course of the decay of radioactivity of L-glutamic acid-U-^{14}C (○) and the incorporation of radioactivity into short-chain fatty acids (●), carbon dioxide (▲), and bacterial hydrolysate (△) in sheep rumen content *in vitro*

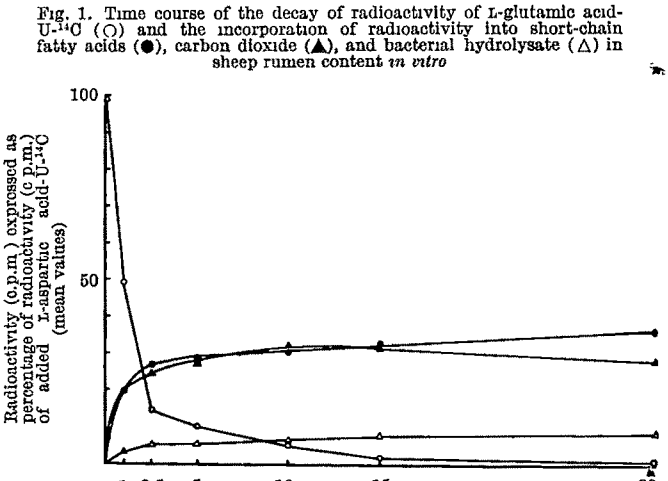


Fig. 2. Time course of the decay of radioactivity of L-aspartic acid-U-^{14}C (○) and the incorporation of radioactivity into short-chain fatty acids (●), carbon dioxide (▲), and bacterial hydrolysate (△) in sheep rumen content *in vitro*

so that entry rates calculated from the initial decay overestimate the rates of production of the amino-acids.

The results, however, leave little doubt of the importance of the free glutamic and aspartic acids of the rumen fluid, the small concentrations observed being a reflexion of their rapid turnover. Some 17 per cent of the radioactivity of glutamic acid could be accounted for in the hydrolysed bacteria. About 10 per cent was as glutamic acid, the rest being mainly in other amino-acids. The corresponding figures for aspartic acid were 8 per cent for total radioactivity in hydrolysed bacteria, about half of which was present as aspartic acid. Net ammonia production was negligible under the conditions examined so that dietary nitrogen metabolized would appear almost quantitatively as microbial nitrogen.

The experiments we have described show that the main fate of glutamic and aspartic acids under physiological conditions in rumen contents is metabolism to carbon dioxide and short-chain fatty acids and that direct incorporation of these amino-acids into bacterial protein is small. Analysis showed glutamic and aspartic acids to occur in high proportion in the bacterial nitrogen. These amino-acids in the microbial protein must come almost exclusively from *de novo* synthesis.

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Distribution Patterns of Proteolytic Enzymes in Normal and Leukaemic Human Leucocytes

Proteolysis is a well-known function of leucocytes[1,2], but literature on the distribution of defined proteolytic enzymes in different types of leucocytes is scarce[3–9]. In this investigation, nine peptidases were estimated in normal and leukaemic leucocytes. Four of them (cathepsin carboxypeptidase, leucine aminopeptidase, imidodipeptidase, iminodipeptidase) were demonstrated in leucocytes for the first time.

Leucocytes were isolated using the method of Lohr and Waller[10]. Proteinase activity was measured by the method of Anson[11]. The exopeptidases were estimated by means of quantitative paper chromatography[12]. For details of assays see Table 1. The assays contained 6·5 million (proteinases) and 10·4 million (exopeptidases) cells per ml. on average. The buffer concentration was 0·025 M. With the exception of glycyl-leucine dipeptidase, which was hydrolysed according to second-order kinetics, the velocities were computed on the basis of first-order rate constants.

Some properties of the enzymes deserved special attention. Most peptidases are fully active in a saponin (0·1 per cent w/v) lysate. Neutral proteinase, however, which undergoes a fifteen-fold increase in activity on addition of saponin[3], is activated a further 6 times by subsequent homogenization (5 min in a Potter–Elvehjem-type homogenizer, cooled by iced water). Cathepsin carboxypeptidase, tested on the cells of chronic myeloid leukaemia, is activated 250 per cent by 10 mM L-cystein. The activity of leucine aminopeptidase (substrate: L-leucine amide[13]) is enhanced 230 per cent by 0·2 mM Mn^{2+}, 35 per cent by 1 mM Mg^{2+}, and 16 per cent by 0·2 mM Co^{2+}, whereas the enzyme splitting L-leucyl-β-naphthylamide (LNAase) is not activated by manganese ions[9] and the enzyme cleaving L-leucyl-glycine is activated by traces of Zn^{2+} (ref. 7).

The results are shown in Table 1. Neutral and acid proteinases, aminotripeptidase, and carboxypeptidase appear to be characteristic enzymes of the granulocytic series. Glycyl-glycine and glycyl-leucine dipeptidases are very active in lymphocytes. The greatest differences are shown by neutral proteinase and glycyl-leucine dipeptidase. The low activities of these enzymes in the cells of chronic lymphoid and myeloid leukaemia, respectively, may be due to contamination by granulocytes (10 per cent) in the case of neutral proteinase and by lymphocytes (4 per cent) in the case of glycyl-leucine dipeptidase; in other words, these enzymes may be absent in lymphocytes and granulocytes, respectively.

Compared with granulocytes, most activities in the myeloblasts of acute leukaemia are unchanged or lowered, except for leucine aminopeptidase and prolidase, the activities of which are significantly elevated. In this respect, also, leucine aminopeptidase differs from LNAase, which was reported to be significantly decreased in acute myeloblastic leukaemia[8]. It is evident, therefore, that leucine aminopeptidase and LNAase are two different enzymes.

The cells of the so-called 'myeloblastic' crisis which terminates chronic myeloid leukaemia are, on a morpho-

Table 1 Proteolytic Activities in Normal and Leukaemic Leucocytes
(Mean values, standard deviations of the means, and numbers of cases)

Enzyme	Healthy donors	Chronic myeloid leukaemia	Acute myeloblastic leukaemia	Terminal crisis in chronic myeloid leukaemia	Chronic lymphoid leukaemia
	μAeq. resp. μmoles/min/10^{11} cells				
Neutral proteinase (*p*H 7·5)*	381 ± 23 (8)	1,250 ± 48 (3)	157 ± 55 (4)	15 ± 15 (2)	35 ± 7 0 (5)
Acid proteinase (*p*H 3·5)*	62·0 ± 4·3 (5)	60·0 ± 31·2 (3)	51·3 ± 29·7 (4)	21·7 ± 17·6 (2)	8·7 ± 3·3 (6)
Catheptic carboxypeptidase†	4·2 ± 1·8 (7)	34·8 ± 10·2 (4)	9·9 ± 5·5 (4)	0 (2)	8·2 ± 4·2 (6)
Leucine aminopeptidase (*EC* 3.4.1.1)‡	282 ± 95 (6)	273 ± 42 (4)	707 ± 105 (4)	262 ± 50 (2)	267 ± 27 (7)
Aminotripeptidase (*EC* 3.4.1.3)§	6,304 ± 1,360 (6)	8,680 ± 2,970 (4)	1,990 ± 449 (4)	2,440 ± 646 (2)	2,850 ± 483 (6)
Glycyl-glycine dipeptidase (*EC* 3.4.3 1.)‖	73·4 ± 24·8 (6)	47·0 ± 11·4 (4)	45·5 ± 5 0 (4)	286 ± 150 (2)	280 ± 119 (8)
Glycyl-leucine dipeptidase (*EC* 3 4.3.2)¶	187 ± 35 (5)	97 ± 27 (4)	267 ± 73 (4)	7,840 ± 2,000 (2)	6,860 ± 2,590 (6)
Imidodipeptidase (*EC* 3 4.3.7)**	169·0 ± 28·2 (6)	91·7 ± 11·4 (4)	222 0 ± 33·8 (4)	208 0 ± 119 0 (2)	147·0 ± 17·5 (6)
Iminodipeptidase (*EC* 3 4 3.6)††	517 ± 127 (6)	560 ± 150 (4)	235 ± 19 (4)	425 ± 88 (2)	563 ± 106 (6)

Conditions of assay (final concentrations):
* 6 mg denatured Hb/ml., homogenization of cell lysates as described in the text, citrate/hydrochloric acid phosphate buffer.
† 10 mM carbobenzoxy-glycyl-L-phenylalanine, citric acid/phosphate buffer, *p*H 5 0
‡ 20 mM L-leucine amide hydrochloride, 0·2 mM Mn^{2+} veronal buffer, *p*H 8 0
§ 50 mM triglycine, phosphate buffer, *p*H 7·0.
‖ 50 mM diglycine, 0·2 mM Co^{2+}, phosphate buffer, *p*H 8·0
¶ 20 mM glycyl-L-leucine, 1 mM Ca^{2+}, 0 1 mM Zn^{2+}, phosphate buffer, *p*H 8 0.
** 50 mM glycyl-L-proline, 1 mM Mn^{2+}, veronal buffer, *p*H 8 0.
†† 50 mM L-prolyl-glycine, 0 02 mM Mn^{2+}, veronal buffer, *p*H 8·0.

logical basis, indistinguishable from those of acute myeloblastic leukaemia. Their enzyme pattern, however, is evidently different (especially for neutral proteinase, leucine aminopeptidase, and glycyl-leucine dipeptidase) and cannot be distinguished from that of lymphocytes. The cells of the terminal crisis, therefore, should not be termed myeloblasts. Perhaps they correspond to a more primitive type of cell, which is functionally related to lymphocytes[14,15].

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PHYSIOLOGY

Effect of Gonadotrophins on Uterine Growth of Hypophysectomized Mice

It was shown by Lamond and Emmens[1] that the effective dose (*ED*) of human chorionic gonadotrophin (HCG) for uterine growth in intact immature Swiss albino (*S.W.* strain) mice was approximately 0·2 I.U. The *ED* is defined as the amount of gonadotrophin, given in one injection, that will result in uteri weighing approximately 12 mg 44–48 h later (that is, approximate doubling of uterine weight). When the mice were hypophysectomized a few minutes before injection, the *ED* was increased to about 2·0 I.U. However, 5–6 h after hypophysectomy even very large doses of HCG had no effect on uterine weight. We have shown that the time of the day when the mice are hypophysectomized influences the *ED* of HCG when the injection is given at the time of hypophysectomy[2]. The mean *ED* after hypophysectomy at 9–10 a.m. was 3·0 I.U., and after hypophysectomy at 3–4 p.m. was 0·6 I.U.

It is generally accepted that follicle-stimulating hormone (FSH) augments the action of HCG on the ovary. The Steelman–Pohley assay[3], using ovarian weights, is based on this assumption. We have used this phenomenon of augmentation of HCG by FSH for the assay of FSH, but have based our determinations on production of uterine growth-promoting substances produced by the ovary. The standard technique involves hypophysectomy before 10 a.m. followed 5–6 h later by injection of 4·5 I.U. of HCG and the unknown in single subcutaneous injections at separate sites. The mice are killed 44 h after injection and uterine weights recorded. Full details of the assay and certain modifications that should allow its use for routine work have been given elsewhere[2]. The assay is sensitive and precise. It seems to be more reliable than that recently described by Igarashi and McCann[4] since the results are not influenced by luteinizing hormone (LH). Moreover, the possibility of confounding due to diurnal release of gonadotrophins or other substances that might influence the uterine weight response in intact animals is eliminated.

The importance of unknown diurnal factors in mice is shown by the results of a series of FSH determinations after hypophysectomy performed either in the morning or the afternoon (Table 1). The *ED* of a number of gonadotrophic preparations, given alone to intact mice and to mice 5 h after hypophysectomy performed in the morning (8.30–10.30 a.m.) or in the afternoon (3–5 p.m.), and the 95 per cent limits of error are shown on the left-hand side of Table 1. On the right-hand side are the *ED*'s of FSH in the above preparations; these were estimated on the basis of augmentation of HCG when both were given 5 h after hypophysectomy. For a particular preparation either all assays were carried out simultaneously, or the *ED*'s are a mean of a number of determinations carried out on different occasions. In general, there have been no significant changes with time in sensitivity in this colony.

The results show wide differences between the preparations. The differences between assays are summarized in Table 2. It is generally believed that the mouse uterus test measures total gonadotrophin and the HCG-augmentation test measures FSH only.

Column 4 in Table 2 indicates the relative activities of the preparations for total gonadotrophin and FSH. On this basis the preparations fall into three categories: (*a*) Large differences—N.I.H.–FSH and Armour FSH are rich in FSH, but relatively inactive when given alone to hypophysectomized mice. (*b*) Small differences—N.I.H.–HPG and Armour LH are rich in FSH and also relatively active when given alone. (*c*) No change—N.I.H.–LH, ox pituitary fraction and pregnant mares' serum (PMS) evidently contain only small amounts of FSH. The first two are also relatively inactive gonadotrophins, whereas PMS is active in hypophysectomized animals.

Column 5 in Table 2 shows considerable differences in FSH potency between the preparations after afternoon as compared with morning hypophysectomy. The preparations again fall into three groups: (*a*) Large increase in potency was shown by the crude ox pituitary fraction. (*b*) Small increases in potency were shown by N.I.H.–FSH, N.I.H.–HPG and Armour FSH. (*c*) No change or a slight decrease in potency was shown by the remainder.

Table 1. Summary of the Results of Assays of Various Gonadotrophic Preparations giving the *ED*'s and their 95 per cent Limits of Error

Substance*	Standard mouse uterine weight test			HCG-augmentation mouse uterine weight test†	
	Intact mice	Mice injected 5 h after hypophysectomy at			
		8.30–10.30 a.m.	3–5 p.m.	8.30–10.30 a.m.	3–5 p.m.
N.I.H.–FSH–*S1*	510‡	≧500‡	≧500‡	5·0 (4·7–5·3)	1·98 (1·6–2·4)
Armour FSH (pig 216–172–9)	182 (70–474)	330‡	330‡	4·5 (3·6–5·6)	2·4 (1·9–2·9)
N.I.H.–HPG–*UE–B*	22·1 (14·4–33·8)	50 (28·6–87·3)	32 (15·0–60·0)	4·3 (3·5–5·3)	2·0 (1·3–3·0)
N.I.H.–LH–*S5*	470‡	≧500‡	≧500‡	≧500‡	≧500‡
Armour LH (horse 216–171–15)	70·2 (47·8–103·0)	65·4 (49·5–86 4)	43·0 (34·0–56·0)	12·9 (10·3–16·0)	17·2 (16·9–17·4)
Pregnant mares' serum (PMS)	0·12 (0·08–0·17)	1·52 (1·45–1·64)	1·64 (1·27–2·13)	1·1 (0·8–1·5)	0·9 (0·6–1·5)
Ox pituitary fraction	13·2 mg (9·5–18·2)	15·5 mg (11·8–20·2)	17·2 mg (13·9–21·4)	16·0 mg (14·1–18·3)	20·3 μg (15·8–26·0)

* All values not indicated are in μg except for PMS which are in I.U.
† Mice injected at an interval of 5 h following hypophysectomy, which was carried out at the times shown.
‡ Approximate values only; insufficient material for accurate determination.

Table 2. CHANGES IN *ED* FROM ONE ASSAY TO ANOTHER

Assay type:	Standard assay in hypophysectomized mice	Standard assay	Standard and HCG augmentation	HCG augmentation
Comparison	a.m. p.m.	Intact mice : hypophysectomized mice (a.m. and p.m. combined)	a.m. hypophysectomized mice : HCG augmentation after a.m. hypophysectomy	a.m. : p.m.
N.I.H.–FSH–*S1*	*NS*	*NS*	100 1	2 1
Armour FSH (pig 216–172–9)	*NS*	*NS*	100 : 1	2 : 1
N.I.H.–HPG–*UE–B*	*NS*	*NS*	10 : 1	2 . 1
N.I.H.–LH–*S5*	*NS*	*NS*	*NS*	*NS*
Armour LH (horse 216–171–15)	*NS*	*NS*	5 : 1	1 : 1·5
PMS	*NS*	1 : 15	*NS*	*NS*
Ox pituitary fraction	*NS*	*NS*	*NS*	800 · 1

NS, Not significant.

The results suggest certain lines of research:

(1) There was no diurnal effect on potency of PMS, given either alone or with HCG. This suggests that there is no diurnal variation in ovarian sensitivity *per se*; thus, there may be diurnal variation in circulating augmentative or inhibitory substances. Until this is settled, it will be impossible to carry out useful studies of specificity for gonadotrophins in intact animals.

(2) The hypotheses that the diurnal fluctuation in response is associated with diurnal release of gonadotrophin and that the time of release is influenced by the photoperiod have been examined (these results will be published separately elsewhere).

(3) It is evident that neither the PMS nor the LH used here contain any FSH that was able to augment HCG. The test as described could be useful to investigators who are attempting to separate the FSH portion of the molecule from the remainder.

(4) Replacing HCG with LH in the augmentation assay would be an important step towards an understanding of the relation between FSH and LH. Our investigations[2] indicate there is no augmentation between sheep FSH and sheep LH in uterine growth of hypophysectomized mice. However, it is possible that misleading results may be obtained by examining the biological action of gonadotrophins from one species in another species. Techniques for hypophysectomy in the calf and lamb are being investigated in this laboratory in order to carry out combined-action studies in homologous species.

(5) We are widening the scope of these investigations by determining the *ED*'s for ovulation in intact and hypophysectomized mice.

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Diurnal Variation in the Spontaneous Ejaculations of the Male Albino Rat

IT is generally accepted that the sexual activity of the male rat is subjected to a diurnal variation. Thus, Beach and Levinson[1] and Larsson[2] have found that male rats in the presence of females are sexually more active under night conditions. In 1961 Orbach[3] demonstrated that the isolated male rat spontaneously ejaculates. The ejaculates yielded appear as plugs of coagulated semen which can be collected provided the animals are prevented from grooming the penis orally. In the course of an investigation I obtained figures which would be suitable for a study of the diurnal variation in such spontaneous ejaculations of the male albino rat.

Eight sexually inexperienced male albino rats (Sprague–Dawley), 6·5 months old at the beginning of the experiments, were prevented from grooming their penes by a 2 cm broad girdle, made from plaster of Paris, around the chest. Ventrally this girdle was firmly attached to a ring of galvanized iron-wire (diameter 1·5 mm) around the neck. The experiments lasted from April 21 until June 8.

The animals were separately caged in a windowless room and subjected to an artificial diurnal rhythm with a dark period extending from 8 p.m. to 8 a.m. A piece of paper was placed below the net-floor of each cage on which the seminal plugs were collected. Each cage was connected with a clock device and equipped with an electromagnet which automatically placed a sheet of paper below the floor every sixth hour, as indicated in Table 1. Consequently, the ejaculates were collected twice during the light and dark periods, respectively. The papers were removed once a day, and the number of plugs on each sheet of paper counted. The plugs were then dried over silica gel to constant weight.

Table 1. NUMBER OF PLUGS DELIVERED AT SPONTANEOUS EJACULATIONS DURING DIFFERENT PERIODS OF THE DAY

Conditions	Period	Rat No.:	No. of plugs I	II	III	IV	V	VI	VII	VIII	Sum
Light	8 p.m.–2 a.m.		29	16	17	16	18	19	17	35	167
Light	2 a.m.–8 a.m.		13	2	18	8	12	14	14	22	103
Dark	8 a.m.–2 p.m.		2	1	1	0	9	4	9	3	29
Dark	2 p.m.–8 p.m.		14	5	8	6	8	9	5	13	68

Table 2. MEAN WEIGHTS OF THE DRIED PLUGS

Period	Mean weight (mg)	*S.D.*
8 p.m.–2 a.m.	13·45	5·96
2 a.m.–8 a.m.	14·47	6·36
8 a.m.–2 p.m.	12·42	6·67
2 p.m.–8 p.m.	12·23	8·82

As seen from Table 1, there is a diurnal rhythm in the spontaneous ejaculations with the highest frequency between 8 p.m. and 2 a.m., the lowest between 8 a.m. and 2 p.m. The distribution of the plugs between different periods is not a random one ($\chi^2 = 112, f = 3, P < 0{\cdot}0005$). The mean weights of the plugs, on the other hand, do not differ significantly between the different periods (Table 2). Consequently, not only the number of ejaculates but also the amount of semen yielded are at a maximum during the first light hours of the day.

As male rats are sexually more active during the dark phase of their light–dark cycle[1,2], the frequency of spontaneous ejaculations found may be explained in the following way. The activity of the male organs is at a maximum during night conditions. In the presence of females the products of the sexual glands are delivered on copulation. When no copulations occur, as in isolated

males, the sexual products accumulate and may be delivered spontaneously, mainly at the end of the active period. It is, however, so far still an open question whether or not the presence of sexually active female rats modifies the sexual behaviour of the males.

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Accumulation of Excessive Doses of Iodide by the Thyroid Gland of Normal, Hypophysectomized and Thyroxine-treated Rats

The accumulation of iodide by the thyroid gland is customarily measured subsequent to the administration of radioiodide and is expressed either as 'thyroid iodide uptake' (as a percentage of the administered dose) or as 'thyroid : serum radioiodide concentration ratio' (T/S ratio). The literature related to this subject-matter has been amply reviewed by Halmi[1] and more recently by Wolff[2]. Both these authors make it clear that besides thyrotrophin (TSH), other factors, such as dietary iodine for example, play an important part in the regulation of thyroidal iodine metabolism. This communication reports work dealing with the accumulation of inorganic iodide by the thyroid gland of animals subjected to various types of treatment and injected with doses of iodide large enough to practically block the organification of the accumulated iodide[3].

The experimental animals and the experimental procedures have already been described elsewhere[3,4]. Preliminary investigations showed that after the injection of large doses of iodide to normal rats, at any given dose-level, thyroid iodide increased progressively with time, reaching a maximum at 24 h. On the other hand, at any time interval, thyroid uptake values increased as the iodide dose was augmented; however, increasing the dose beyond 100 μg per rat—a dose large enough to block organification of iodide transported into the gland[2,5]—was followed by relatively small increments in thyroid uptake. In the experiments described here, an iodide uptake period of 6 h was arbitrarily chosen.

Rats were divided into different groups as follows: Group I, normal untreated rats; Group II, hypophysectomized rats in which iodide was injected (*A*) 3 days, and (*B*) 10 days after hypophysectomy; Group III, hypophysectomized rats in which iodide uptake was investigated 12 days after the operation and which were injected with TSH (Primothyron, Schering *A.G.*, Berlin) in doses of 0·5 U.S.P. U per rat per day for (*A*) 5 consecutive days, starting on the seventh day after surgery, and (*B*) 12 days, starting on the day following surgery; Group IV, normal rats treated for 14 consecutive days with *l*-thyroxine (25 μg per rat per day). All animals were killed for determination of thyroid iodide uptake 6 h after the injection of 0·1, 1·0 or 10·0 mg of iodide. One μc. of iodine-131 was injected along with the two smaller iodide doses used and 10 μc. with the largest dose used. The results of these experiments are shown in Table 1.

Whereas the thyroid iodide uptake by the normal rats was quite similar at the three dose-levels used, in all other groups increasing the dose resulted in notable increases in the amounts of iodide accumulated. In the hypophysectomized rats of Groups II (*A*) and (*B*), the removal of the hypophysis was reflected in a markedly reduced thyroid iodide accumulation with a dose of 0·1 mg I^-/rat; but, paradoxically enough, with the largest iodide dose used (10·0 mg I^-/rat), thyroid iodide uptake reached levels superior to those encountered in the comparable normal group. To check whether or not the lack of circulating TSH was responsible for this phenomenon, hypophysectomized rats were treated with TSH for 5 or 12 days prior to the uptake. A thyroid stimulation effect was apparent from a comparison of thyroid iodide uptake values of Group II (*B*) and Groups III (*A*) and (*B*); however, here again, although the thyroid iodide uptakes after a dose of 0·1 mg I^-/rat were but a fraction of the normal, with the highest iodide dose the thyroid iodide uptake exceeded that of the normal. Iodide uptake in the thyroxine-treated group following a dose of 0·1 mg I^-/rat was about four times higher than in the hypophysectomized rats of Group II (*B*), thus suggesting that thyroxine treatment does not suppress TSH secretion completely (as was pointed out elsewhere[4]). As the dose was increased to 10·0 mg I^-/rat, however, uptake in the hypophysectomized group was higher than in the thyroxine-treated group. With the lowest iodide dose used, the thyroids of the thyroxine-treated rats accumulated as much iodide as did the glands of rats 3 days after hypophysectomy or of TSH-treated hypophysectomized rats. Nevertheless, as the iodide dose was augmented, the uptake by the thyroid glands of the thyroxine-treated rats was much lower than in either of the latter two groups.

It might be surmized from the foregoing that, unless the noted results were due to the hyperthyroid state of the thyroxine-treated rats, some factor of adenohypophysial origin and other than TSH may play an important part in the control of thyroidal iodine metabolism. On the basis of present-day knowledge of thyroid physiology we can offer no other explanation for the above phenomena. Various additional possibilities were, however, also considered. 'Thyroid iodine uptake' is in reality a balance between iodine transported into the gland and iodine released from it. It may be assumed that in the normal rat an increase in iodide dose beyond a certain level results in no further changes in thyroid uptake because at this high iodide dose-level the rate of entry equals the rate of exit, or that the amount of iodide

Table 1. Effect of Hypophysectomy, of Thyrotrophin (TSH) Administration to Hypophysectomized Rats, and of Thyroxine Treatment of Normal Rats on 6 h Thyroid Iodide Uptake of Large Doses of Iodide

Group No.	Experimental condition	Thyroid iodide uptake					
		I^- dose 0·1 mg/rat		I^- dose 1·0 mg/rat		I^- dose 10·0 mg/rat	
		μg I^-/T*	% N†	μg I^-/T*	% N†	μg I^-/T*	% N†
I	Normal rats (12)‡	2·2 ± 0·4	100	2·0 ± 0·3	100	4·3 ± 1·4	100
II	Hypophysectomized rats						
(*A*)	3 days after surgery (4)	0·24 ± 0·02	11	1·2 ± 0·4	60	10·6 ± 1·8	246
(*B*)	10 days after surgery (10)	0·08 ± 0·01	4	0·6 ± 0·1	30	5·3 ± 0·9	123
III	TSH-treated hypophysectomized rats§						
(*A*)	5 days of treatment (12)	0·32 ± 0·05	15	1·3 ± 0·3	65	6·8 ± 1·1	158
(*B*)	12 days of treatment (8)	0·46 ± 0·07	20	1·4 ± 0·5	70	8·9 ± 1·6	200
IV	Thyroxine-treated rats (8)	0·32 ± 0·06	15	1·3 ± 0·2	65	4·7 ± 1·0	100

* Iodide (μg) per thyroid; values are averages ± *S.D.*
† Per cent of normal uptake (values are approximates).
‡ Numbers in parentheses refer to number of animals per group at each dose-level.
§ Iodide was injected 12 days after hypophysectomy.

transported into the gland reaches a maximum while the exit of iodide remains constant. It is generally known that in the hypophysectomized rat the rate of accumulation of iodide is diminished due to lack of TSH stimulation, as is shown by considerably lower C_{mt} values (C_{mt} being the 'unidirectional clearance of radioiodide by 1 mg of thyroid tissue per minute'[1]). For these thyroids to accumulate more iodide than normal glands would require a marked inhibition of its release from the gland. It was actually shown that the K_{TB} (exit rate constant for thyroidal radioiodide[1]) of hypophysectomized animals is somewhat lower than normal[1,2]. However, if this were due to lack of TSH, then in the TSH-treated hypophysectomized rats the changes in iodide accumulation with increased iodide dose should have approached or at least paralleled those of normal. Diffusion into the gland was also considered, and the uptake values of all glands were corrected by the method proposed by Halmi[6], that is, by subtracting 40 per cent of the value for serum iodide (in mg/100 ml.) from that for thyroidal iodide (in mg/100 mg thyroid tissue). The application of these corrections did not affect the general trend of the results and, moreover, T/S ratios larger than unity were always observed. The increase in uptake with an increase in dose was more restrained in the thyroxine-treated group than in any of the other groups, except for the normal group. Thus it might be tentatively concluded that (1) in the presence of the anterior pituitary gland both entry of iodide into and its release from the thyroid gland are carefully controlled—and apparently not only by TSH—and (2) that when the anterior pituitary is ablated, this control is lost and the amount of iodide entering the gland increases in direct proportion to the increase in the dose of iodide given.

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Epileptiform Seizures induced by Hypothalamic Stimulation in the Rat: Resistance to Fits following Fits

ELECTRICAL self-stimulation procedures in which animals operate pedal-switches to stimulate their own brains are sometimes disrupted by epileptiform seizures. These may appear as convulsive major attacks, or as brief pauses during which the subjects 'freeze' for about half a minute before they resume pedal pressing[1]. In a series of experiments that involved self-stimulation of the hypothalamus in more than thirty rats, we observed that the seizures occurred almost entirely at the beginning of the experimental sessions, and pedal pressing became more regular and rapid after the first few minutes. Fig. 1 is a typical record of this effect.

There are a number of possible causes for this progressive reduction in seizure activity during self-stimulation. A procedural consideration is that stimulus intensities were administered in descending order of magnitude in successive bouts. Also, since fits may be punishing[2], self-stimulating subjects may learn to hold the pedal down for briefer stimulus trains, or to respond at transiently slower rates, so as to avoid producing further fits from overstimulation. It is also possible that the occurrence of a seizure leads to a temporary change within the brain that affords protection against further fits. The following experiment tested these possibilities by administering intracranial stimulation in schedules that were not under the rat's control. In other respects the procedure followed was similar to that described elsewhere[3].

Six rats which had had overt convulsive attacks during previous self-stimulation trials were given a stimulus series of one-per-second, 0·5-sec, fifty-cycle trains from an automatic stimulator in four sessions on each of 2 days. Sessions were scheduled to follow each other at intervals of 30 min, 1 h, and 2 h, in counter-balanced order, and a session will be referred to by the intersession interval which preceded it. The sessions themselves were identical, and consisted of three bouts of 200 stimuli, each bout being followed by a 2-min rest period. The stimulus intensity for each subject was 10 or 20 μamp above the lowest intensity which induced major seizures in preliminary trials. These convulsive thresholds lay between 50 and 90 μamp, and were 10–30 μamp higher than the thresholds for self-stimulation.

Stimulation occurred in a glass tank, and during each bout subjects were closely watched for the onset of convulsions, at which point the stimulating current was switched off. The number of stimuli administered and the duration of clonic activity were recorded, and the bout was resumed 3 min after the seizure had ended.

Table 1. DISTRIBUTION OF FITS OVER BOUTS AND SESSIONS, THEIR MEAN DURATION, AND THE MEDIAN NO. OF STIMULI NEEDED TO PRODUCE THEM

Ranges are standard errors of the mean

Sessions	Bout	Total fits	Duration of fits (sec)	Median stimuli needed
First	1	12*		
	2	1	40±1·9	38·5
	3	1		
After 2 h	1	10		
	2	0	35±2·4	40·5
	3	0		
After 1 h	1	6		
	2	0	33±2·8	55·0
	3	0		
After 0·5 h	1	0		
	2	0	—	—
	3	0		

* Includes two fits which occurred in the same bout. One bout yielded no fits.

Table 1 shows that only two of the thirty recorded fits occurred outside the first bout of a session, and that on only one occasion was there more than one fit in the same bout. Since stimuli were of constant intensity, rate, and duration, the progressive disappearance of fits during self-stimulation can therefore be ascribed to the onset of a central resistant state, and need not depend on changes in the stimulating current. The approximate duration of this resistant state can be inferred from the distribution of fits over sessions: the complete freedom from fits in the half-hour sessions had almost disappeared in the 2-h sessions (only two fit-free first bouts in twelve), and the six fits occurring in the 1-h sessions suggest that, with the current strengths used, about half the protective effect

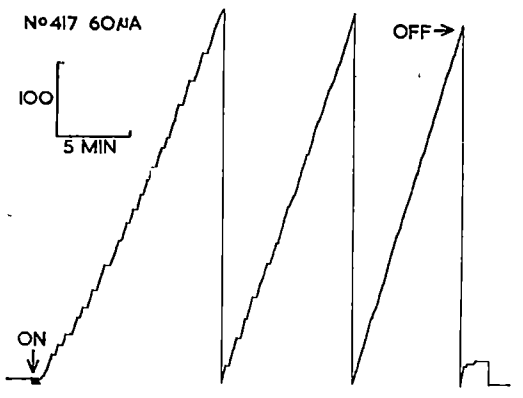


Fig. 1. Cumulative record of 1,500 lever presses during self-stimulation. The first part of the recording was interrupted by a series of non-convulsive seizures, giving the trace a saw-tooth appearance, but the later part of the record is smooth

was still present after 1 h. Table 1 also reveals that the 1-h fits were significantly less severe, in terms of duration, than those occurring in the initial sessions ($t = 2{\cdot}2$, $P < 0{\cdot}05$), and their production required significantly more shocks than the 2-h fits (Mann–Whitney $U = 11$, $P < 0{\cdot}05$).

Clinical evidence of post-ictal resistance to fits is necessarily difficult to obtain, but a recent report describes an epileptic patient in whom self-induced seizures conferred a temporary freedom from spontaneous attacks[4]. The neurological state underlying a resistance of this kind could explain why a fit stops[5], and why identical foci in different patients may or may not give rise to overt epilepsy[6].

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PHARMACOLOGY

Effect of Promethazine Hydrochloride on Hand–Eye Co-ordination

The potential danger to driving and other tasks of barbiturate and antihistamine drugs has been emphasized[1]. While the former have been widely investigated, the central effects of antihistamine drugs are not fully realized, although they are easily accessible to the general public.

An investigation has been carried out into the effect of promethazine hydrochloride ('Phenergan') on visuo-motor co-ordination.

The apparatus consisted of a rotating drum (1 rotation/53 sec) covered with an insulating layer in which 400 holes had been cut. On the 'disk-dotting' principle, and using a steering wheel of 18 cm diameter, the subject followed the irregular course of the holes, so establishing electrical contact and recording the score on a digital computer.

Four healthy male students, aged between 21 and 23 years, took part in a double-blind cross-over experiment in which promethazine hydrochloride (50 mg) was compared with a placebo at 2-weekly intervals. Each subject was fully familiarized with the technique in order to minimize learning effects. They abstained from stimulants and cigarettes during the experiment.

Before, and at 1, 2 and 3 h after administration of the drug or placebo, measurements were recorded over eight rotations of the drum, with an interval of 1 min between each rotation.

The mean values of the highest score of each determination at any one time in the four subjects were obtained and the results are shown in Fig. 1. Promethazine hydrochloride produced a significant fall in score ($P < 0{\cdot}05$) compared with the placebo at 3 h, but no significant difference was seen at 1 and 2 h.

The mean of each eight determinations was compared with the highest value of the same for each occasion in each subject. A highly significant correlation was found between them ($r = 0{\cdot}882$, $P < 0{\cdot}001$).

Although antihistamine drugs are known to produce varying degrees of central nervous depression, few controlled trials have been carried out on specific functions. Impairment of visual flicker discrimination by diphenhydramine (25 mg) was demonstrated by Roback, Krasno and Ivy[2], and Payne[3] found that meclozine (50 mg) significantly impaired memory for verbal material. On the other hand, Colquhoun[4] was unable to detect significant impairment of vigilance or short-term memory after administration of meclozine (50 mg) although it was marked if the drug was given together with 32 g ethyl alcohol.

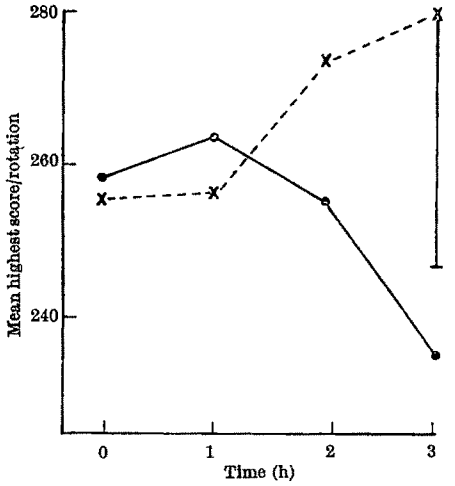


Fig. 1. Changes in mean highest score/rotation at 0, 1, 2 and 3 h after administration of promethazine hydrochloride (50 mg) (——) and placebo (- - - -) in four subjects. The 95 per cent confidence limits (1-tail test) were shown for placebo at 3 h

The present results demonstrate that promethazine hydrochloride significantly impairs hand–eye co-ordination. The dose used was sufficient to produce subjective symptoms of central depression in all the subjects, and further experiments are planned to investigate more fully the effect of smaller doses of this and other antihistamine drugs. This is of importance in view of the ease with which these drugs can be obtained by the general public, and of the possible hazards involved in driving and other complex tasks.

The apparatus used in this experiment was easy to construct and the subjects readily became proficient in its use.

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Effect of Malachite Green, Congo Red and Safranin on Cell Division in Gemmae of *Allium cepa*

Some evidence has been published that certain organic dyes induce inflammatory conditions and even give rise to carcinogenic processes. All these substances belong either to the aromatic order or to heterocyclic compounds containing aromatic rings. The reports published on the harmful effects of these dyes deal as a rule with cases of diseases caused by dyes used in factories and laboratories. The evidence given in these reports is not entirely conclusive, because in production processes the workers come into contact with initial or intermediate products which exert a toxic influence. This is particularly so because many of the intermediate products are amino- or nitro-derivatives of the aromatic order. It is, therefore, evident that in order to solve this problem, experimental investigations on dyes suspected of a toxic or carcinogenic effect are necessary.

Since we are interested in the problem of the effect of carcinogenic compounds on the cell nucleus and its division, it was from this aspect that we commenced our experiments. The gemmae of the underground portion of the onion shoot (*Allium cepa* L.) were used for these experiments.

In carrying out experiments with this material, it was possible to take into consideration only water-soluble dyes. For this reason, from the dyes suspected of carcinogenic properties, we considered Congo red, malachite green and safranin to be the most suitable for our experiments.

We prepared aqueous solutions of these dyes in concentrations of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} per cent. The morphological effector in our experiments was the gemmae of the onion root (*Allium cepa* L.). All the plants were divided into three groups according to the type of dye to be investigated.

Each of these groups was then divided into five groups according to the concentration of the dye, one group being the control group. The plants of the control group grew in distilled water. Since the period of growth for the root lasted only 5 days we did not use any medium, the food reserves in the cotyledons being quite sufficient for that length of time. The onions were placed on cellulose tissue in Petri dishes and covered with 150 ml. aqueous solutions of the dyes. Five days later, ten roots from each of the groups were cut off. The roots were cut 5 mm from tip and prepared by the paraffin method, after which microtome sections 6μ thick were cut. The sections thus prepared were used for the counting of cell divisions. In our calculations, the various phases of division were taken into consideration as well as all deviations from normal mitotic division. The values obtained from each concentration were compared with the values noted in the control group by means of the χ^2 test. As a zero hypothesis, it was assumed that there were no significant differences between the values investigated and therefore these values should not differ from the mean arithmetical values of the group compared. Significant differences were marked '+' (the level of significance being taken to be $\alpha = 0{\cdot}01$). In order to avoid a more detailed description of the findings, we have presented them in table form. Table 1 shows the various phases of division and indicates the normal and pathological divisions. The figures given in the table are the mean values of 10 readings. They were calculated as the mitotic index for approximately 200 cells. In the last column the statistical significance of the observed differences is given. The total number of normal and pathological divisions are presented in Fig. 1. In order to ensure an even distance between the concentrations on the axis, an axis = 7 + log of the concentration was used.

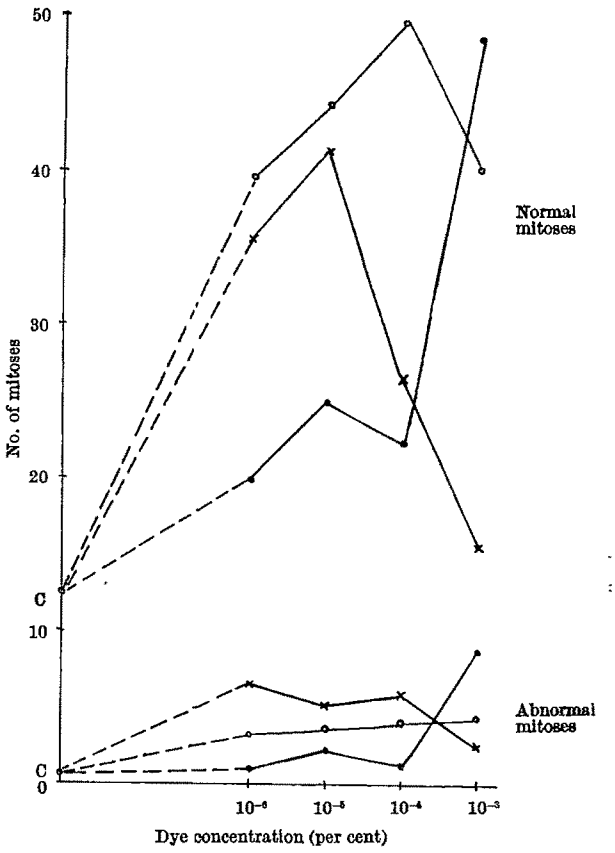


Fig. 1. Curve showing the total number of mitoses in *A. cepa* roots under the influence of malachite green, Congo red, and safranin. ×, Malachite green; ●, Congo red; ○, safranin

Though the dyes used in our experiments are generally considered to be non-toxic, there have been reports that

Table 1. Number of Mitoses in *Allium cepa* Roots growing in Aqueous Solutions of Malachite Green, Congo Red and Safranin

Dye	Dye concentration in per cent	Prophase		Metaphase		Anaphase		Telophase		Total number of mitoses		Values χ^2	
		N.M.	*A.M.*	*N.M.*	*A.M.*	*N.M.*	*A.M.*	*N.M.*	*A.M.*	*N.M.*	*A.M.*	*N.M.*	*A.M.*
Malachite green	10^{-3}	10·3	0·1	3·9	0 8	0·4	1·1	1·0	0·5	15·6	2·5	−3·42	+10 13
	10^{-4}	18·0	0·2	5·2	1·4	1·6	2·8	1·8	1·2	26·6	5·6	+50 84	+38·11
	10^{-5}	25·9	0·1	8·5	1·3	2·6	3·0	4·3	0·8	41·3	5·2	+154·17	+34·32
	10^{-6}	22·3	0·2	5·8	2·8	2·3	2·9	5·2	0·7	35·6	6·6	+110·94	+47·68
Congo red	10^{-3}	34·5	0·9	9·3	3·1	1·6	3 7	3·2	1·1	48·6	8·8	+36	+69·06
	10^{-4}	14·4	0·0	3·8	0 6	2·0	0 8	1·8	0 0	22·0	1·4	+26·14	−2·34
	10^{-5}	17·8	0·1	4·9	0 7	1·1	1·1	1·1	0·3	24·9	2 2	+41·12	+7·76
	10^{-6}	11·9	0·0	6·0	0·6	1·4	0·3	0·6	0·1	19 9	1·0	+16·9	−0·52
Safranin	10^{-3}	28·2	0·3	7 1	0·6	1·4	1·4	3·5	2·1	40·2	4·4		
	10^{-4}	28·8	0·0	10·1	0·4	4 2	2 4	6 6	1·1	49 7	3·9	+222·48	+22 66
	10^{-5}	25·0	0·0	10·0	0·17	4 0	1·7	5·3	1·7	44·3	3·6		
	10^{-6}	22·2	0 0	9·6	1·1	3 8	1·3	4 0	0·9	39·6	3·3	+120 9	+16·9
	Control	7 3	0 2	1 6	0 0	0·8	0·1	2·8	0·4	12 5	0·7		

N.M., Normal mitoses; *A.M* , abnormal mitoses.

Congo red may lead to neoplasms, for example, cancer of the bladder.

Malachite green is strongly toxic to the rabbit, and safranin causes inflammation of the skin and may lead to the growth of malignant neoplasms of the bladder[1].

Investigations on the mitotic activity of various chemical compounds are usually carried out on three models. The model most frequently used is the gemma of the underground shoots of plants from the Liliaceae and Papilionaceae families[2,3]. A modification of this method is used when the investigations are carried out on excised roots[4]. Rapidly dividing animal tissue and tissue culture are also used[1,5–7]. Investigations on the effect of carcinogenic substances, anti-mitotic factors and X-rays have been carried out on these models[3,8]. Keyl and Werth[9], in their cytological investigations on salivary gland chromosomes, found that malachite green caused changes in the structure of those chromosomes.

In our department we have investigated the effect of various salts of the ferrous group (cobalt, nickel and iron) on mitotic processes[2]. In all these investigations, we observed changes in the nucleus, cytoplasm and in cell division. One of the most significant symptoms was a marked disturbance in the mitotic processes of the cells. For this reason, in our investigations, we took into consideration only the effect of aqueous solutions of the dyes on mitotic processes. In each of our experiments it was found that aqueous solutions of the dyes had a stimulatory effect on the mitotic processes and gave rise to a considerable number of abnormal cell divisions.

On the basis of these experiments, it was possible to draw the following conclusions: (1) Some of the dyes used in histological techniques and belonging to the aromatic order or to heterocyclic compounds containing an aromatic ring have an effect on cell division. (2) This effect is manifested in stimulation of cell division, that is, in a higher mitotic index. (3) After exposure of the plants to the action of aqueous solutions of the dyes, a greater number of abnormal divisions is observed. (4) The character of these changes corresponds to those observed when investigating other carcinogenic compounds.

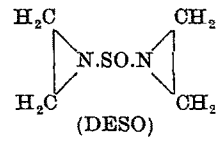
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Hydrolysis of Di(aziridin-1-yl)sulphoxide to Ethyleneimine

Di(aziridin-1-yl)sulphoxide (DESO), which was synthesized as a potential anti-tumour agent, has been shown to possess the property of liberating ethyleneimine in the presence of phosphate ion[1]. This communication reports a more detailed investigation of this effect. Ethyleneimine assay involved the colorimetric measurement (at 420 mμ) of the chloroform extractable dye obtained by reaction of the base with 1,2-naphthaquinone-4-sodium sulphonate in barbitone buffer (0·1 M, *p*H 9·6) The production of ethyleneimine from DESO was verified by comparing the absorption curves of the dyes derived from ethyleneimine and DESO (absorption maxima 303 and 425 mμ). On incubating DESO (2 mg/ml., 37° C) for 2 h in barbitone buffer over the *p*H range 6·4–8·0 there was never more than 3 per cent hydrolysis, but at *p*H 6·5 in phosphate buffer there was 74 per cent conversion to ethyleneimine. DESO was also quite stable on incubation in water, but addition of phosphate buffer resulted in the rapid production of ethyleneimine[1].



(DESO)

In 2·5 M phosphate buffer, *p*H 6·5, DESO (2 mg/ml.) yielded 100 per cent of its ethyleneimine in 10 min. Using 1 M and 0·5 M buffer, 83 and 67 per cent respectively of the available ethyleneimine was liberated in the same period, indicating hydrolysis to be dependent on phosphate ion concentration. On incubating the sulphoxide (0·5 mg/ml., 0·0038 M), in barbitone buffer containing graded amounts of phosphate, the amount of DESO converted during 2 h was proportional to the logarithm of the phosphate concentration when the latter was 0·3–6 mg/ml. (0·0032–0·063 M) (Fig. 1). At ion concentrations of 6–30 mg/ml. (0·32 M) the yield of ethyleneimine was not increased to more than 85 per cent and the *p*H of the solution was reduced.

The rate of ethyleneimine formation, in phosphate buffer, increased with diminishing *p*H and a linear rate of reaction was obtained up to the time when 40–50 per cent of ethyleneimine radicles had been removed from the parent compound (Table 1). It thus appears that one ethyleneimine ring is more readily liberated, although breakdown as a whole depends on phosphate ion concentration.

The mechanism of the phosphate effect has not been established, but a definite reaction between DESO and phosphate is implied, otherwise catalytic amounts of the latter would permit ethyleneimine liberation and the relation shown in Fig. 1 could not exist. It may be that reaction occurs in some way with the H_2PO_4' form, the amount of which also increases as *p*H is reduced.

As regards other ions, sulphate and chloride (up to 0·5 M) had no effect, but sulphite and thiosulphate (0·067 M) caused DESO (2 mg/ml.) to yield 18 and 24 per cent

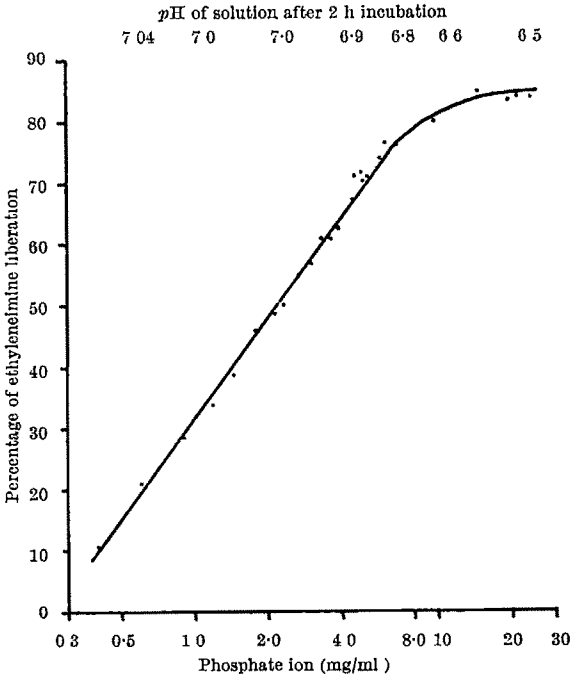


Fig 1 Ethyleneimine production by di(aziridin-1-yl)sulphoxide (0 5 mg/ml.) when incubated (2 h) with various amounts of phosphate in 0·1 M barbitone buffer (0 1 M *p*H 7)

Table 1. Rate of Ethyleneimine Production by Di(aziridin-1-yl)-sulphoxide (1 mg/ml.) when Incubated (37° C) in Phosphate Buffer (0·067 M: *p*H 5·3–8·0)

Time incubated (min)	Percentage of available ethyleneimine liberated			
	*p*H 5·3	*p*H 6·3	*p*H 7	*p*H 8
10	24	20	12	3
20	42	36	23	6
30	54	49	32	9
40	62	57	41	11
50	67	62	47	14
60	69	65	54	16

respectively of ethyleneimine by 2 h. *NNN'N'*-diethyleneurea, which is structurally related to DESO (carbon replacing sulphur), only yielded ethyleneimine in alkaline solution and then only in small amounts (6 per cent in 3 h, *p*H 8).

While it is generally agreed that the chemical reactivity of ethyleneimine derivatives forms the basis of their tumour-inhibitory activity, little is known about the *in vivo* circumstances which may contribute to or inhibit the biological effect. It is possible that the present data explain why DESO fails to inhibit the growth of experimental tumours, since ethyleneimine lacks tumour-inhibitory activity[2].

I thank Dr. H. Jackson for his advice in the preparation of this article.

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Serum Concentration of Phenylbutazone in Tests for Antiphlogistic Activity and under Clinical Treatment

Owing to marked individual variations in the rate of metabolic transformation in man, the daily doses of phenylbutazone used in therapy show a relatively wide range. Burns *et al.*[1] found a biological half-life for phenylbutazone ranging from 1·5 to 6 days, and correspondingly found plateau plasma concentrations ranging from 60 to 150 mg/l. in different individuals. After an initial dose of 800 mg orally followed by 200 mg four times daily, Wilson *et al.*[2] found plasma concentrations from 50 to 100 mg/l. and consider these concentrations necessary for therapeutic effect. Currie[3] found 80–110 mg/l. at the beginning of the therapy and later under maintenance dose-levels of 46–80 mg/l. Bruck *et al.*[4] are of the opinion that, in order to avoid side-effects, plasma concentrations should not exceed 100 mg/l., and during treatment they maintained concentrations of between 50 and 90 mg/l. They propose that treatment should begin with 200 mg per day orally, and that the dose should increase by 100 mg daily until the response is satisfactory.

On an mg/kg base these doses lie considerably below those necessary for the demonstration of antiphlogistic effects in animal experimentation. In experiments in this laboratory, these doses amounted to 25–50 mg/kg when a maximal reduction of several forms of experimental oedema in rats was to be achieved[5,6].

Since it seemed possible that the difference in the dose might be compensated for by the much shorter half-life of phenylbutazone in the rat, we decided to compare the serum concentrations reached after oral application of doses effective in the different tests for antiphlogistic activity in the rat with those determined in patients under treatment with phenylbutazone.

Phenylbutazone was determined spectrophotometrically using the method of Burns *et al.*[1]. Fig. 1 shows the serum concentrations after oral application of 10, 25, and 50 mg/kg in male and female rats. The concentrations present during 5½ h (the duration of the test for antiphlogistic activity) agree closely with those obtained under clinical treatment for the two higher doses. The lower serum concentrations after a dose of 10 mg/kg correspond to the submaximal effect in the test for antiphlogistic activity[5,6]. We are inclined, therefore, to regard the effect of phenylbutazone on the different models of inflammation used in our laboratory (kaolin, aerosil and carrageenin) as a true correlate of clinical antiphlogistic activity, the more so as a similar sensitivity of peripheral processes to drugs should exist even in different species.

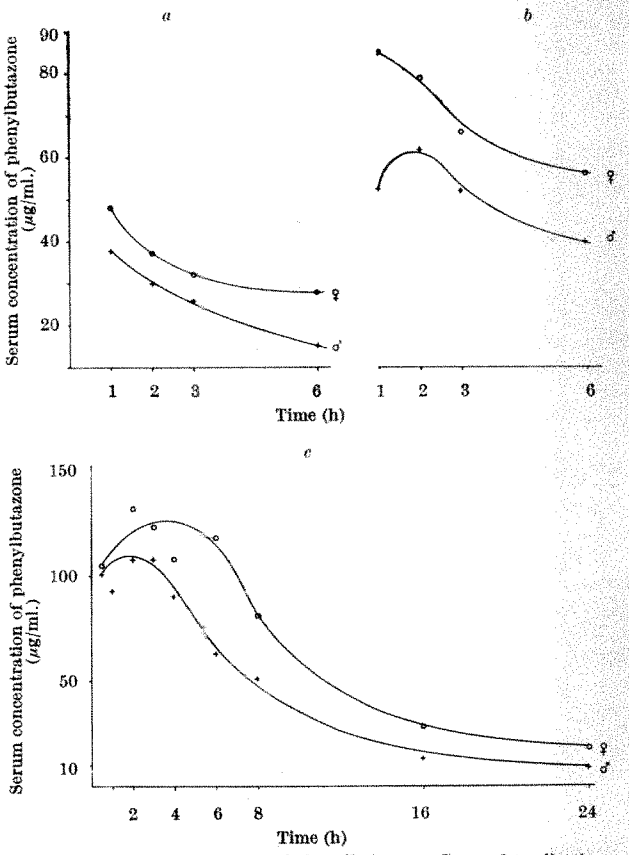


Fig. 1. Serum concentrations of phenylbutazone after oral application to male and female rats (Leo strain). Dosage: *a*, 10 mg phenylbutazone/kg; *b*, 25 mg phenylbutazone/kg; *c*, 50 mg phenylbutazone/kg

It can be seen from Fig. 1 that the concentrations obtained in female rats were always higher than those in the male ones. This sex difference cannot be explained by a different metabolic rate alone, since the half-lives determined for both sexes lie rather close together (5·2 h for the males, 6 h for the females), and since the difference could not be abolished by pretreatment with *SKF* 525-*A*. Besides the difference in half-life, a lower volume of distribution and a higher haematocrit were found in our female rats, all three factors contributing to the higher phenylbutazone serum concentrations in this sex.

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HAEMATOLOGY

Gm(b) Factor in the γ Globulin of Non-human Primates

Recent investigations in man have shown that the Gm factors of γG globulin are linked to non-segregating antigenic determinants which define at least four different classes of heavy polypeptide chains in human γG globulin[1]. Studies in man have also suggested that the Gm(b) factor is not unique, but rather is pleomorphic and may vary in different human races[2]. Certain of the Gm genetic factors including Gm(b) have been recognized in the serum of some non-human primates[3,4]. It therefore became of interest to assess the Gm(b) factor as it occurred in non-human primates, to evaluate any pleomorphism and to investigate its relationship to the H-chain sub-group with which the Gm(b) factor is known to be associated in man.

Table 1. Inhibition of Gm(b) Typing Systems by γ Globulin of Different Primates

Anti-Gm(b) agglutinator from	γ Globulin from	Degree of agglutination with inhibiting γ-globulin at concentration (mg/ml.) 1·0	0·5	0·25	0·12	0·06	0·03	0·15	Sal.
Rheumatoid human	Man*	0	0	0	0	1	2	2	2
	Gorilla†	0	0	0	0	1	2	2	
	Chimpanzee*	0	0	0	1	2	2	2	
	Baboon*	1	2	2	2	2	2	2	
	Old world monkey*	1	2	2	2	2	2	2	
	New world monkey*	2	2	2	2	2	2	2	
	Tamarin*	2	2	2	2	2	2	2	
Immunized monkey	Man	0	0	0	0	1	2	2	2
	Gorilla	2	2	2	2	2	2	2	
	Chimpanzee	2	2	2	2	2	2	2	
	Baboon	2	2	2	2	2	2	2	
	Old world monkey	2	2	2	2	2	2	2	
	New world monkey	2	2	2	2	2	2	2	
	Tamarin	2	2	2	2	2	2	2	
Non-rheumatoid human	Man	0	0	0	0	0	0	1	2
	Gorilla	0	0	0	0	0	1	2	
	Chimpanzee	0	0	0	0	0	1	2	
	Baboon	0	0	0	0	0	0	1	
	Old world monkey	0	0	0	0	0	1	2	
	New world monkey	1	2	2	2	2	2	2	
	Tamarin	2	2	2	2	2	2	2	

* Isolated from pooled serum.
† Isolated from individual serum.

Seven representative species from five different primate families are represented in these investigations: *Hominidae* (man), *Pongidae* (gorilla, chimpanzee), *Cercopithecidae* (old world rhesus monkey, baboon), *Cebidae* (new world spider monkey) and *Callithricidae* (tamarin). Serum pools from each species represented the contribution of 3–12 different animals with the exception of gorilla serum from a single animal. Gamma globulins were isolated by zone electrophoresis in starch. Gm typing was performed by an inhibition of agglutination technique as previously described[5] utilizing three different anti-Gm(b) agglutinators: (*a*) that from a rheumatoid human, (*b*) that from a rhesus monkey which had been immunized with human Gm(b+)γG heavy chains and the serum of which was then absorbed with a human Gm(a+b−) serum, and (*c*) that from a non-rheumatoid human. Each system used red cells coated with an appropriate incomplete anti-D, and gave concordant results in the typing of 21 Gm(b+), 8 Gm(a+b−) and 1 Gm(b+f−) human sera from the White and Negro races. Table 1 presents results of typing the various primate γ globulins. As shown, the rheumatoid agglutinator was inhibited by human and gorilla γ globulins to an equivalent degree, somewhat less by chimpanzee γ globulin, and no inhibition was seen by the other γ globulins. Inhibition of the monkey anti-Gm(b) agglutinator was obtained only by human γ globulin, while investigations with the non-rheumatoid human agglutinator gave strikingly different results. Marked and quantitatively similar inhibition was obtained by γ globulins from man, gorilla, chimpanzee, old world (rhesus) monkey and baboon, while the new world (spider) monkey and tamarin failed to inhibit even at 1 mg/ml.

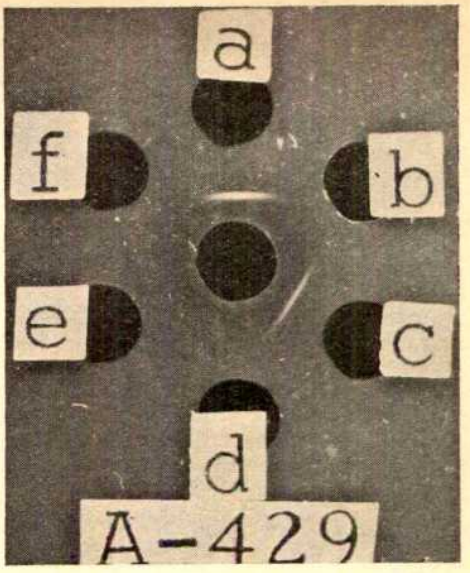


Fig. 1. Reaction of monkey anti-Vi antiserum (centre well) with (*a*) Gm(b+) myeloma protein at 1 mg/ml.; (*b*) gorilla γ globulin at 10 mg/ml.; (*c*) human γ globulin at 10 mg/ml.; (*d*) Gm(a+) myeloma protein at 1 mg/ml.; (*e*) monkey γ globulin at 10 mg/ml.; (*f*) chimpanzee γ globulin at 10 mg/ml.

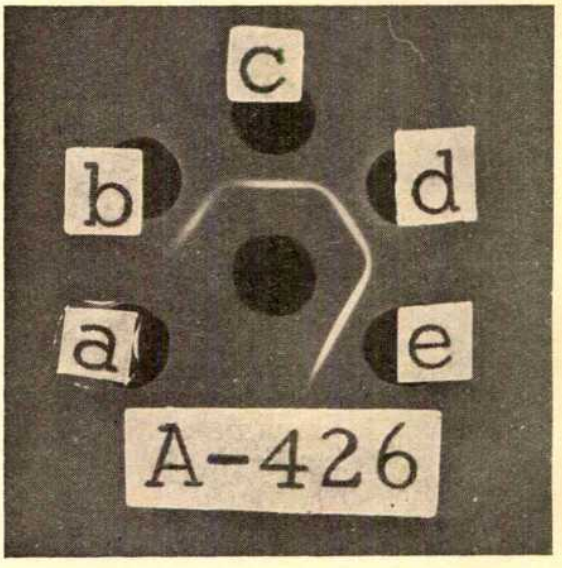


Fig. 2. Reaction of monkey anti-Vi antiserum absorbed with 8 mg per ml. antiserum of (*a*) human γ globulin; (*b*) chimpanzee γ globulin; (*c*) gorilla γ globulin; (*d*) monkey γ globulin; (*e*) tamarin γ globulin; a Gm(b+) myeloma protein at 1 mg/ml. (centre well)

A precipitating antiserum prepared in the monkey against Gm(b+) human γG H chains was specific for the Vi H-chain sub-group after appropriate absorption. This sub-group has previously been shown to be associated with the Gm(b) factor in human γG globulin[1]. Search for the Vi H-chain sub-group determinant with this antiserum was performed in a number of ways. First, double diffusion in agar against γ globulins at concentrations of from 1 to 20 mg/ml. failed to reveal precipitin lines, as shown in Fig. 1. Also, quantitative precipitin investigations with monkey and chimpanzee γ globulins failed to detect precipitated antigen. In order to search for the presence of cross-reacting but non-precipitating Vi antigen, inhibition studies were also done. Aliquots of anti-Vi antiserum were absorbed with quantities of the various γ globulins equal to more than twice the amount of human

γ globulin necessary to absorb completely anti-Vi activity. Typical results are shown in Fig. 2, in which Vi-specific activity remained after absorption. In addition, investigations utilizing inhibition of quantitative precipitation of human γ globulin at the equivalence point by the various primate γ globulins also failed to demonstrate Vi specific antigens in the non-human primates.

These studies would suggest that the Gm(b) factor is not only a mosaic of determinants varying between human races and in certain non-human primate families, but is also probably composed of more than a single determinant in the γG globulin of a single individual. Clearly, the Caucasian Gm(b) has one determinant which is not shared with non-human primates and which was detected in these investigations by an anti-Gm(b) raised in a rhesus monkey. One might anticipate that Gm(b) positive non-human primates may have a similar determinant in their Gm(b) 'area' which is specific for that particular primate family. In addition, at least one Gm(b) determinant found in Caucasian and Negro Gm(b) positive γ globulin occurs in the γ globulin of the *Pongidae* and *Cercopithecidae*, as is evident from their inhibition of the Gm(b) specific haemagglutination system utilizing an agglutinator from the non-rheumatoid human. This probably does not represent simple cross-reactivity in that the inhibition was quantitatively similar to that by human γ globulin. In addition, at present it is conceived that Gm specific agglutinators from non-rheumatoid donors are a uniquely specific form of anti-Gm agglutinator. It is impossible, however, to exclude cross-reactivity between similar but not identical Gm(b) determinants. There is no clear explanation of the variable degrees of inhibition seen in studies with the Gm(b) specific rheumatoid agglutinator. Such reagents may, in fact, be detecting several determinants of the Gm(b) mosaic, which may in turn appear in varying degrees in the non-human primates. Alternatively, cross-reactivity may have influenced results with this reagent to a significant degree.

The failure to demonstrate the Vi antigen to accompany Gm(b) determinants in the *Pongidae* and *Cercopithecidae* is of some interest. It is, of course, impossible to exclude totally the presence of this antigen. It is known that different anti-Vi-specific antisera may detect different areas of the γG globulin molecule[6]. If this indicates polymorphism of the Vi determinant, the use of the Vi specific monkey antiserum may have precluded demonstration of its presence. The most that can be said is that this determinant as it is recognized in man does not seem to occur in association with a Gm(b) determinant recognized by a human agglutinator in these primate γ globulins. This would support the original concept that the H-chain sub-group determinants are distinct from, but linked to, the Gm characters and occur in the same area of the H polypeptide chain, rather than the alternative suggestion that they are an epistatic configuration the structure of which is necessary for the appearance of the Gm(b) factor.

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HISTOCHEMISTRY

Ribonuclease and Deoxyribonuclease Substrate Film Methods: a Pitfall in the Interpretation of Results

DURING the course of histochemical investigations into various metabolic disorders, the substrate-film methods for the demonstration of ribonuclease and deoxyribonuclease[1,2] were examined. Satisfactory thin films were obtained by dipping chemically cleaned slides into the DNA or RNA gelatine mixtures at 60° C and then shaking off the excess liquid. The films were allowed to stand vertically at 60° C for 5 min before wiping clean the bottom quarter of the slide. This procedure gave stable films which did not lift or distort during the subsequent operations of fixation in formalin, incubation and staining. The gelatine was obtained from B.D.H. ('Gold Label' grade), and the DNA and RNA from L. Light.

The apparent distribution of DNase and RNase in tissue sections shown by the methods cited[1,2] led to the conclusion that not all the activity could be attributed to the respective enzyme. It was thought that quite a considerable proportion of the apparent activity was due to proteolytic enzymes dissolving the gelatine film, even though formalin fixed, thus releasing the substrate non-specifically. To test this hypothesis, substrate films of RNA and DNA were prepared in the normal manner and 0·1 ml. water, 0·1 ml. ribonuclease 0·01 per cent, and 0·1 ml. trypsin 0·01 per cent were placed separately on each film and the slides were incubated at room temperature for 1 h. The slides were then washed and stained for 10 min with 2 per cent toluidine blue.

The water controls left both films completely untouched and ribonuclease digested only the RNA film. Trypsin completely digested both films. From these results it is concluded that the present substrate film techniques for ribonuclease and deoxyribonuclease can lead to false results unless the gelatine used is found to be resistant to tryptic digestion after formalin fixation. Much of the apparent activity using B.D.H. 'Gold Label' gelatine is due to the action of proteolytic enzymes. Further work is in progress incorporating inhibitors in the gelatine substrate films and using agar as an alternative supporting medium.

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IMMUNOLOGY

Mouse Thymic Iso-antigens

Old, Boyse and Stockert reported the presence of a common iso-antigen (*LT*) in certain leukaemias of two strains of mice, and in thymus of only one of these strains and one unrelated strain[1]. Later, the *LT* iso-antigen was found in normal thymus of eight out of twenty-four strains[2]. The *LT* iso-antigen was not detected in the normal tissues examined other than thymus[1,2].

Following our initial report of a strong antigen associated with thymic lymphocytes of *AKR* mice[3], thymocytes from eighteen strains of mice were found to possess either the θ-*AKR* or the θ-*C3HeB/Fe* iso-antigens on their surface[4]. These thymic antigens were distinct from *LT*. In the four to six strains of mice that were tested further, neither splenic lymphocytes nor lymph node lymphocytes were cytolysed by iso-antisera that cytolysed thymocytes.

Absorption experiments indicated that splenic and lymph node lymphocytes possessed a low percentage of the θ-iso-antigens present on thymocytes. Among the strains tested, no exceptions were found[4]. Results were sufficient to establish the immunological distinction of *AKR* thymocytes[5].

This communication extends the foregoing findings to eighteen strains or sub-strains of mice. Use of an *AKR* iso-antiserum almost fifty times more potent than previously used[4] has permitted increased precision. Since again no exceptions were found, immunological distinction of thymocytes seems confirmed as a general phenomenon in mice.

The potencies of iso-antisera for immune cytolysis of lymphocytes were determined in a small-scale assay system[4]. Absorptions of iso-antisera by lymphocytes were carried out by a quantitative adaptation[4] of the method of Möller[6] and Amos *et al.*[7]. These two methods should give complementary results, since Möller and Möller have shown that cytolytic potencies are directly related to the content of iso-antigens on the cell surface[8].

Iso-antisera to thymocytes were previously prepared by six weekly intraperitoneal injections of viable thymocytes, plus a single subcutaneous injection of thymocytes incorporated in complete Freund–McDermott adjuvant[4,5]. Immunization of groups of three *C57BL*/6 mice by various schedules showed that adjuvant actually depressed the immune response (Table 1, groups *C* and *G*), perhaps because of the relatively high dose injected (0·2 ml.). A single intraperitoneal injection of viable thymocytes produced detectable sensitization (group *A*).

Table 1. Effectiveness of Immunization of *C57BL*/6 Mice by *AKR* Thymocytes

Group of *C57BL*/6 mice	No. of weekly intraperitoneal injections of *AKR* lymphocytes	No. of subcutaneous injections of adjuvant	Interval between injections and bleeding (days)	Cytolytic potency* of serum against *AKR* thymocytes
A	1	—	10	(24% a.c.)†
B	3	—	10	40
C	6	—	10	2,580
D	—	1	10	<2
E	—	1	31	<2
F	—	1	52	<2
G	6	1	10	860

* Cytolytic potency is expressed as 100/cytolytic titre.
† The highest cytolysis obtained was 24 per cent above the control-level.

Table 2. Immune Cytolysis of Thymic, Splenic and Lymph Node Lymphocytes from Various Strains of Mice by *AKR* Iso-antiserum against *C3HeB/Fe* Thymocytes

Mouse strain	Cytolytic potencies* against lymphocytes from Thymus	Spleen	Lymph nodes
AKR	<2	<2	<2
RF	<2	<2	<2
RFM/Un	<2	<2	<2
C3HeB/Fe	794	(19% a.c.)†	(16% a.c.)
C3H/DiSn	844	(28% a.c.)	<2
C3H/St	614	(19% a.c.)	(17% a.c.)
A/He	398	(18% a.c.)	(17% a.c.)
BALB/c	773	(16% a.c.)	(14% a.c.)
C57BL	873	<2	(17% a.c.)
C57BL/6	429	(22% a.c.)	<2
C57BL/10	794	<2	<2
C57L	574	(27% a.c.)	<2
C58	601	(27% a.c.)	(17% a.c.)
DBA/1	1,000	<2	(14% a.c.)
DBA/2	510	<2	<2
ICR Swiss	429	<2	(20% a.c.)
SWR	482	<2	<2
129	794	<2	<2

* Cytolytic potency is expressed as 100/cytolytic titre.
† The highest cytolysis obtained was 19 per cent above the control-level.

Potent *AKR* iso-antiserum to *C3HeB/Fe* thymocytes was prepared by the most effective schedule of injections (Table 1, group *C*). This iso-antiserum cytolysed thymocytes from mice possessing the θ-*C3HeB/Fe* iso-antigen at high potencies; in contrast, splenic or lymph node lymphocytes were not cytolysed in excess of 28 per cent above the control value (Table 2). Equivalent data were previously reported for cytolysis of lymphocytes from mice that possess the θ-*AKR* iso-antigen (namely, *AKR* and *RF* mice) by *C3HeB/Fe* iso-antiserum[4]; lymphocytes from the *RFM/Un* sub-strain of *RF* mice have now been found to react in a similar manner to those of the parent strain.

The absorptive capacity of splenic lymphocytes was between 6 and 21 per cent of that of thymocytes (Table 3). Equivalent data have previously been reported for *AKR* mice[4]. Since it is known that a critical concentration of iso-antigens must be present on the cell surface before cytolysis can occur[8,9], the present results of cytolysis and absorption experiments are compatible.

Table 3. Absorption of *AKR* Iso-antiserum against *C3HeB/Fe* Thymocytes by Thymic and Splenic Lymphocytes from Various Strains of Mice

Mouse strain	Absorptive capacity* of lymphocytes from Thymus	Spleen
AKR	<5	<1
RF	<5	<1
C3HeB/Fe	100	20
C3H/DiSn	110	7·3
C3H/St	112	21
A/He	57	14·3
BALB/c	164	13·3
C57BL	114	14·9
C57BL/6	139	13·0
C57BL/10	141	12·0
C57L	94	7·3
C58	—	17·3
DBA/1	110	9·6
DBA/2	80	6·4
ICR Swiss	98	19·6
SWR	100	9·6
129	114	9·3

* Calculated relative to absorption by the same number of *C3HeB/Fe* thymocytes.

Crosses were made between *AKR* and *C3HeB/Fe* mice. Thymocytes from the F_1 offspring were cytolysed both by iso-antisera to the θ-*AKR* iso-antigen and by iso-antisera to the θ-*C3HeB/Fe* iso-antigen. The cytolytic potencies were, on average, 39 per cent lower than against thymocytes from the parent strain, which could only be cytolysed by one or other of the two iso-antisera (Table 2, and ref. 4). Similar results were obtained with thymocytes from F_1 offspring of a mating between *RF* and *C57BL*/6 mice. Thymocytes from (*AKR* × *C3HeB/Fe*)F_1 mice absorbed respectively 70 and 66 per cent as much of the two iso-antisera as thymocytes from the respective parents. These data are consistent with a theoretical absorption at an intermediate value, possibly 50 per cent, which would be expected if the θ-*AKR* and θ-*C3HeB/Fe* iso-antigens represented alleles of a single locus that has two sites. Inbred mice are homozygous with respect to one or other of these alleles, and both antigens are expressed in the heterozygous state.

With regard to chemical nature, the θ-*C3HeB/Fe* iso-antigen of thymus was non-dialysable, and could not be recovered in active form after treatment[10] with lipid solvents, such as chloroform–methanol (2 : 1), at 0° C. Treatment with sodium deoxycholate at concentrations between 1 and 30 mg/ml., followed by exhaustive dialysis to remove the deoxycholate, did not increase the antigenic activity of the supernatant obtained after centrifugation at 35,000*g*. On the basis of unit nitrogen content, the microsomal fraction[11] of a homogenate of *C3HeB/Fe* thymus was nineteen times more potent than the whole homogenate, when tested for absorption of an *AKR* iso-antiserum prepared against *C3HeB/Fe* thymocytes. These data suggest that the θ-*C3HeB/Fe* iso-antigen is a lipoprotein.

In the mouse, presence of the *LT* iso-antigen in certain strains that possess the θ-*C3HeB/Fe* iso-antigen indicates that a single inbred strain may possess more than one thymic antigen[4]. In man, the presence of auto-antibodies directed against both thymus and muscle in one-third of a series of patients with myasthenia gravis[12] suggests the presence of iso-antigens shared between thymus and muscle, although other explanations are possible. Both in the mouse and in man much further work seems required to delineate the presence, distribution and significance of thymic antigens.

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Mouse Nervous Tissue Iso-antigens

MUCH work has been done on experimental auto-immunization with nervous tissues, and the relation of the resulting pathology to that found in demyelinative diseases of the human nervous system[1]. Furthermore, the existence of organ-specific lipid haptens has long been recognized; recently, Joffe, Rapport and Graf have identified one of these haptens as a galactocerebroside[2]. No strain-specific iso-antigens were described by these authors[1,2].

We recently described two strain-specific iso-antigens, θ-*AKR* and θ-*C3HeB/Fe*, one or the other of which is present at a high concentration in adult brain and thymus of all mouse strains that were tested[3,4]. In new-born mice of the four strains investigated, these antigens were found at a high concentration only in thymus; neonatal brain contained between 1 and 3 per cent of the antigen concentration found in adult brain. Thus, the two antigens appeared to originate either in thymus or in closely related cellular precursors. These θ-iso-antigens were present at much lower concentrations in lymph node lymphocytes, splenic lymphocytes, appendix, lung and liver. Some leukaemias from *AKR* mice possessed a high content of the θ-*AKR* iso-antigen, while others, apparently of non-thymic origin, possessed a low content. These data were obtained by use of immune cytolysis and of a quantitative absorption procedure[4].

In the present investigation, the same methods but more potent iso-antisera were used. Results of absorption experiments are expressed as the absorptive capacity per unit wet weight of brain. Since the iso-antisera used showed high specificity in their absorption by different tissues, absorptive capacity is taken to parallel the content of θ-iso-antigens[4].

The appearance of θ-iso-antigens in brain of three strains of mice (Fig. 1) paralleled the histological[5,6] and physiological[7] maturation of mouse brain. The θ-iso-antigens remained at very low levels for the first 4 days after birth. Between 10 and 20 days of age, the antigen content rose from approximately 15 to 62 per cent of the adult-level, which was reached at 5–6 weeks of age (Fig. 1).

In addition, groups of two *C3HeB/Fe* mice aged 14, 18, 33, 57 and 81 weeks were investigated for antigen changes. All mice were male except for the youngest group. The mean relative content of θ-*C3HeB/Fe* iso-antigen per unit wet weight of brain was 100, 99, 87, 93 and 101 per cent, respectively. Thus, no significant change in antigen content was observed during adulthood.

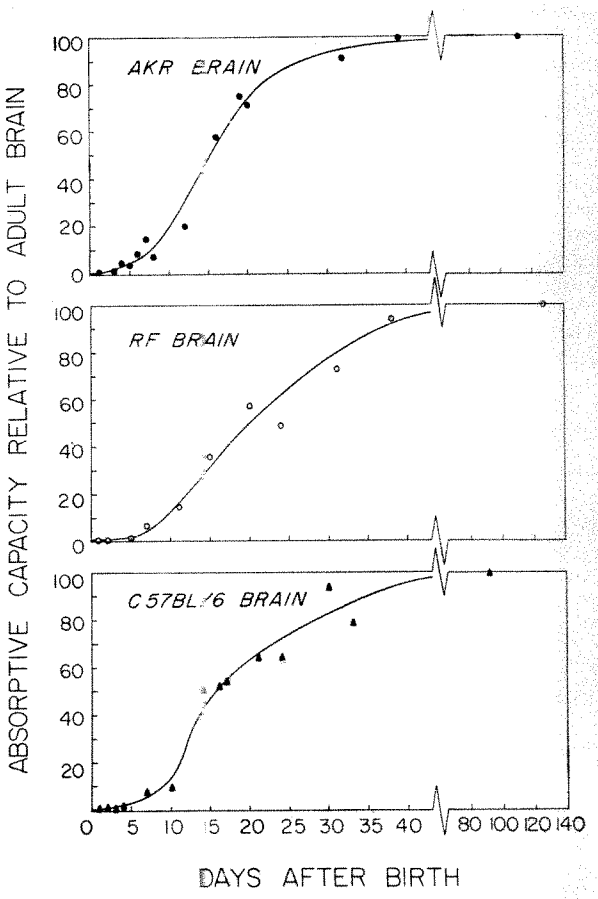


Fig. 1. Development of the θ-iso-antigens in brain of three strains of mice. The absorptive capacity per unit wet weight of brain was determined for *AKR* and *RF* mice with *C3HeB/Fe* iso-antiserum to *AKR* thymocytes, and for *C57BL/6* mice with *AKR* iso-antiserum to *C3HeB/Fe* thymocytes. Most points represent brain pooled from two mice

Previously, we reported[4] that immunological maturation of brain occurs in four strains of mice: *AKR*, *C57BL*, *ICR* Swiss and *A*. This finding has now been extended to eight additional strains: *C3HeB/Fe*, *C3H/DiSn*, *C57L*, *C57BL/6*, *C58*, *DBA/1*, *RF* and *SWR*. Neonatal brain averaged 0·8 per cent of the content of θ-iso-antigen in adult brain of these eight strains. Since no exceptions have been found, the presence of θ-iso-antigens in mouse brain, and their development from low levels at birth to high levels in young adulthood, appear to be general phenomena in mice. These results suggest the possibility that analogous iso-antigens may exist in other species including man, and may be involved in auto-immune diseases of the nervous system[1].

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Comparative Investigation of Immune Globulins of Various Vertebrate Classes

During the past few years our knowledge of the immunoglobulins of man and mammals has been substantially extended. The method of making antiglobulin sera developed by Milgrom *et al.* in 1956 (ref. 1) has played a decisive part in this particular connexion. As is well known, this method makes it possible to prepare antisera which react, in addition to γ-globulins (IgG, IgA, IgM), with complemental factors, if any[2]. Since our knowledge of non-mammalian immunoglobulins is limited[3–10], I prepared (using a modification of Milgrom's method[2]) antisera to the globulins of mammals (horse, cattle, hog), birds (duck, goose), turtles (*Testudo hermanni* Gmelin), and fishes (*Cyprinus carpio* L., *Tinca tinca* (L.), *Perca fluviatilis* L., and *Ictalurus nebulosus* Le Sueur). The serum concentration used to bring about coupling with erythrocytes was chosen so that, for the mammals, only antibodies were detectable against the γ-globulins during immunoelectrophoresis. In this connexion, distinct precipitate lines of the IgG and IgM globulins were always observed. Moreover, for both horse and hog a weak line was observed corresponding to the IgA globulin. The antisera prepared against the globulins of goose and duck showed wide identity during immunoelectrophoresis. Two parallel lines were found that could be assigned to the IgG and IgM globulins (Fig. 1*A*). Clearly visible is a splitting up of the IgG line in the anodic region.

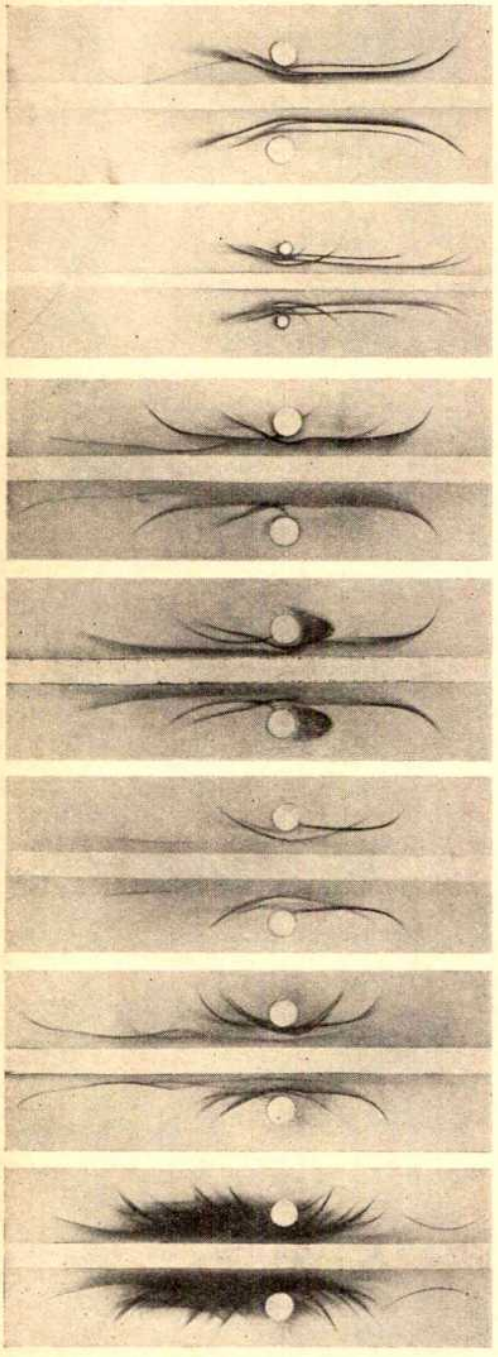


Fig. 1. Immunoelectrophoresis of 'immunoglobulins' (see text) from: *A*, duck; *B*, *Testudo*; *C*, *Cyprinus*; *D*, *Tinca*; *E*, *Ictalurus*; *F*, *Perca*; *G*, normal immunoelectrophoresis from *Perca*. Anode to the left

The immunoelectrophoretic analysis of the antiserum against turtle globulins yielded 4–5 precipitin lines (Fig. 1*B*). Of these, the IgG line shows, at both ends, a splitting that cannot be simply attributed to ageing of the serum. In addition to the typical IgM line two distinct lines were also present in the α_2 region; it is possible that these correspond to complemental factors.

Whereas the analysis of the antisera against mammalian, bird, and turtle globulins shows a certain conformity, the fish globulins differ both from the others and from one another to a considerable extent. It is true that lines corresponding to the IgG globulin are thus found for *Cyprinus*, *Tinca*, and *Ictalurus*, but they are lacking for *Perca* (Figs. 1*C*–*F*). Also, the immunoelectrophoresis with one antiserum against the complete serum proteins of *Perca* confirms the absence of a typical IgG fraction (Fig. 1*G*). Here, only a faint but entirely uniform fraction is found in the cathodic region.

The immune sera prepared using Milgrom's method precipitate for fishes, quite a large number of fractions extending into the α-globulin region, and for *Perca* and *Cyprinus* even into the pre-albumin region. Although further investigations will be required in order to clarify which of these fractions are genuine immunoglobulins, the present results already give some indication of the strong heterogeneity of the immunoglobulins for fishes.

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PATHOLOGY

Leptomeningeal Sarcomata and Gliomata induced in Rabbits by Rous Sarcoma Virus

Much has been written in recent years about the ability of Rous sarcoma virus (RSV) to induce malignant neoplasms in mammals.

Heterozygotic albino mice[1], rats[1–6], hamsters[7,8], guinea-pigs[9], and monkeys[10] were found to be susceptible to the Carr-Zilber[3] strain or Schmidt-Ruppin strain of RSV (ref. 1). Recently, Bryan[11] strain and Mill–Hill (Harris)[12] strain of RSV were found to be oncogenic for hamsters[13,14].

Zilber[15] was the first to inoculate rabbits with Rous sarcoma virus. Numerous fibrous nodules and punctate haemorrhages in various organs were observed following repeated subcutaneous inoculation of Carr-Zilber strain RSV. Usually the fibrous nodules underwent reabsorption[16]. Later, Ahlström[17] subcutaneously inoculated rabbits

Table 1. NEW-BORN RABBITS INOCULATED INTRACEREBRALLY WITH RSV, SCHMIDT-RUPPIN AND BRYAN STRAINS

Experiment No.	Inoculum titre P.F.U.* × 10^7/0·15 ml.	No. of weaned rabbits	Appearance of neurological symptoms (average days)	No. of rabbits with leptomeningeal sarcoma and glioma
Schmidt-Ruppin strain RSV from chicken wing-web's sarcomata. Pool No. 44				
1	3·4	4	28	4
2	1·7	4	28	4
Bryan strain RSV from chicken wing-web's sarcomata. Pool No. 19.				
3	8·5	4	25	4
Bryan strain RSV from chicken wing-web's sarcomata. Pool No. 35.				
4	8·1	3	23	2
5	0·9	3	—	—
Controls				
—	PBS†	3	—	—

The viral inoculum was titrated in chorio-allantoic membrane of embryonated hen's eggs.
Values are expressed in plaque-forming units (P.F.U.).
* Plaque-forming units in embryonated hen's eggs chorio-allantoic membrane.
† Phosphate buffer saline *p*H 7·2 used as diluent.

less than 4-weeks old with Schmidt-Ruppin[1] strain RSV. Fibrosarcoma-like tumours developed at the site of inoculation, but these regressed spontaneously after some weeks. Several of the rabbits treated in this way showed lymphogenic cysts filled with a clear fluid.

The present investigation was undertaken to determine the effects of intracerebral inoculation of RSV, Bryan[11] and Schmidt-Ruppin[1] strain, into new-born rabbits.

Both strains of RSV were prepared by the technique of differential centrifugation[18] from wing-web's sarcomata induced in 2–3-week-old white Leghorn chickens. The cell-free virus preparations were then stored in 0·153 M potassium citrate at −70° C and prepared for inoculations as previously described[8]. The titres of the final concentrations used in the inocula are shown in Table 1.

New-born rabbits (New Zealand White) were obtained from the animal production unit of the National Institutes of Health, and when they were less than 24 h old they were inoculated with 0·15 ml. of viral suspension in the mid-portion of the right cerebral hemisphere. A high degree of mortality was experienced with these animals during the neonatal period. After 3 weeks, subcutaneous fibrous nodules were discernible at the site of inoculation in the surviving animals. At this time the rabbits showed sluggishness, somnolence, lateral posture of the head and left or right hemiparesis (Fig. 1). Some animals were left to die, and some were killed in a moribund condition. Two were killed in fair condition. The results are summarized in Table 1.

At autopsy, the brain was found to be enlarged and an increase in volume of cerebrospinal fluid was noted in rabbits inoculated with the RSV (both strains). Hydrocephalus was present in most of the animals. Gross examination showed medium-size nodules scattered throughout the meninges and focal areas of malacia within the cerebral tissue. Under the microscope the superficially located nodules could be seen to consist of large elongated cells with an oval vesicular nucleus, basophilic cytoplasm with thick processes. These areas appeared to be meningeal

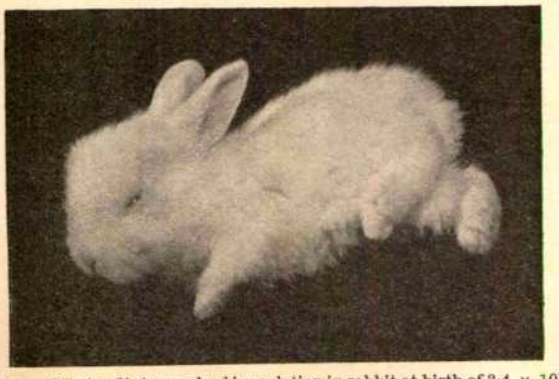


Fig. 1. Effects of intracerebral inoculation in rabbit at birth of 3·4 × 10^7 P.F.U. of Rous sarcoma virus, Schmidt-Ruppin strain. Age of the animal, 31 days. Note lump on the head at the site of inoculation, left hemiparesis and ptosis of eyelid

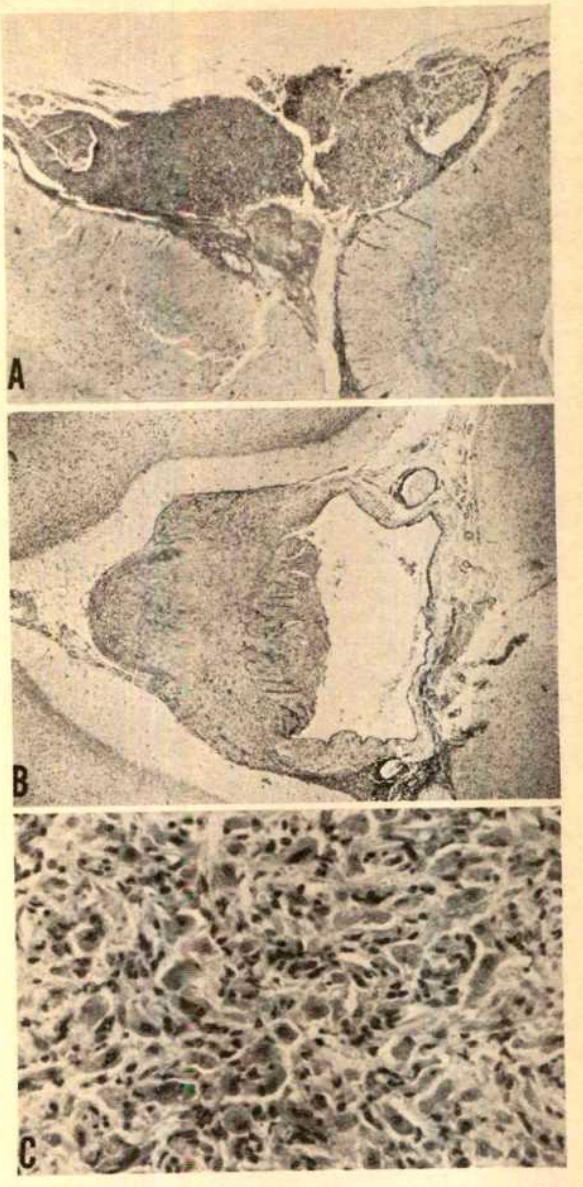


Fig. 2. (Same animal as in Fig. 1.) (*A*) Parasagittal leptomeningeal sarcoma. Haematoxylin and eosin (× *c.* 20). (*B*) Glioma in the periventricular subependymal tissue of the right hemisphere. Haematoxylin and eosin (× *c.* 17). (*C*) Cell morphology of the glioma. Haematoxylin and eosin (× *c.* 240)

sarcomata (Fig. 2*A*). The intracerebral tumours were located in the periventricular subependymal tissue and consisted of round vesicular cells, with basophilic cytoplasm, and thin fibrillar processes. These areas could be classified as gliomata (Figs. 2*B*, 2*C*).

In most of the animals small tumours were observed in the lungs, and in one instance in the liver. Control rabbits inoculated with the suspending medium alone (phosphate buffer saline) failed to show any evidence of intracranial tumours or of pathological changes after 3 months.

It has already been pointed out[8,13] that high doses of RSV are needed to produce oncogenesis in the brain of the hamster. This is also the case for the rabbit. Approximately 10^7 P.F.U. of Schmidt-Ruppin and 10^8 P.F.U. of Bryan strain RSV are needed to produce tumours in the brain of the rabbit.

The presence of tumour growth in the lungs of all rabbits, and in the liver of one treated rabbit, is probably not a case of true metastasis and may be due to viral induced neoplasms in these organs brought about by virus escaping from the brain.

The reason for the regression of the subcutaneous tumours induced in rabbits by Zilber[16] may be due to the difference in the strain of the RSV used. This was not so in the case of Ahlström's experiments[17], for he noted regression of the fibrosarcoma-like lesions of the rabbits using the Schmidt-Ruppin strain of RSV. Using the latter strain we obtained a malignant progression of the induced neoplasia which led to the death of the host. To explain these different results, it is possible that the large doses of virus used in our work may not be the only reason. The choice of the brain as a target organ may be of some importance. The induction of malignant neoplasms by RSV in rabbits has not previously been reported. Brain tumours have not been induced in this host by any virus. The use of these large animals, carrying a primary malignant tumour, should be of interest in cancer research.

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Retardation of Parasitaemia, Prolongation of Life or Survival of Lactating Mice in Infections with *Trypanosoma cruzi*

Krampitz[1] found an endogenous synchronization of the propagation of host and parasite in *Trypanosoma duttoni* infections of mice. Experimental infections with *T. duttoni* at certain periods of pregnancy have resulted in an exceedingly intense and prolonged parasitaemia in mice.

Mice infected with *T. cruzi* have also shown an increase in the number of parasites during pregnancy; however, the increase was only slight compared with that in female control animals.

Lactating mice, however, display a very peculiar behaviour. Development of the parasites is retarded in them; the mice live longer or survive. When given a dosage of $1{\cdot}3 \times 10^6$ trypanosomes per mouse immediately following birth, the life of the animals is found to be about one-third longer than that of normal mice. Infection on the tenth day after birth significantly increases the time of survival. When extremely high doses of trypanosomes ($1{\cdot}3 \times 10^6$) were administered, one-tenth of the lactating mice survived. Lower doses of trypanosomes (namely 700×10^3 to 250×10^3), which would be fatal for normal mice, produce similar conditions. Here, it can be expected that up to one-half the lactating animals will survive. The results are well confirmed statistically.

White female mice, 3–4 months old (nulliparae), of the weight class 33–35 g of the strain *NMR* 1/Tübingen were used for our experiments, as well as trypanosomes of the strain *WBH* (*T. cruzi*, Wellcome Brazil–Hamburg)[2], which had been kept for approximately 1 year by mouse passages. All injections of parasites were given intramuscularly. The mouse litter was constantly adjusted to 10. Parasites obtained from venous tail blood were counted daily in a Thoma haemocytometer. Approximately 250 animals have been used so far in the experiments, which were frequently repeated. The number of mice in the various experimental groups was always kept the same (10) in order to comply with the statistical basis of the experiments.

Further experiments are being undertaken at present, in particular to clarify the cause of these strange phenomena. Therapeutical implications cannot be excluded.

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RADIOBIOLOGY

Plasma Concentration and Excretion of Calcium-47, Strontium-85, Barium-133 and Radium-223 following Successive Intravenous Doses to a Healthy Man

Several investigations have been made of the turnover of radioactive calcium and strontium in man. Qualitatively their metabolism is similar, as is to be expected from their close chemical relationship, but quantitative data reveal biological discriminations which occur in certain organs. Corresponding similarities and discriminations in the turnover of barium and radium have been investigated, but experimental data refer almost entirely to laboratory animals.

In the present investigation, a healthy adult male, sixty years old, was given not only barium and radium but also calcium and strontium so that the metabolism of all four alkaline earths could be compared in the same individual. By this means, biological variation between different individuals was eliminated. Each radioactive isotope was given intravenously on a separate occasion (Table 1), but all four were administered within a 10-week period to minimize any possible temporal changes in turnover. The present preliminary report gives the results for the plasma concentration and excretion following the four successive intravenous injections of the separate radioactive isotopes.

A weighed portion from a solution of the respective carrier-free radioactive solution was made up to 10 ml. in isotonic saline. 4 ml. of a sterile solution were injected into the antecubital vein of the right arm and the remainder was used for the preparation of radioactive standards. Urine and faeces were collected and serial 20-ml. blood samples were taken by veni-puncture from the left arm at 10 min, 3 and 12 h and about 1, 2, 4, 6 and 8 days after each intravenous dose.

For ^{47}Ca, ^{85}Sr and ^{133}Ba, the γ-ray intensity in the principal photopeak (Table 1) was measured with a scintillation γ-ray spectrometer by comparing the counting-rate in the peak with that from a standard solution. In the case of ^{223}Ra, the method of assay for solutions of faecal ash was essentially the same, but in samples of low activity, for example, in plasma, the total α-particle activity in a chemically separated fraction was compared with that from a standard.

The results obtained for the plasma concentrations of the separate radionuclides up to 8 days after injection are

Table 1. Characteristics of Injected Isotopes

Isotope	Half-life	Radiation emitted	Principal γ-ray energy (MeV)	γ-ray abundance (%)	Intravenous dose (μc.)	Date of injection
^{47}Ca	4·7d	β-γ	1·31	77	1·94	26.2.65
^{85}Sr	65·0d	γ	0·51	100	0·54	9.2.65
^{133}Ba	7·5y	γ	0·36	70	2·25	12.1.65
^{223}Ra and daughter products	11·7d	$\alpha\beta$-γ	0·27	~20	0·72	23.3.65

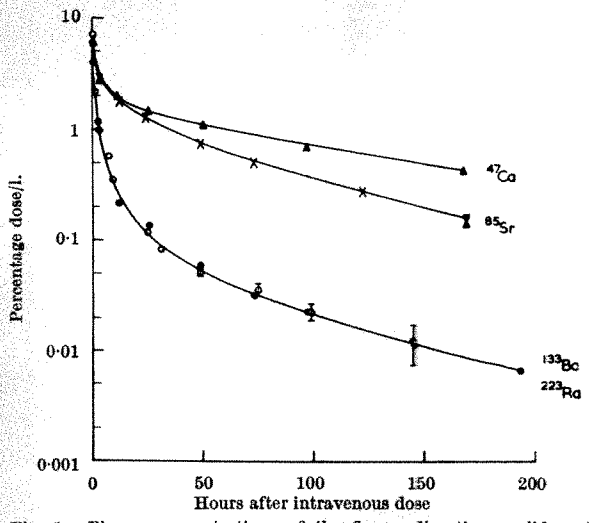


Fig. 1. Plasma concentrations of the four radioactive nuclides at various times after the respective intravenous dose

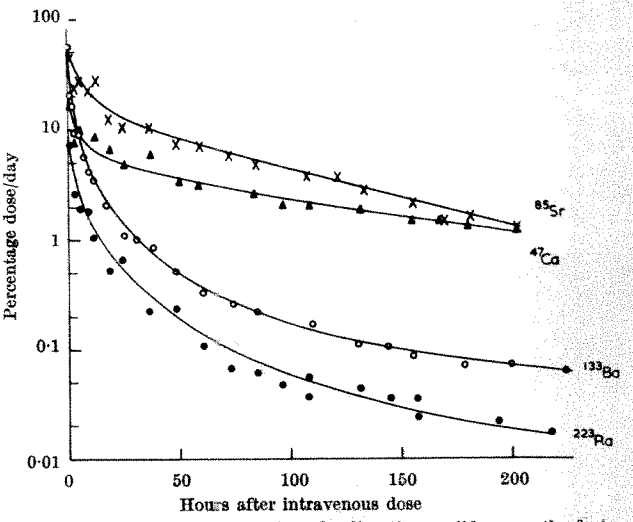


Fig. 2. Rate of urinary excretion of radioactive nuclides over the first 8 days after the respective dose

shown in Fig. 1. The concentrations of ^{133}Ba and ^{223}Ra were indistinguishable over the period of measurement and after a few hours they were more than an order of magnitude less than those for ^{85}Sr and ^{47}Ca.

It is of special interest to note that 8 years ago plasma concentrations of ^{85}Sr were measured up to 50 h after an intravenous dose in the same experimental subject[1]. The results of these earlier measurements are in good agreement with the present ones for which the precision was much greater.

The present values for the plasma concentrations of ^{223}Ra are in general agreement with those observed by Atherton *et al.*[2] following an intravenous dose to a chronically sick man forty-two years old. Bauer, Carlsson and Lindquist[3] measured the plasma concentrations in a woman twenty-five years old following intravenous injections of ^{45}Ca and ^{140}Ba. The fall in plasma concentration with time was in general agreement with the results for ^{47}Ca given in Fig. 1, but for ^{140}Ba corresponding values were about three times greater than the present ones for ^{133}Ba. This discrepancy may well be due to difficulty in the estimation of the β-particle contribution of ^{140}Ba in a mixture of ^{45}Ca, ^{140}Ba and ^{140}La.

The urinary output rate for the four separate radionuclides over the first 200 h after the dose is shown in Fig. 2. Values are shown in Table 2 of the renal clearance, defined here as the ratio of urinary output rate to the contemporary plasma concentration. The renal clearance for strontium was 3·2 times that for calcium in good agreement with earlier measurements on the same man[4] and with clearance ratios reported for different subjects[4,5]. So far as we know, the results for the renal clearance of ^{133}Ba and ^{223}Ra (mean values 10·2 and 3·4 l. per day, respectively) are the first measurements to be reported for man. There is some evidence of a fall in the renal clearance of both ^{133}Ba and ^{223}Ra after the first 24 h (Table 2). This fall could be due to a change in the ion-binding of these nuclides in plasma.

Total excretion (urine plus faeces) is shown in Fig. 3. This was ^{47}Ca < ^{85}Sr < ^{133}Ba and ^{223}Ra; about 85 per cent of the injected dose of the latter pair were excreted in the first 8 days. Values are also given for the ratio of the total faecal to the total urinary output in the first 8 days after the dose. These results show that gastro-

Table 2. RENAL CLEARANCE (L. PER DAY) OF ^{47}Ca, ^{85}Sr, ^{133}Ba AND ^{223}Ra AFTER INTRAVENOUS DOSE

Hours after dose	^{47}Ca	^{85}Sr	^{133}Ba	^{223}Ra
2	3·7	12·4	10·6	3·1
10	3·4	10·5	13·0	4·4
24	3·3	9·4	12·6	4·6
72	3·3	9·1	7·5	2·9
120	3·4	12·2	7·9	2·7
168	3·5	12·0	9·7	2·8
Mean	3·4 ± 0·1	10·9 ± 0·7	10·2 ± 1·6	3·4 ± 0·4

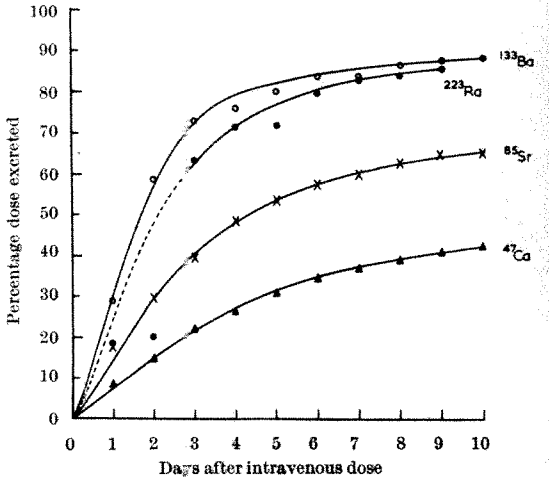


Fig. 3. Percentage of the respective dose excreted over the first 10 days. Ratio of total faecal to total urinary excretion after 8 days

^{47}Ca	^{85}Sr	^{133}Ba	^{223}Ra
0·48	0·25	9·0	36

intestinal clearance of the intravenous dose predominates for ^{133}Ba and especially ^{223}Ra.

A further report will appear elsewhere giving additional results, particularly of the retention measurements. These are still in progress for ^{85}Sr and ^{133}Ba. We expect reliable estimates of the body content of the former isotope to be obtained over the next year and of the latter over several years.

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Formation of Ribonucleic Acid during the Recovery of the Deoxyribonucleic Acid Synthetic System of Bacteria damaged by Ultra-violet Light

A requirement for RNA and protein synthesis in recovery of the DNA synthetic system of *Escherichia coli* damaged by ultra-violet light (UV) has been demonstrated[1–4]. At that dose of ultra-violet light producing the maximum possible delay in DNA synthesis without non-restorable inactivation, an approximate doubling of RNA occurs before initiation of DNA synthesis[4]. If chloramphenicol, an antibiotic which blocks protein synthesis, is added before the doubling of RNA, DNA synthesis proceeds, after the completion of the restoration period, at a reduced, non-accelerating rate directly related to the increase in RNA between the time of exposure to ultra-violet light and the time of adding chloramphenicol[4].

The dye, acriflavine, at appropriate concentration blocks DNA synthesis in the UV-damaged cell but allows formation of RNA in limited amount and at a reduced rate[4,5]. The RNA synthesized is primarily the soluble variety with density gradient sedimentation characteristics comparable with rapidly labelled or messenger RNA; little RNA is incorporated into ribosomal fractions[6]. Acriflavine was added to a UV-exposed culture of *E. coli* (strain *WP2*) after different periods of post-irradiation incubation at 37° C. The results of this experiment are shown in Fig. 1. It was observed that the rate of synthesis of DNA in acriflavine increases with the duration of the post-irradiation restoration period before addition of the dye, as was found with chloramphenicol[4]. Restitution of DNA synthesis is correlated with recovery of capacity for RNA synthesis in the presence of the dye equivalent to the UV-exposed culture incubated without the dye (Table 1) and with the formation of the 30*S* and 50*S* ribosomal fractions[6]. A comparable pattern of effect has been found for chloramphenicol on RNA synthesis (but not on formation of ribosomal fractions) in UV-exposed cultures. These results suggest that restorative processes in the UV-exposed cell restitute some damage, presumably to the DNA, with which the dye interacts to block DNA synthesis and the synthesis of the RNA involved in formation of the ribosomal fractions. It would appear that, for restoration of synthesis to occur, some synthetic event blocked by acriflavine, which is located randomly in the population in regard to time elapsed from initiation of the restorative process, must occur before addition of the dye. That this event involves protein synthesis is suggested by the comparable results with chloramphenicol[4]. Thus, when the dye is added subsequent to the synthetic event for a given bacterium, synthesis of RNA involved in formation of ribosomal fractions proceeds (Table 1) but initiation of DNA formation requires a restorative period subsequent to addition of the dye (Fig. 1).

Table 1. Effect of Acriflavine, added after Different Periods of Incubation following Exposure to Ultra-violet Light, on Subsequent RNA Synthesis with *E. coli* Strain *WP2*

Time of AC addition (min)*	Relative amount RNA with incubation† Post-irradiation incubation (min) 30	45	60	75	90	105	120
0	1·23	1·30	1·45	1·52	1·53	1·57	1·52
10	1·22	1·39	1·52	1·62	1·64	1·62	1·62
20	1·25	1·39	1·64	1·67	1·75	1·75	1·75
30	1·39	1·43	1·66	1·87	1·89	2·12	2·06
40	1·41	1·56	1·72	1·94	2·12	2·25	2·38
No AC	1·41	1·61	1·83	1·96	2·07	2·31	2·66
No UV, no AC	1·76	2·26	2·77	—	—	—	—
No UV, + AC	1·62	2·14	2·63	—	—	—	—

* See the legend for Fig. 1 for details of this experiment. The cultures were exposed to ultra-violet light (45 sec) and then incubated at 37° C. At the indicated times after exposure to ultra-violet light, acriflavine (AC) was added and incubation continued (please read across). The culture labelled 'No AC' had no acriflavine added. The culture labelled 'No UV, no AC' was an unirradiated control culture with no acriflavine in the incubation medium. The culture labelled 'No UV, + AC' was an identical culture with acriflavine in the incubation medium.

† Relative amount of RNA at the time of sampling after the indicated period of incubation over that at the time of exposure to ultra-violet light. This amount (1·0) was 150 μg per ml. of culture.

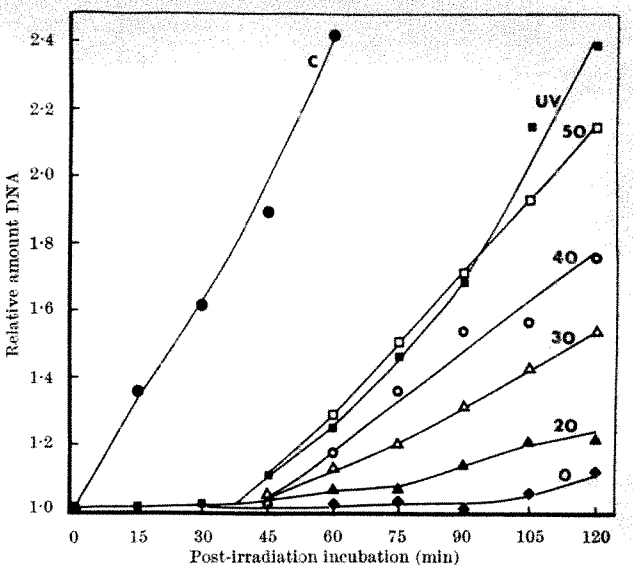


Fig. 1. DNA synthesis in acriflavine added after different periods of incubation at 37° C after exposure to ultra-violet light of a culture of *E. coli* strain *WP2* (tryptophan requiring). The exposure was for 45 sec at a dose rate of 10 ergs/mm²/sec as measured by a Hanovia ultra-violet meter at the position of the cells. The time of addition of acriflavine (0·5 μg/ml.) to each culture after exposure to ultra-violet light is denoted (in minutes) by the number labelling each curve. The curve labelled '*UV*' denotes a culture exposed to ultra-violet light and incubated without addition of acriflavine. The curve labelled '*C*' represents an unirradiated control culture incubated without addition of acriflavine. Acriflavine at the concentration used does not significantly inhibit the formation of DNA in an unirradiated culture. The amount of DNA at the time of initiation of incubation in 1 ml. of the culture was 20 μg. The techniques used have been described[7]. All incubations were in minimal medium supplemented with 20 μg per ml. of L-tryptophan

The synchrony of initiation of DNA synthesis in UV-exposed cultures[4] could be explained if it were assumed that the completion of a sequential cycle of repair of the damage done by the ultra-violet light to the bacterial chromosome is necessary for resumption of DNA synthesis. Evidence has been presented that the decrease in rate of DNA synthesis caused by addition of chloramphenicol prior to complete recovery involves the inhibition of synthesis of part of the DNA[4]. The results with chloramphenicol (and acriflavine) could be explained if at some site on the circular chromosome, randomly situated in the bacterial population in relation to the site of initiation of restoration of damage done by the ultra-violet light, protein synthesis were necessary for the continuation of restoration. Thus, only when the restorative process has passed the chromosomal site requiring protein synthesis by the time of chloramphenicol addition would repair of the chromosome be continued to completion and the initiation of DNA synthesis occur.

The suggestion has been made that complementary RNA formation on the DNA template is involved in restitution of the UV-damaged DNA synthetic system[4,7]. That RNA synthesis (but not protein synthesis) is required for restoration of the UV-damaged DNA synthetic system subsequent to the requirement for protein synthesis already described can be supported by the following experiment in which chloramphenicol and 6-aza uracil are added to UV-exposed cultures of *E. coli* after 30 min of post-irradiation incubation and removed after 50 min (Fig. 2). Chloramphenicol (which inhibits protein but not RNA synthesis)[8,9] in a UV-exposed culture during this period allows completion of restoration, as indicated by initiation of DNA synthesis at the same time as observed with a UV-exposed culture incubated without chloramphenicol. However, 6-aza uracil (which inhibits both RNA and protein synthesis in bacteria)[3,10] in a UV-exposed culture[3] during this period results in a delay in initiation of DNA synthesis for 10 min after removal of the bacteria from the analogue, a period equal in duration to that period of time between addition of the analogue and the initiation of DNA synthesis as observed with UV-exposed cultures

Table 2. DELAY IN DNA SYNTHESIS WITH UV-EXPOSED *E. coli* STRAIN *WP2* INDUCED BY DIFFERENT PERIODS OF INCUBATION WITH 6-AZA URACIL DURING THE RESTORATION PERIOD

Time of addition of 6-aza uracil (min)*	0	10	20	30	40
Projected time of initiation of DNA synthesis†	72	66	58	51	41

* See the legend to Fig. 2 for details of this experiment. The cultures were exposed to ultra-violet light (45 sec) and incubated at 37° C. 6-aza uracil was added at the indicated times after exposure and incubation continued. The bacteria were removed from this medium by filtration after 40 min of post-irradiation incubation and resuspended for further incubation in fresh medium free of the analogue.

† Determined by projection to a relative DNA concentration of 1·0 (see Fig. 2) of a straight line through four points representing relative amounts of DNA determined at 10-min intervals of sampling after initiation of measurable DNA synthesis. The rates of DNA synthesis observed in these cases were comparable with that observed for the 6-aza uracil incubated culture as reported in Fig. 2.

incubated without the analogue. That the increased delay in DNA synthesis caused by prior incubation with the analogue is related in duration to the period of interruption of the post-irradiation recovery process is demonstrated by the results of another experiment (Table 2). In this the analogue was added after different periods of incubation after exposure to ultra-violet light and removed at the time of initiation of DNA synthesis in the UV-exposed culture incubated without the analogue. That chloramphenicol blocks the formation of ribosomes but allows formation of RNA to occur[8,9] suggests the possibility that the RNA which is involved in restoration of the UV-damaged DNA synthetic system is complementary RNA formed at the site of repair. The assumption that a 'node' of RNA synthesis on the DNA template at the time of exposure to the ultra-violet light, randomly situated in relation to the origin of the chromosome, is the site of post-irradiation initiation of the RNA synthesis involved in restoration and that protein synthesis is required for this node to pass the chromosomal origin (or some other site) would be in harmony with the foregoing observations. The results with acriflavine (Table 2) suggest that this protein formation somehow triggers the formation of ribosomal RNA.

The nature of the molecular mechanism by which RNA synthesis effects restoration of the UV-damaged DNA synthetic system cannot be defined at the present time. It has been suggested that such complementary RNA might function in some undefined manner in the restitution of the DNA after enzymatic excision of a region of the DNA strand containing a UV-induced lesion, such as the thymine dimer[4]. This hypothesis would imply that the formation of DNA strands in the region left after excision of the thymine dimer region occurs sequentially along the chromosome and is dependent on RNA synthesis. Evidence for such synthesis of DNA during the restoration period has been presented[11]. However, the fact that restoration of damage done by ultra-violet light occurs in a thymine-requiring strain deprived of thymine during the restoration period[1–3] casts doubt on the assumption that restoration requires sequential resynthesis of DNA in the excised regions of the chromosome during the restoration period. This would not preclude resynthesis of the excised regions on addition of thymine and initiation of DNA synthesis, as a result of the previously completed restoration process involving RNA synthesis. The suggestion has been put forward that complementary RNA derived from the opposite DNA strand functions as a template in by-passing sites of UV-damage to the DNA strand[7].

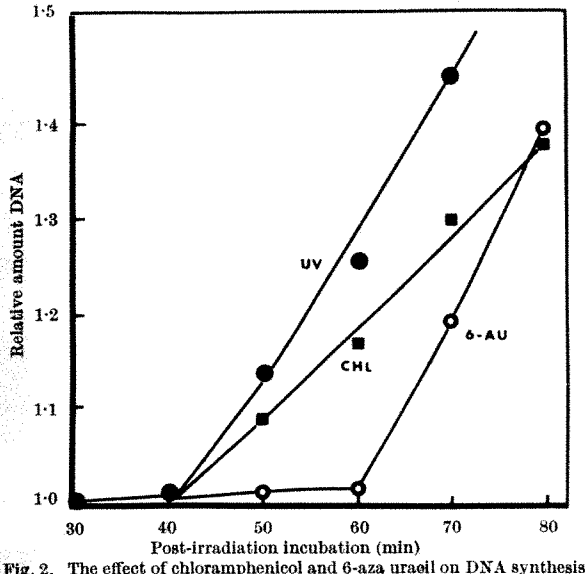


Fig. 2. The effect of chloramphenicol and 6-aza uracil on DNA synthesis in UV-exposed cultures of *E. coli* strain *WP2* (tryptophan requiring). These antimetabolites were added to separate UV-exposed (45 sec) cultures after 30 min of post-irradiation incubation in tryptophan-containing minimal medium at 37° C. The resulting concentration of chloramphenicol (*CHL*) was 40 μg per ml. and that of 6-aza uracil (*6-AU*) was 50 μg per ml. The curve labelled '*UV*' denotes DNA synthesis in a UV-exposed culture incubated without addition of either of the antimetabolites. After 50 min of post-irradiation incubation, the three cultures were separated from their growth medium using membrane filters, washed with warm (37° C) tryptophan-supplemented minimal medium and resuspended to the same volume in the same warm medium. This operation took less than 2 min. Incubation was then continued. The techniques used have been described[7]. The amount of DNA in 1 ml. of culture at the time of initiation of incubation was 24 μg

These results suggest the possibility that complementary RNA is an essential component of the DNA synthetic system in the unirradiated bacterial cell and that post-irradiation RNA formation is necessary for repair or replacement of sites damaged by ultra-violet light in this RNA. This possibility might underlie the observation[4] that a doubling of RNA occurs before initiation of DNA synthesis in cultures exposed to that dose of ultra-violet light which produces maximum delay in initiation of DNA synthesis without non-repairable inactivation. It could be assumed that at this dose almost complete replacement of the RNA involved in restoration is necessary. The reduced duration of the delay in initiation of DNA synthesis with lower doses of ultra-violet light[4] would be related to the possibility that with lower doses of ultra-violet light, the RNA involved need only be partially replaced and that the sequential restoration mechanism is able to pass rapidly over regions of undamaged RNA. All these considerations imply that complementary RNA formation on the DNA template encompasses (or controls) gross replication of RNA in the cell. The concept that prior gross RNA formation somehow limits subsequent DNA synthesis in the growth cycle of the bacterial cell is supported by a recent investigation[4,12]. Thus, involvement of protein formation in initiation of the cycle of DNA synthesis may be indirect and dependent on the necessity of protein formation for continuation of RNA synthesis past the chromosomal origin and for the formation of ribosomes.

Some implications of these findings for the understanding of mutation in bacteria induced by ultra-violet light will be presented elsewhere[6,10].

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BIOLOGY

Myo-inositol-2-t Incorporation by Germinating Pollen

Pollen of pear (*Pyrus communis*) germinated *in vitro* in 10^{-3} M *myo*-inositol-2-*t* rapidly incorporates label into its 70 per cent ethanol-insoluble polysaccharides[1]. Analysis of pectinase hydrolysates recovered nearly all this label in D-galacturonic acid and L-arabinose residues. Other investigations[2,3] have shown that this conversion is highly specific and that the original carbon sequence in inositol is conserved. Hexose constituents present in these 70 per cent ethanol-insoluble pollen residues, principally D-glucose and D-galactose, are essentially unlabelled.

This communication offers autoradiographic evidence to show that *myo*-inositol-2-*t* taken up by germinating pollen and incorporated into alcohol-insoluble tissue residues is initially localized in the pore region of the pollen grain; with germination the label appears in pollen tube membranes.

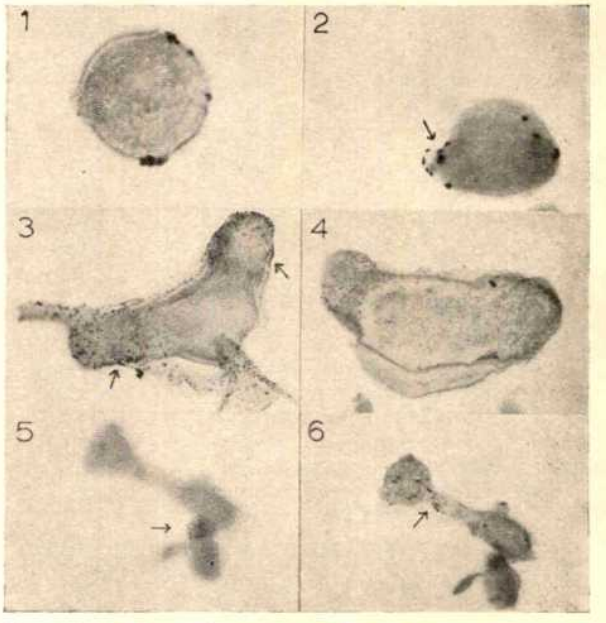
Pollen of *Oenothera organensis*, from the Botanical Garden, University of California, Berkeley, and of *Pyrus communis* var. 'Packham Triumph', from Mina T. Firman Co., Chelan, Washington, was used in these experiments. In a typical run, *Pyrus* anthers collected during the spring of 1963 and stored over desiccant at $-15°$ C for 5 months were returned to room temperature and shaken in a small straight-walled polyethylene container. The pollen grains clung to the plastic container walls and the anther residue was discarded. Pollen samples of about 50 mg were scraped from the plastic container walls and sprinkled on the surface of a solution in a 4·5-cm diam. Petri dish containing 0·4 M sucrose, 10^{-3} M calcium phosphate (*p*H 7), 10^{-3} M sodium borate, and 4×10^{-3} M *myo*-inositol-2-*t* (34 μc./mg). Incubation at 26° C for 0·5–3 h resulted in germinated pollen grains with tubes up to 100μ in length. Following incubation, each portion was fixed in 15 ml. of a 3 : 1 mixture of ethanol and glacial acetic acid. Within 30 min, most of the pollen settled to the bottom and excess fixative, together with most of the labelled media, could be drawn off with a Pasteur pipette. Fixed tissues were re-suspended in fresh fixative and allowed to settle several times to remove all traces of soluble radioactivity. After the final wash, the pollen was transferred to paraffin for sectioning.

Under Kodak 'Safelight' filter Wratten 1, sections were laid on pre-warmed slides coated with photographic emulsion (plate type *V*1060, Kodak, Ltd., England), covered with a thin sheet of 'Teflon', and pressed firmly against the emulsion by applying light pressure with a hand roller. Slides were then stored in light-tight boxes for 2–4 weeks. After storage, each slide was dipped into 1·25 per cent cellulose acetate emulsion and drip-dried at least 3 h to secure the pollen sections in place during subsequent steps of photographic development and staining[4]. Stained, developed slides were examined microscopically for comparison of morphological features and occurrence of silver grains sensitized by tritium in the film directly beneath the sections. Photographic records were made with a 'Polaroid-Land' camera using 'Polapan' 200 type 42 film.

In Figs. 1 and 2, pollen of *Oenothera*, grown for 30 min at 26°, reveals a highly localized distribution of tritium in pore regions. All three pores of *Oenothera* incorporated tritium, although the planar sections seen in these figures prevent one from viewing the third pore. Similar results were obtained with pollen of *Pyrus*. Evidence that considerable metabolism occurs in pollen grains even before tubes become microscopically visible has been obtained with *Petunia* pollen[5]. Heslop-Harrison[6] has published interesting electron micrographs in which he notes a distinct modification of the plasmalemma underlying the pore area. As these areas develop during the formation of pores, an underlining plate of endoplasmic reticulum forms. There is no wall growth over these pore areas during subsequent development and maturation of the pollen grain. Electron micrographs prepared by M. Ledbetter (personal communication) also clearly reveal the presence of a reticulate plasma membrane which penetrates the intine layer in the pore region of mature *Saintpaulia* pollen. The present observations merely reveal incorporation of tritium into tissue structures resistant to the extractive procedures used during tissue fixing and staining. However, one can assume from our previous investigations that the structures involved include labelled pectic substance derived from metabolism of *myo*-inositol-2-*t*.

As germination proceeds and the tube elongates, tritium localizes in the non-extractable portions of the tube membrane (Fig. 2) and accumulates in an annular pattern about the circumference of the pore at the tube base. In Figs. 2 and 3, sections through sucrose-germinated pollen of *Oenothera* reveal a concentration of tritium (indicated by arrows) coincident with the basal wall regions a short distance beyond the point where the tubes emerge from the pore. Similar observations made in *Pyrus* pollen can be seen in Figs. 5 and 6, in which the same view is presented at two focal depths. In Fig. 5, a high concentration of tritium is revealed by oblique section through the base of one pollen tube, while in Fig. 6 a second pollen tube in approximately longitudinal section reveals the same pattern close to the juncture of tube and pore. These accumulations of tritium might represent regions in which pectin is deposited at the time of germination. Alternatively, these accumulations might result from pectin formation in the pore surface prior to germination, in which case eruption of the pore membrane and elongation of the pollen tube would create an annular ring of tritium-labelled tissue about the pollen tube at its basal end.

In one experiment with pollen of *Oenothera* and *Pyrus*, sucrose was replaced with three molar equivalents of D-glucose in the germination medium. As seen in Fig. 4, non-extractable tritium from *myo*-inositol-2-*t* metabolism appeared throughout cytoplasmic regions, especially in



Figs. 1–6. Fig. 1. Autoradiograph of a fixed section of *Oenothera organensis* pollen which has been germinated at 29° C in a solution containing 0·4 M sucrose, 0·001 M calcium phosphate (*p*H 7·0), 0·001 M sodium borate and 0·004 M *myo*-inositol-2-*t* (34 μc./mg) for 30 min. Fig. 2. Same as Fig. 1 but germinated about 50 min. Fig. 3. Same as Fig. 1 but germinated about 90 min. Fig. 4. Same as Fig. 3 except sucrose was replaced by 1·2 M D-glucose. Fig. 5. Same as Fig. 1 but with *Pyrus communis* pollen germinated for 60 min. Fig. 6. Same section shown in Fig. 5 but taken at a different focal depth

the regions behind each pollen tube tip. Glucose, but not sucrose, is readily utilized by germinating *Pyrus* pollen for biosynthesis of pectic substance (unpublished observations). In the present experiment, it appears that glucose does not interfere with movement of *myo*-inositol into cytoplasmic regions or incorporation of *myo*-inositol into cytoplasmic structures which resist extractive procedures used during fixation but does prevent subsequent mobilization of tritium-labelled metabolites into cell-wall structures. Additional experiments are necessary before detailed evaluation of the glucose effect can be attempted.

In summary, autoradiographic experiments in which pollen grains were germinated in a sucrose-rich media containing *myo*-inositol-2-*t* reveal a highly localized pattern of tritium incorporation into non-extractable tissue structures in the pore region shortly after the pollen is placed in the labelled medium. During subsequent tube development, tritium is incorporated into structures of the walls and membranes of fixed pollen tube sections. From related investigations, it appears that these tritium accumulations represent regions of deposition of pectic substances.

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An Adaptive Butyrase Enzyme System in the Alga *Prototheca zopfii*

Anderson[1] reported that *Prototheca zopfii*, which is a colourless alga, was able to assimilate most of the monosaccharides, and all the fatty acids with the exception of formic, isovaleric and pyruvic acids. Pyruvate could, however, become assimilated if the *p*H were lowered to 4·5, and this also affected the rate of oxidation of the carbon energy source.

During confirmation of this work, *P. zopfii* (Strain 263/5) from the National Collection of Algae and Protozoa, Botany School, Cambridge, was grown in liquid culture consisting of a carbon energy source at a dilution of 0·1 per cent in a basal medium suggested by Whickerham[2]. Glucose, ethanol, and the sodium salts of lactic and butyric acid were investigated. The inoculum was derived from a culture adapted to glucose utilization by continual sub-culture on a 0·1 per cent glucose supplemented (Whickerman, 1951) medium. All cultures were incubated at 25° ± 0·5° C and aeration improved by sloping the tubes containing 5 ml. of medium to angle of 10° from the horizontal. Preparation of the inoculum was carried out by centrifuging out the cells from liquid culture at 4,000 r.p.m. for 20 min and washing the cells with sterile distilled water. 10×10^5 cells were used as the inoculum for each culture. Growth was estimated using an EEL nephelometer and obtaining cell population by comparison of the readings to a standardized graph relating nephelometer readings to gross cell density in the culture. All the results for the four carbon substrates used are recorded in Fig. 1.

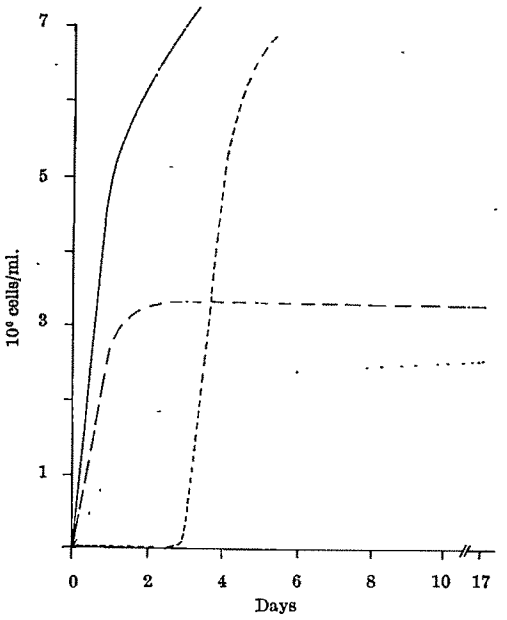


Fig. 1. Graphical representation of the growth of *Prototheca zopfii* on four carbon substrates: glucose (——), ethanol (– · – · –), lactate (. . . . .), and butyrate (– – –) when incubated at 25° C

Glucose, ethanol and lactate were all utilized immediately, although each with a different intensity of final cell production. Since *P. zopfii* was adapted to glucose and grew immediately and assimilated ethanol and lactate, this indicated the presence of constitutive enzyme systems for these two organic compounds. For butyrate, there was a lag period of 3 days before growth commenced. Growth was then as rapid as for the utilization of glucose, indicating that this strain of *P. zopfii* has a very efficient adaptive butyrase enzyme system.

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Repellency of the Mandibular Gland Scent of Worker Honey Bees

The mandibular glands of worker honey bees secrete 10-hydroxy-2-decenoic acid, the chief lipoid component of larval food[1,2], but although this substance is odourless, the secretion as a whole has a strong smell resembling that of the mould on blue-veined cheese. It is now shown to be repellent to foraging bees.

A 50 × 18 mm glass specimen tube containing sucrose syrup was exposed in an apiary and a few bees trained to visit it. When sufficient bees were visiting the tube it was replaced by three clean tubes symmetrically arranged on a table that could be revolved. Following Ribbands[3], the repellency of a substance was tested by placing it in two of the tubes and a control substance in the third. The table was turned frequently so that no tube had a constant position relative to the general surroundings. Each bee that landed was immediately driven off. A bee landing on the tube with the control substance was counted as positive and one landing on either of the tubes with the test substance negative. Each test was con-

tinued until either the number of positives exceeded the number of negatives by 5, which showed preference for the control tube ($P = 0.031$), or the number of negatives exceeded the number of positives by 6, which signified nothing. The materials used were crushed heads of foraging bees in ethylene glycol, crushed thoraces of the same bees in ethylene glycol, and ethylene glycol alone. Solution in glycol was necessary to retard evaporation of the scent, which otherwise became imperceptible in a few minutes. In other experiments the number of bees landing on each of a pair of tubes was counted until a total of ten landings had occurred; then a fresh pair of tubes was provided and the experiment repeated. Tests were made with clean empty tubes, clean tubes containing sucrose syrup, tubes from which bees at another feeding place had been actively taking syrup and tubes that these other bees had emptied of syrup and, though still visiting, were leaving, unsatisfied. Each bee that landed on a tube during the tests was immediately driven off, and if the tube was a clean empty one it was replaced by another.

Tubes with thoraces were preferred ($P < 0.001$) to tubes with heads (Table 1); this was because the heads were actually repellent and not merely less attractive than the thoraces, as tubes with glycol alone were preferred ($P < 0.001$) to those with heads. The mandibular-gland scent was fairly certainly responsible for the repellency of the crushed heads, as no other part of the head has a readily noticeable smell. Tubes that bees had emptied of syrup and were leaving, unsatisfied, were more attractive than tubes from which bees had been actively gathering syrup ($P < 0.02$, Table 2). It is unlikely, therefore, that bees warn others away from an exhausted food source by depositing mandibular gland scent on it.

Table 1. Repellency of Mandibular Glands

No. of bees landing on tubes in each test. (Nos. in parentheses are for two tubes)

Contents of tubes	Test No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
10 crushed heads in ethylene glycol	(8)	(4)	(1)	(1)	0	0	0	0	1	(10)	(1)	(0)	(0)	(0)	(1)	(1)	(1)
10 crushed thoraces in ethylene glycol	13	9	6	6	(6)	(6)	(6)	(6)	(7)								
Ethylene glycol alone										15	6	5	5	5	6	6	6

Table 2. Absence of Repellency at Exhausted Food Source

Tube *A*	Tube *B*	Mean of $(a-b)$*	*S.E.* of mean	No of tests
Clean, containing syrup	Clean, empty	1·00	0·72	12
Containing syrup on which bees had been feeding	Clean, containing syrup	0·80	0·79	10
Emptied of syrup by bees; bees had still been landing but were finding no syrup	Clean, empty	5·22	0·69	13

* a = No. of landings on A. b = No. of landings on B

The repellency of honey-bee mandibular-gland scent to foragers is surprising; the stingless bee, *Scaptotrigona postica*, marks points along routes to food sources with its mandibular gland secretion[4], which is physically similar to that of honey bees[5], and in males of many bee species the secretion is a sexual attractant[6]. Foraging bees usually have more secretion in their mandibular glands than bees taken from the hive[7], but this is probably evidence of diminished use rather than more active secretion, as it is the younger bees that feed the secretion to larvae, and the older ones (that is, those most likely to be foraging) have smaller secretory cells in their glands[8]. Probably the scent is present in newly discharged larval food and soon evaporates; if so, it may deter nurse bees from giving more food to larvae that have just been fed. Maschwitz[9] found that mandibular-gland secretion evoked an aggressive reaction from bees at a hive entrance, and Shearer and Boch[10] obtained a similar reaction with 2-heptanone, which they had identified in the secretion. Mandibular-gland scent is much less effective in producing hostility than the scent that is contained in a fold in the membrane at the base of a bee's sting[9], but it seems likely that it does play some part in colony defence. Possibly its main function is to repel robbers; this could explain its repellency to syrup-collecting bees.

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An Estuarine Fauna in a Freshwater Lake in South Africa

An expedition from the Department of Zoology and Entomology, Rhodes University, recently visited Lake Sibayi in Northern Zululand, Natal. The lake is completely separated from the sea by a line of forest-covered sand-dune which is 600 ft. high at the highest point. During July the lake water was homogeneous throughout, thus there was no stratification with regard to temperature (17°–18° C) or chlorides. The chloride content of the water in July, which is the dry season, was 135 p.p.m. This is high, and the lake can be regarded as being chloride-enriched. The source of chloride is possibly the cyclical salt carried in the air from the sea and precipitated in the basin, the sea being only half a mile from the lake at its nearest point. However, assuming that the chloride is derived from the sea and that the salts present in the lake are in the same proportion as in sea-water, the salinity would be in the region of 0·25 parts per thousand. This is extremely low and the lake must perforce be regarded as a freshwater environment.

A normal freshwater fauna was found, as typified by chironomids, oligochaetes, freshwater pulmonates (*Bulinus*, *Biomphalaria*, *Lymnaea*) and the prosobranch *Melanoides*, freshwater fish (*Clarias*), mayflies, Odonata, belostomatids, dytiscids and hydrophyllids. In addition there is a typical estuarine component as shown by amphipods, tanaids, isopods, polychaetes and both adults and zoeae of *Hymenosoma orbiculare* Desm. The amphipods were found bearing well-developed eggs. Tinley[1] has recorded the estuarine fish, *Gilchristella aestuarius* (Gilchrist and Thompson), and *Atherina breviceps* Cuvier.

Of the animals so far identified, the crab *Hymenosoma orbiculare*, an isopod *Cyathura carinata* (Kröyer) and two of the four species of amphipods have been reported from Natal estuaries[2,3].

The discovery of estuarine animals in a freshwater lake requires an explanation. Clearly the presence of such a fauna indicates an estuarine origin for Lake Sibayi. Bathymetric soundings in the lake indicate the presence of a north–south running trough with a so far recorded maximum depth of 38 m. The floor of this trough is at least 30 m below present sea-level and this indicates that its formation occurred at a time when the sea was at a lower level than it is at present. This hypothesis is supported by geological evidence of a lowered sea-level of 30–40 m during a Pleistocene glacial phase[4]. After the sea-level rose again the mouth of the estuary closed to form the present lake.

We consider that this is a unique situation not previously described in South Africa and that the estuarine animals found in Lake Sibayi represent the remnants of an estuarine fauna. From this the question arises whether the fauna has slowly adapted to increasingly freshwater conditions or whether the species found can normally live in fresh waters but are excluded by other factors. Experi-

mental investigations in an endeavour to solve this problem are being carried out in our laboratories.

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Composition of the Blood in Onychophora

The primitive terrestrial arthropods of the Class Onychophora are of especial zoological interest. As comparatively little is yet known of their physiology it seems worth recording some measurements of the blood ion-levels in *Peripatopsis moseleyi* and also the composition of a satisfactory physiological saline based on these ion-levels (Tables 1 and 2).

Table 1. Concentration of Ions in Blood of *P. moseleyi* in m.equiv./l.

	Na	K	Ca	Mg	Cl
Average of 7 animals	109*	5·6*	< 20†	< 1 not detected	—
Individual specimens	94·5	3·46	—	—	69·5
	93·2	3·9	6·2	< 1·2	72·0
	96·3	5·14	7·2	—	71·5
	88·2	4·37	2·4	—	74·5
Average of 4 specimens	93·0‡	4·2‡	5·3‡	< 1·2‡	71·9§

The *p*H of freshly drawn blood is 7·3‖.

Methods: * EEL flame photometer; presence of other ions not allowed for.
† Ethylenediamine tetraacetic acid titration.
‡ Unicam flame spectrophotometer *S.P.*900.
§ Electrometric titration.
‖ Beckman drop electrode.

Table 2. Composition of Peripatus Ringer's Solution

	Stock solutions M	Stock solutions g/l.	ml.	Final m.equiv./l.
NaCl	0·54	31·6	198·0	106·9
KCl	0·54	40·2	9 25	5·0
$CaCl_2 \cdot 6H_2O$	0·36	78·2	9·20	6·6
$MgCl_2 \cdot 6H_2O$	0·36	36·6	2·0	1·4
$NaHCO_3$		2·0	2·5	0·6
NaH_2PO_4 anh.		0·1	5·0	0·1

Distilled water added to make up to 1,000 ml. Final *p*H is 7·3.
Glucose: 0·4 g added to 100 ml. before use.

The analyses were based on whole blood, but the ions contained in blood cells can contribute little to the total since haemocytometer counts for six animals indicate only an average of about 5,000 cells/mm³. Two counts by Tuzet and Manier[11] give an average of 2,000 cells/mm³. As the blood cell volume must be less than 0·5 per cent of the blood volume, even the potassium contribution of the cells would be at most only 5 per cent of the whole blood potassium-levels, while that of other ions would be negligible. The blood does not clot.

Dissected preparations of the neuromuscular system remain viable in the physiological saline for at least 12 h at 20° C.

Simultaneous measurements of blood osmotic pressure were not made, but a level very close to 110 mmole/l. sodium chloride, the average of values found by Picken[1] for species of *Peripatopsis*, would be expected if inorganic ions account for the major part of the total concentration.

The animals normally live in moist microclimates and show a preference for high humidities[2]. In dry air they lose water rapidly owing to evaporation from the numerous tracheal openings[3,4]. Those used for the present determinations were kept beforehand under rotting wood on damp cotton-wool, and it will be noted that there is relatively little variation between individuals in the ionic composition. This implies that in favourable conditions the body-fluid concentration is accurately regulated. Certain of the regulatory mechanisms involved are known. Water which might otherwise be lost in the excretion of nitrogenous waste is conserved by the secretion of uric acid into the gut[5]. The nephridia produce hypotonic urine and are probably emptied infrequently[1,5]. In some species water can be taken up from moist surfaces by eversible coxal sacs[6]. Despite these conservatory devices there is likely to be a net loss of water from the body if the animals are exposed to drying conditions. It is interesting to note therefore that the blood concentration is very low in comparison with that of terrestrial arthropods of comparable size belonging to the classes Crustacea, Myriapoda, Arachnida and Insecta[7–10]. It is possible that this low tonicity may be of advantage to an animal occasionally liable to suffer desiccation, since a given water loss will concentrate the body fluids by a smaller absolute amount than if the concentration were higher. Consequently if the volume of any cells is to be maintained constant, the necessary adjustments to the internal osmotic activity and ion-levels will also be of smaller magnitude than otherwise would be the case.

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Effects and After-effects on Planarians of Reversals of the Horizontal Magnetic Vector

The freshwater planarian, *Dugesia dorotocephala*, was investigated at Evanston, Illinois, to learn whether it displayed orientational responses to abrupt experimental reversals of the horizontal vector of magnetism comparable with responses recently reported for marine mud-snails, *Nassarius obsoletus*, at Woods Hole, Massachusetts[1]. The study of the planarians extended over the period from April 12 to September 25, 1962, which included nearly 6 synodic months.

Observations were made between 9 and 11 a.m. on 25 days of the first month, 22 days of the second, and 24, 27, 21 and 16 days of the succeeding synodic months, respectively. Each morning two observers independently determined the mean turning rate over a 1-in. course for 15 paths of initially northbound planarians under each of eight experimental conditions in a fixed three-light field in a manner previously described[2,3]. One of the observers alternated the assaying of worm paths in the Earth's field (controls) with worm paths in each of four experimentally reversed fields (0·05, 0·2, 1·0 and 4·0 gauss) presented in shuffled order. The other observer performed the same kind of experiment except for using different field strengths (0·1, 0·5, 2·0 and 6·0 gauss). Each observer worked alternately with the 0·05- to 4·0-gauss and the 0·1- to 6·0-gauss series. The observers were uninformed of the order of presentation of the reversed magnetic strengths and of whether their alternating series commenced with a control or with an experimentally reversed field. Five worms remained in the small orientation vessel and were

repeatedly used to obtain each complete series of 120 paths, and then these worms were discarded. The entire investigation involved assay of 32,400 paths using 1,350 different worms.

One additional variable was present in the experiment. Whether on any given day the experiment was performed in an equi-potential right–left electrostatic field, a consequence of the presence of interconnected, large, vertical aluminium plates to right and left[4], or simply was performed in the unmodified right–left electrostatic gradient existing at the time, was determined by lot.

Fig. 1 shows the difference between mean planarian path while in each of the eight reversed field strengths and the average of all the controls in the same series. The series which were run in the equi-potential right–left field (solid circles) and those run in the unmodified electrostatic field (open circles) are shown separately, together with standard errors of means ($N > 100$). The similarity of these two emphasizes the general reproducibility of the results during this period and indicates that at least over the greater portion of the experimental range no significant influence of the right–left electrostatic gradient on the response was observable. However, at the two ends of the range, the presence of the plates suggestively sharpened a mean response to the 0·05-gauss field and, on the contrary, essentially abolished a mean response to the 6-gauss one.

The dotted curve in Fig. 1 illustrates, for comparison, the corresponding relationship between strengths of reversed fields, within the same range, reported[1] for southbound mud-snails which were observed at Woods Hole, Massachusetts, during July and August of the same year by other observers. Neither group of observers was informed of the results being obtained by the other one. The form of the relationship, though exhibiting about a 1° displacement, is remarkably similar for the two species. With the 1° displacement it is evident that a maximum in snail response (in this case left-turning) occurred near 0·1 gauss. This is consistent with the published report[5] that the mean turning behaviour of north- and south-directed snails differs significantly one from another. On the other hand, the presence of a minimum in mean turning response of planarians at 0·1 gauss is consistent with the previous report[3] that the mean paths of north- and south-directed planarians are indistinguishable from one another even though both are highly significantly different from those of east- or west-directed worms. The existence of the minimum at 0·1 gauss instead of 0·5 gauss suggests an adaptation of planarians to the strength of the horizontal vector at the specific local

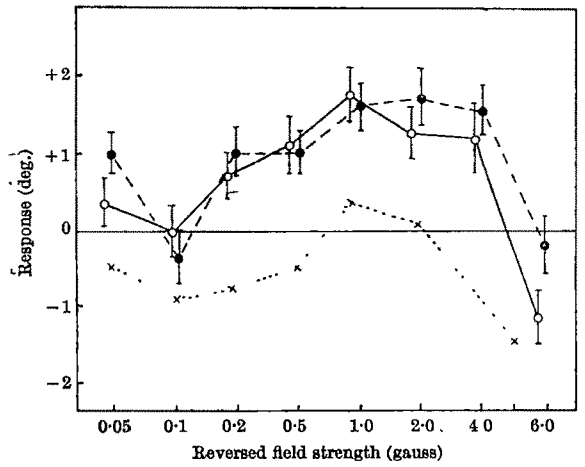


Fig. 1. Response of planarians, expressed as path difference from the controls, to each of a series of strengths of reversed horizontal magnetic vectors while otherwise in either the natural field (open circles) or a right–left equipotential electrostatic field (solid circles), and for mud-snails (× - - - ×) studied elsewhere during the same year (ref. 1). Standard errors of means ($N > 100$) are shown

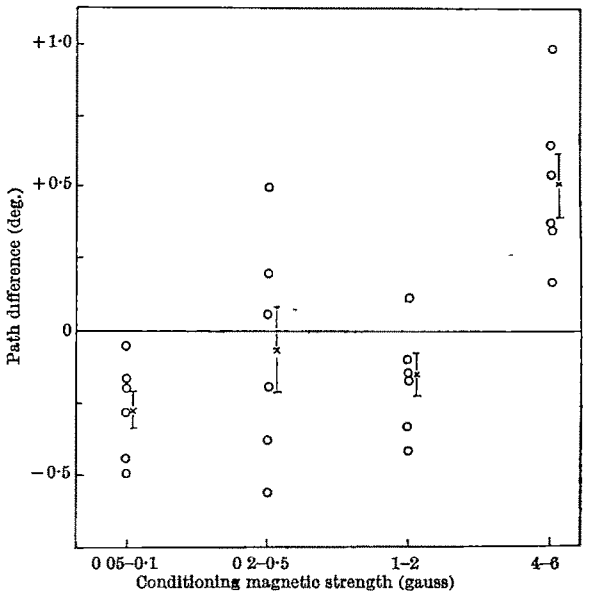


Fig. 2. The differences, for each of 6 synodic months, of the paths of north-directed control planarians from the mean of all control paths for that month, during 5-min periods following termination of 5-min exposures to reversed horizontal magnetic vectors at each of four strength-ranges. Means and their standard errors ($N = 6$) are also shown

geographic latitude in contrast to total field strength, just as had been demonstrated for mud-snails[1].

The data were next examined to learn whether exposures to the reversed horizontal magnetic vectors were followed by any after-effects. The duration of exposure to an experimentally reversed magnetic field averaged about 5 min, the time required to observe fifteen paths, and similarly the assay for any after-effect of an experimentally altered field-strength expressed as altered behaviour of the following controls, extended over about a 5-min period immediately after removal of the experimental field.

The data were reduced to synodic monthly units and the mean path of the controls in the Earth's field for each month was correlated with the strength of the reversed magnetic field that immediately preceded it. Data for controls when first in a series were discarded. The monthly unit was selected in view of the well-established monthly variation in response of both snails and planarians to very weak magnetic fields. Also, such a unit period was long enough to randomize in large measure any influences of other, uncontrolled weather-system-related geophysical variations on the phenomenon under investigation.

In Fig. 2 the mean path of the control worms is plotted for each of the 6 synodic months of data in relation to strength of the immediately preceding reversed experimental magnetic field. Mean path for each of the four control groups is expressed as the path difference from the mean path for all the controls of that month. It is evident that following those field strengths that deviated by no more than a factor of 4 (0·2–2·0 gauss) from the Earth's total field of about 0·5 gauss, the control values did not differ significantly from the mean for all controls. However, following the weakest fields (0·05 and 0·1 gauss) there was an after-effect in the form of a significantly increased counter-clockwise turning. Following the strongest fields (4 and 6 gauss) there was increased clockwise turning. Analysis of variance of the four populations of values yielded $F = 9{\cdot}9$ ($P < 0{\cdot}001$), indicating that the controls following the four strength-ranges of magnetism were, with very high probability, not random samples of values from a homogeneous population.

It is noteworthy that the after-effect shown by the planarians is qualitatively of the same general character as that reported previously for mud-snails[1], namely,

increased clockwise turning following fields stronger than the Earth's and increased counter-clockwise turning following fields weaker. This similarity obtained despite the fact that the snails had been south-directed while the planarians in the present investigation were north-directed.

Inspection of Fig. 1 reveals that the worms turn less far to the right in response to the 4- to 6-gauss fields than to the 1- to 2-gauss ones, and right-turning response to the 0·05- to 0·1-gauss fields averages about the same as to the 4- and 6-gauss ones. Therefore, the highly significant differences in after-effects among these ranges as shown in Fig. 2 cannot be attributed simply to a tendency of the worms to maintain in the subsequent control field the same path in the orientation apparatus that resulted from direct response to the experimentally reversed magnetic fields. Some other explanation must be sought. It is postulated that the phenomenon reflects acclimation or accommodation of the magnetoreceptive mechanism toward the altered strengths. This behaviour is interpreted as further evidence suggesting that the organism is, normally, physiologically adjusted in some manner to the strength of geomagnetism, even to specific vector strengths.

The general similarities of responses of the planarians to those obtained earlier for mud-snails suggest that responsiveness to the horizontal magnetic vector may be a fundamental and widespread biological property.

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Distribution of the Genus *Nephrops* (Crustacea Decapoda Macrura) in the Indo-Pacific Region

A survey of the northern part of the South China Sea recently carried out by R.V. *Cape St. Mary*, of the Fisheries Research Station, Hong Kong, has shown that considerable numbers of *Nephrops* spp. (Crustacea Decapoda Macrura) are present in this region. Four species have so far been obtained: *N. thompsoni* Bate, *N. sagamiensis* Paris, and two which are new[1]. The most abundant species is *Nephrops thompsoni* Bate, previously known only from the original specimen obtained by the *Challenger* Expedition and a second specimen from Formosa[2]. One of the new species is almost equally abundant.

The widespread occurrence of these lobsters has been revealed during the systematic trawling survey of the South China Sea being carried out as part of the programme of R.V. *Cape St. Mary*. Further prospecting cruises are being undertaken to determine whether or not *Nephrops* is present in commercially exploitable quantities.

In the northern part of the South China Sea *Nephrops* has been found only on soft muds off the edges of the continental shelf, mainly between 100 and 200 fathoms, although one species was obtained at 450 fathoms. Attempts to catch *Nephrops* on oceanic banks have so far been unsuccessful. In November 1964, R.V. *Cape St. Mary* obtained *N. thompsoni* at 94–98 fathoms off North Borneo. The original record of this species was from the Philippines between Manila and Samboang. The results so far obtained indicate that *N. thompsoni* is probably present all around the continental slopes of the South China Sea.

The European *Nephrops norvegicus* (L.) is now extensively exploited by the north-west European and Mediterranean fisheries, where it has become a luxury product sold under such names as scampi, langoustine and Dublin-Bay prawn. Until recently the tropical species of *Nephrops* has not been exploited, but a commercial fishery based on the New Zealand species, *N. challengeri* Balss, is now being developed[3,4]. *N. norvegicus* is abundant in depths of 25–50 fathoms and its fishery presents no special problems. The Indo-Pacific species occur mainly at depths of more than 100 fathoms where few local vessels are yet able to fish.

No systematic surveys have been undertaken for *Nephrops* in tropical waters, but the recent results obtained in the South China Sea by R.V. *Cape St. Mary* indicate that this genus may be more abundant and widespread than has been supposed. Two new species have recently been found in Australian waters[5,6], and there are numerous scattered records from the Indian Ocean. The purpose of this communication is to draw the attention of fishery research workers in the Indo-Pacific region to the possibility that quantities of *Nephrops* may be present at suitable localities and depths, and that further efforts should be made to investigate their distribution and biology. Any further information concerning the distribution of adult *Nephrops*, or their larvae, in the Indo-Pacific region would be welcomed by me.

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ENTOMOLOGY

Ascorbic Acid in the Larval Stages of *Plusia signata* (Lepidoptera : Noctuidae)

Day[1] found that in the larva of *Tineola*, the columnar but not the goblet cells contain vitamin C. Dadd[2] reported that vitamin C is essential in the development of *Schistocera gregaria*. Gilmour[3] found ascorbic acid widely distributed among insects and synthesized by them. An investigation of different larval instars of *Plusia signata* was undertaken to determine the distribution of ascorbic acid in different tissues in these stages.

The histological presence of ascorbic acid was demonstrated by the silver method as modified by Bacchus[4], and its estimation was performed by the indophenolxylene extraction method of Robinson and Stotz[5].

Histological sections showed that the presence of ascorbic acid was greatly marked in different tissues during different instars. First instar larvae which had hatched about 1 h before, and which had not yet started feeding, showed even impregnation throughout the gut epithelium and blood cells. The cells of the spinning glands exhibited very little impregnation, in fact, only localization in the supra-nuclear regions of the cells. Fat bodies and nerve cells also contained small amounts of ascorbic acid. In the second and third instars, the accumulation of ascorbic acid appeared to have increased only in the mid-gut epithelium and blood cells. The cells of the spinning glands in these stages exhibited very slight increase of ascorbic acid, but the impregnated cells now showed an even distribution throughout: the ascorbic acid was not

limited only in the supranuclear zone as in the previous stage. During fourth and fifth instars, the cells of the mid-gut epithelium, cells of hepatic caecae and the epithelial cells of the hind-gut showed heavy impregnation. Cells of fore-gut epithelium and nerve cells did not indicate any increase in the ascorbic concentration. Heavy impregnation of the spinning gland cells was now found mostly towards the lumen surface of the cells. The fat body and blood cell also showed considerable concentrations of ascorbic acid in these latter instars. A comparative investigation of different instars showed that there was a gradual increase in the amount of ascorbic acid in different tissues as the larva grew, except in the case of nerve cells. Estimation of ascorbic acid during different instars based on the extracts of whole larvae also indicated such gradual increase (Table 1).

Table 1

Instars	mg/g
First instar larva	0·08
Second instar larva	0·35
Third instar larva	0 49
Fourth instar larva	0·71
Fifth instar larva	0·82

From both histological examination and extract estimation, it is evident that high concentration of ascorbic acid is found in the larval life. This ascorbic acid does not appear to be purely of dietary origin, as in the first instar larva, which had not started feeding, an appreciable quantity of ascorbic acid was found in the tissues. The gradual increase in the amount of ascorbic acid from first instar to the last suggests that ascorbic acid is required during the process of metamorphosis during the formation of adult organs.

I thank Dr. S. Keshava for his advice and various facilities.

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MICROBIOLOGY

Inhibition of Yeast Growth by Streptomycin

For the isolation of a pure culture from yeast cultures heavily contaminated with bacteria, sub-cultures were made into media containing penicillin and streptomycin. With certain yeasts we observed that yeast growth as well as bacterial growth was inhibited. Preliminary observations suggested that the streptomycin was the inhibitory agent. This observation contradicts earlier opinion which considered fungi, including yeasts, to be insensitive to the action of streptomycin[1,2]. Based on this opinion of insensitivity to streptomycin, some media designed for the isolation of pathogenic[3] and saprophytic[4] yeasts include streptomycin, at a concentration of 40 μg/ml., to inhibit bacterial growth.

However, partial inhibition of yeast growth by streptomycin at the high concentration of 100–10,000 μg/ml. (ref. 5), and also partial inhibition of a variant of *Torula utilis* by 13 μg/ml. of streptomycin[6], have been reported. This communication describes experiments which were designed to measure quantitatively the streptomycin-sensitivities of yeast strains from the National Collection of Yeast Cultures which appropriate screening had suggested to be sensitive to this agent.

The streptomycin-sensitivities of the yeast strains were assessed in M.Y.G.P. (ref. 7) except for the osmophilic strains which were assayed in osmophilic medium (malt extract 0·3 per cent, yeast extract 0·3 per cent, sucrose 40 per cent, dextrose 5 per cent, peptone 0·5 per cent). The final *p*H of both types of medium was 6·9–7·0. Streptomycin sulphate was added aseptically to the medium just prior to inoculation. As the nature of the medium has been demonstrated to have a significant effect on the activity of streptomycin towards yeasts[5], a complex medium was deliberately chosen for assay so that inhibition of yeast growth in the assay system would then reflect the probability of inhibition of yeast growth occurring during laboratory isolations. Assays were performed in 10 ml. amounts of medium, and each tube was inoculated with a standard loopful of a 3-day yeast culture ($4·5–11 \times 10^4$ yeast cells per tube). Incubation was at 25° C for 72 h on an automatic shaker (3 cm amplitude, 80 cycles per min). The degree of growth at the end of this period was assessed visually.

Table 1. Sensitivity of Yeasts to Streptomycin

Yeast	N.C.Y.C. No.	Growth in presence of streptomycin sulphate (μg/ml.) 0	10	20	50	100
Candida utilis	193	+	+	+	+	+
Endomycopsis fibuliger	13	+	+	Sl†	–	–
Hansenula anomala (*O*)*	522	+	+	+	+	+
Hansenula subpelliculosa (*O*)	558	+	+	+	+	+
Saccharomyces acidifaciens	464	+	+	+	–	–
Saccharomyces carlsbergensis	511	+	+	+	Sl	–
Saccharomyces carlsbergensis	519	+	+	+	–	–
Saccharomyces carlsbergensis	520	+	+	+	+	–
Saccharomyces cerevisiae	83	+	+	+	+	Sl
Saccharomyces elegans	128	+	+	+	–	–
Saccharomyces ludwigii	364	+	+	+	–	–
Saccharomyces rosei	492	+	+	–	–	–
Saccharomyces rouxii (*O*)	381	+	+	+	+	–
Saccharomyces rouxii (*O*)	581	+	+	+	+	+
Schizosaccharomyces octosporus (*O*)	427	+	+	–	–	–

* (*O*) Denotes osmophilic strain.
†Sl denotes slight growth.

Table 2. Effect of *p*H on Inhibitory Action of Streptomycin towards Yeasts

Yeast	N.C.Y.C. No.	*p*H	Growth in presence of streptomycin sulphate (μg/ml.) 0	10	20	50	100
Saccharomyces elegans	128	4·7–5·0	+	+	+	+	Sl
		5·7–6·0	+	+	+	–	–
Saccharomyces acidifaciens	464	4·7–5·0	+	+	+	–	–
		5 7–6·0	+	+	+	–	–
Saccharomyces rosei	492	4·7–5·0	+	+	Sl	–	–
		5·7–6·0	+	+	Sl	–	–
Saccharomyces carlsbergensis	519	4·7–5 0	+	+	+	+	–
		5·7–6·0	+	+	+	Sl	–

The results summarized in Table 1 appear to be the first demonstration that streptomycin at such low concentrations can completely inhibit the growth of a variety of yeasts during the incubation period. Furthermore, the degrees of sensitivity of the various species tested suggest that the level of streptomycin incorporated into media for isolation of yeasts may inhibit some yeast strains as well as bacteria. Media used for the isolation of yeasts are of lower *p*H than the assay media, but Table 2 reveals that reduction of *p*H to 5·7–6·0 has little effect on the degree of sensitivity of these yeast strains to streptomycin, and even at *p*H 4·7–5·0 some yeast strains could still be inhibited by the concentration of streptomycin in these media.

In the light of these observations, therefore, it would appear unwise to incorporate streptomycin routinely in any medium designed for the isolation of yeasts.

We thank Dr. A. H. Cook for his advice and encouragement.

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Sporogen—an 'Inductor' for Bacterial Cell Differentiation

CELLULAR differentiation has proved to be one of the most fascinating subjects for investigations in the field of biology. Since Spemann's[1] discovery of the phenomenon of induction by organizers, numerous investigations have been directed towards the isolation and identification of inductor substances in morphogenesis. Efforts to obtain such genotropic substances and to analyse the mechanisms of their actions have so far been unsuccessful. In my investigations, bacterial sporogenesis has been taken as a model system for studying the biochemical aspects of intracellular differentiation.

My colleagues and I reported earlier[2] that an endogenous factor was present in the early stationary phase of growth of *Bacillus cereus* strain *T*. This factor played an active part in triggering the vegetative cells to the sporulating stage. It has now been fractionated and a biologically active substance isolated by means of the method which we used to detect biological activity in our previous communication[2]. The trivial name of 'sporogen' has been given to the crystalline material.

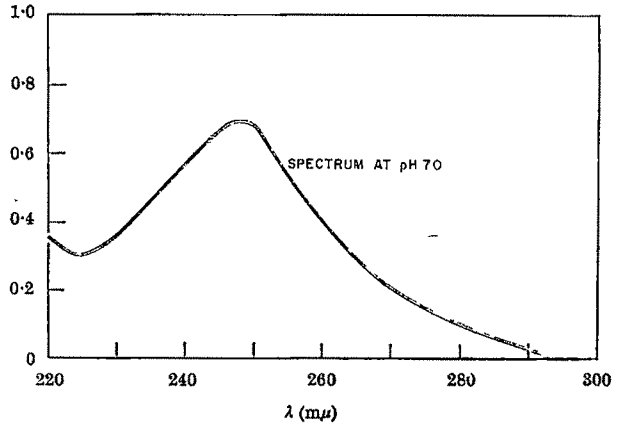


Fig. 1. Ultra-violet absorption spectrum of sporogen

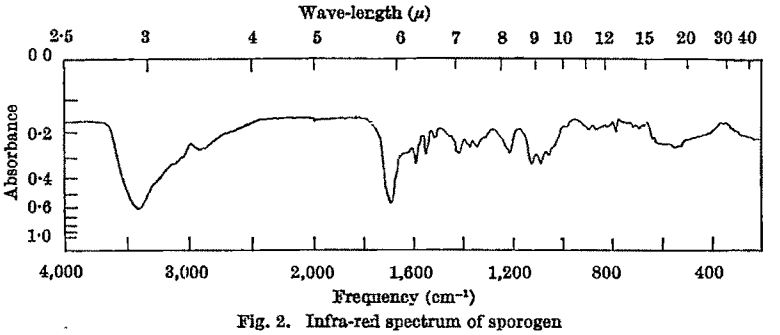


Fig. 2. Infra-red spectrum of sporogen

The procedure for the isolation of sporogen from 5 lb. (wet weight) of *B. cereus* strain *T* cells was as follows. The cells were suspended in 2·5 l. 0·01 M potassium phosphate buffer at *p*H 6·8. The suspension was mixed with an equal volume of glass beads (100μ in diameter obtained from Minnesota Mining and Manufacturing Co.), and the cells disintegrated by passing the mixture through an Eppenbach Colloid mill. The glass beads were removed by decanting off the fluid after allowing the mixture to stand for a while; the cell debris and unbroken cells were obtained by centrifugation. The clear supernatant fluid was adjusted by adding hydrochloric acid so that it had a *p*H of 2·0; at this *p*H a precipitate formed and this was removed by centrifugation. The solution, golden yellow in colour, was adjusted to *p*H 5·5, this time using potassium hydroxide, and left overnight at 4°–5° C. The small amount of precipitate which formed was removed and the solution was evaporated to dryness under reduced pressure; a residue weighing 100 g was obtained. This material was suspended in 5 l. of a mixture of ethanol formic acid and water in the proportion 90 : 5 : 5 and shaken thoroughly at room temperature overnight. The suspension was filtered off and the filtrate once again evaporated to dryness under reduced pressure; 10 g of residue was obtained. This was treated with a minimal volume of 0·2 N acetic acid and fractionated on a column (50 cm × 5 cm) of 'Sephadex' *G*-25 pretreated with 0·2 N acetic acid. The fractions were monitored by measuring the absorption at a wave-length of 249 mμ in a Beckman spectrometer. The active fractions were pooled, and once the solvent had been removed under reduced pressure, the residue was further fractionated on a cellulose column using a mixture of butanol–acetic acid–water (4 : 1 : 1) as the developing solvent. The fractions were analysed by paper chromatography using the same solvent mixture. Biological activity was exhibited by the fraction with an R_F of 0·35. This fraction was collected, the solvent removed by evaporation under reduced pressure and the residue crystallized from aqueous solution (yield 19 mg). The crystalline substance obtained melted between 162° and 165° C and had an elementary composition as follows: C, 42·78 per cent; H, 4·89 per cent; N, 18·63 per cent; O, 33·7 per cent (by difference). It had a characteristic ultra-violet absorption spectrum with an absorption maximum at 249 mμ (Fig. 1). The infra-red spectrum of the compound is presented in Fig. 2.

The chemical nature of the substance is at present under investigation, but little is known about the mechanism of its action. As a working hypothesis, adopting Jacob and Monod's model[3] for induced enzyme synthesis, it may be taken for granted that sporogen derepresses the regulators controlled by the 'sporogenes', thereby releasing the operator gene which in turn switches on the structural genes responsible for the synthesis of macromolecules which is required for the initial steps in sporulation.

I thank Dr. H. Orin Halvorson for his advice, and Mr. Francis Engle for his assistance. I also thank Dr. J. G. Garver of the University of Wisconsin for making available his facilities for growing large batches of organisms.

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GENETICS

Synchrony of Chromosome Duplication

IN some recent publications statements have been made on the marked asynchrony of homologous pairs of autosomes with respect to duplication of the chromosomes—as studied with tritiated thymidine[1–3]. In an earlier publication, in which quantitative analysis of grain counts on chromosomes has been performed, we have reported that no evidence for a marked asynchrony was found[4].

In view of these apparently contradictory reports, the question was re-examined. The results, which are described here, indicate that there is no detectable

difference between the homologues of the chromosomes investigated. It is possible to pick cells in which a truly striking difference exists between homologues of a pair, but when the painful analysis of grain counts over a significant number of cells is performed, these differences appear no more than the extreme ends of a rather wide distribution. It is not legitimate, therefore, to deduce asynchrony unless supported by statistical evidence.

The material examined included blood cultures from a normal female (case 1), a normal male (case 2) and a female with a large iso-X chromosome (case 3); also skin cultures from a normal female (case 4) and from an XXY male (case 5). The cultures were labelled with tritiated thymidine at the end of the S period so that the cells to be analysed spent only about 30–40 min in the S period. The culture, labelling and autoradiography methods were described previously[2].

Each chromosome in group I (Nos. 1, 2 and 3) was grain counted. The total number of grains over all chromosomes was also counted in each cell, and separate counting was undertaken on the 'hot' X chromosomes in cases 1, 3, 4 and 5 (ref. 5). The total number of cells analysed was 226, and the total number of grains counted was more than 84,000; the total over the group I chromosomes was more than 16,000.

In the analysis of our results we will use the following notation:

a, b, the grains over two homologues in a cell
n, the total grains in the cell excluding the 'hot' X
T, the total number of cells
Σ, a summation made over the T cells
$H = \Sigma(a+b)$, the total grains over the two homologues in the T cells
$N = \Sigma n$, the total grains in the T cells excluding 'hot' X's
$M = H/N$, the mean proportion of grains over the two homologues
p, $(1-p)$, the expected proportion of grains over each homologue
w, the statistical weight given to a cell

Although not distinguishable morphologically, the two homologues are different in so far as each was originally derived from a different parent, but they do not necessarily have different labelling patterns. If we call the two homologues A and B, then, due to the considerable statistical variation, we cannot assign the observed grain counts a and b in any individual cell uniquely to A or B, unless a and b are consistently very different. This is not the case except for the pair of X chromosomes. Differences in labelling intensity may be separated from the statistical noise, but it is misleading to attempt this by assigning the greater of a and b to one homologue and the smaller to the other. This could lead to misclassification in up to half the cases, and the average difference would be difficult to interpret. A better approach is to average the square of the difference $(a-b)^2$, since an analysis of variance can then be made.

We denote p and $1-p$ the expected proportion of grains over the two homologues; p may have any value between 0 and 1; $p = 0{\cdot}5$ corresponding to equal labelling. For the moment we will assume that all the chromosomes in our set of cells have a consistent labelling pattern and that all the observed differences are due to random statistical variations. Some cells have, however, been exposed to the label for a longer period and so have more grains; we shall take n the total number of grains as a measure of exposure and assume these grains to be distributed on average in the same proportions over the chromosomes, independent of labelling intensity.

With these assumptions, the variances of $(a + b)$ and $(a - b)$ about their respective mean values will be the same. In calculating these variances it is necessary to weight the observations. We have used the weights appropriate to a binomial distribution, each cell being given a weight w, inversely proportional to its grain count; that is, the inverse of the expected variance of $(a + b)$:

$$w = \frac{N}{N-H} \cdot \frac{N}{H} \cdot \frac{1}{n}$$

Now the expected value of $(a + b)$ is Hn/N and that of $(a - b)$ is $(2p-1)Hn/N$. We denote by Y_s and Y_d the weighted sums of squares of the differences of $(a + b)$ and $(a - b)$ from their expected values. Therefore:

$$Y_s = \Sigma w\,(a + b - Hn/N)^2$$

$$Y_d = \Sigma w\,(a - b - (2p - 1)\,Hn/N)^2$$

Expanding out of these expressions and using the identities for H and N, we obtain:

$$Y_s = \frac{N}{N-H}\left(\frac{N}{H}\,\Sigma\,(a + b)^2/n - H\right)$$

$$Y_d = \frac{N}{N-H}\left(\frac{N}{H}\,\Sigma\,(a - b)^2/n - (2p-1)^2 H\right)$$

The assumption made here requires the sums of squares Y_s and Y_d to be estimates of the same variance and, equating these, $(2p-1)^2$ and p can be calculated.

Table 1. Details of the Grain Count Analysis of the Homologous Autosomes of Group I

For explanation of the symbols, see list in text

Culture and chromosome		T	N	H	$\Sigma(a+b)^2/n$	$\Sigma(a-b)^2/n$	$(2p-1)^2$
1	1	33	6,759	415	29·2969	5·4188	+0·063 ± 0·053
XX	2	33	6,759	658	68·8533	3·9157	−0·014 ± 0·027
	3	33	6,759	544	48·5145	4·4122	−0 007 ± 0·038
2	1	32	11,011	621	39·5196	2·2913	−0·063 ± 0 047
XY	2	32	11,011	952	88·5394	7·1653	+0·011 ± 0·028
	3	32	11,011	729	53·6880	2·7028	−0·056 ± 0·041
3	1	25	3,198	187	14·0715	2·5261	−0·056 ± 0·118
iso-X	2	25	3,198	272	28·6682	3·2196	−0·100 ± 0·099
	3	25	3,198	198	16·2613	2·3813	−0·132 ± 0·135
4	1	66	40,084	2,620	186 0968	12 6237	−0 013 ± 0 022
XX	2	65	39,520	2,116	121·2073	4·5564	−0 030 ± 0 018
	3	61	37,775	1,648	78 9804	3 3870	−0·064 ± 0·028
5	1	72	23,722	1,811	152 1511	13 0099	−0·006 ± 0·024
XXY	2	72	23,722	1,913	169 5604	8·0801	−0·047 ± 0·024
	3	72	23,722	1,393	92·5665	6 0463	−0 058 ± 0·031

Table 1 shows the results for the chromosomes 1, 2 and 3 and from five different cultures. It will be seen that except for two of the fifteen cases examined, the estimate of $(2p-1)^2$ is negative. The squared quantity $(2p-1)^2$ must essentially be positive, and the findings in Table 1 therefore indicate that the basic assumption that Y_s and Y_d are equal is not justified. This shows that the variance of the differences $(a-b)$ is less than the variance of the sums $(a+b)$ from its mean.

This unexpected finding indicates that we have no precise way of estimating p. However, it must be remembered that any value of p other than 1/2 involves a smaller variance for the differences between the homologues within the cells than between cells.

The considerable biological variation implies that some large differences in labelling intensity will be expected to be seen and therefore these must not be given undue significance.

Thus the analysis of our data gives not only no evidence for a difference in the number of grains over the pairs of homologues of the chromosomes in group I, but, in fact, shows that the pairs of homologues have on average a smaller difference than would be expected from the observed variance from cell to cell in this narrow 'end of S' cohort of cells.

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Manchester.

[1] Lima-de-Faria, A., Blanchi, N. O., and Nowell, P., *Conf. Mammalian Cytology and Somatic Cell Genetics*, Puerto Rico, November 1964.
[2] Bader, S., Miller, O. J., and Mukherjee, B. B., *Exp. Cell Res.*, **31**, 100 (1963).
[3] Kikuchi, Y., and Sandberg, A. A., *J. Nat. Cancer Inst*, **32**, 1109 (1964).
[4] Gilbert, C. W., Muldal, S., Lajtha, L. G., and Rowley, J., *Nature*, **195**, 869 (1962).
[5] Rowley, J., Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Fraccaro, M., and Kaijser, K., *Nature*, **197**, 251 (1963).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 31

CIBA FOUNDATION FOR THE PROMOTION OF INTERNATIONAL CO-OPERATION IN MEDICAL AND CLINICAL RESEARCH (at The Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5 p.m.—Prof. C. P. Leblond, F.R.S. (McGill University): "Role of the Golgi Complex in Secretion" (18th (Annual) Ciba Foundation Lecture).

UNIVERSITY OF LONDON (at the London School of Economics, Houghton Street, London, W.C.2), at 5 p.m.—Prof. R. J. Harrison-Church: "Some Geographical Aspects of West African Development" (Inaugural Lecture)*.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "The Problems of Radiation Pressure and Associated Mechanical Forces".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. F. Hoskins: "The Definition and Properties of the Delta Function".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. M. W. Thring: "The Status and Training of Engineers and Technologists". (Last of three Cantor Lectures on "The Place of the Technologist in Modern Society".)

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. J. Kohn: "The Use of Balsa Wood in Association with Reinforced Plastics".

Tuesday, February 1

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. H. Humphrey: "Cell Damage by Complement" (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Prof. P. L. Shinnie: "The Place of Archaeology in African Studies".*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Dr. E. C. A. Horner: "Olefin Copolymers".

Wednesday, February 2

COLOUR GROUP (GREAT BRITAIN) (at Strand Electric and Engineering Co., Ltd., 29 King Street, London, W.C.2), at 3 p.m.—Prof. W. D. Wright: "Colour Tolerances—Yesterday and Today"; Dr. Vickerstaff: "Advances in Colour Recipe Prediction"; Mr. J. G. Holmes: "Temperature Variables in Coloured Light Signals"; Dr. W. S. Stiles, F.R.S.: "Significant Advances in the Field of Colour Vision".

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Sir Alister Hardy, F.R.S.: "The Development of the Plankton Recorder Method".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. M. A. Champney: "The Electron Beam in Electronics Research and Production".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. R. O. Redman, F.R.S.: "The Surface of the Moon" (Peter Le Neve Foster Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "Determination of Purity in High-purity Organic Materials".

Thursday, February 3

ROYAL SOCIETY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 2.30 p.m.—Discussion Meeting on "Structure and Function of Lysozyme", organized by Dr. M. F. Perutz, F.R.S.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. Rodnight: "Phosphoprotein Metabolism in the Brain". (Seventh of fifteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 5.30 p.m.—Mr. H. E. C. Powers: "Crystallization: a Study in Molecular Architecture". (Further lecture on February 4.)*

CHEMICAL SOCIETY (in the Chemistry Lecture Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Dr. C. B. Colburn: "The Difluoramino Free Radical and Its Derivatives" (Centenary Lecture)

INSTITUTE OF REFRIGERATION (at the National Colleg… lating, Refrigeration and Fan Engineering, South… S.E.1), at 6 p.m.—Mr. P. G. Valentine: "… Store Construction".

Frid…

UNIVERSITY OF L… Du Cane Road…

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. W. S. Metcalf: "Radio Astronomy".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. E. A. Mason: "The Chemical Industry of Japan".

INSTITUT FRANÇAIS DU ROYAUME-UNI (at Queensberry Place, London, S.W.7), at 8.15 p.m.—French Scientific Films.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Peter Beighton: "Easter Island People".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (preferably with a special interest in the biological sciences) IN HISTORY AND PHILOSOPHY OF SCIENCE—The Secretary, The Queen's University, Belfast, Northern Ireland (February 4).

ASSISTANT LECTURER IN BIOCHEMISTRY IN ST. SALVATOR'S COLLEGE—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (February 5).

LECTURER and an ASSISTANT LECTURER (with special interest in either physical geography and biogeography or in regional studies) IN GEOGRAPHY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (February 7).

LECTURERS or ASSISTANTS IN PHILOSOPHY—The Secretary of the University Court, The University, Glasgow (February 7).

LECTURER (preferably with a special interest in the development of computer software) IN THE COMPUTER UNIT—The Registrar (Room 39, O.R.B.), The University, Reading (February 8)

GRADUATE (with a degree in chemistry, biochemistry or an allied biological subject and a wide general interest in biology) TO ASSIST IN CANCER RESEARCH—The Secretary, King's College Hospital Medical School, Denmark Hill, London, S.E.5 (February 12).

LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY—The Secretary, The University, Aberdeen (February 12).

LECTURER or ASSISTANT LECTURER (with interests in soil mechanics) IN ENGINEERING—The Registrar, The University, Manchester, 13, quoting Ref. 8/66 (February 12).

LECTURER IN THE BIOCHEMISTRY DEPARTMENT—The Secretary, Royal Free Hospital School of Medicine (University of London), Hunter Street, London, W.C.1 (February 14).

LECTURER or ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 14).

RESEARCH ASSISTANT (graduate in chemistry or the biological sciences) IN THE DEPARTMENT OF BOTANY to work with Dr. A. J. Peel on transport physiology in phloem tissue using the aphid technique—The Registrar, The University, Hull (February 14).

SECOND CHAIR OF GEOGRAPHY—The Registrar, University College of Swansea, Singleton Park, Swansea (February 14)

ASSISTANT LECTURER IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (February 15).

CHAIR OF PURE MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (February 15).

UNIVERSITY DEMONSTRATOR (with an honours degree in an appropriate pure science (for example, biochemistry or physiology) or agricultural science, and preferably teaching and/or research experience) IN ANIMAL NUTRITION—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (February 15).

ASSISTANT LECTURER IN ARCHAEOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (February 16).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF CHEMISTRY IN THE FACULTY OF SCIENCE to work on an electron spin resonance instrument shortly to be installed in the Department and concerned with the study of inorganic complexes—The Registrar, The University, Manchester, 13, quoting Ref. 9/66 (February 16).

LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Leicester (February 16).

LECTURER/ASSISTANT LECTURER IN PHYSICAL CHEMISTRY; a LECTURER/ASSISTANT LECTURER IN APPLIED MATHEMATICS; and a RESEARCH OFFICER to assist in the operation of programmes for the College IBM 1620 tape/disc/card system—The Registrar, University College of Wales, Aberystwyth (February 17).

LECTURER or ASSISTANT LECTURER (preferably with research experience, interests, or qualifications in any of the following fields: parasitology, ecology, or small mammal ecology and systematics) IN THE DEPARTMENT OF ZOOLOGY, Ahmadu Bello University, Northern Nigeria—The Inter-University Council, … Bedford Place, London, W.C.1 (February 17)

… SCHOOL OF ENVIRONMENTAL SCIENCES—The Registrar, …ham Hall, Norwich, NOR 88C (February 19).

… IN THE DEPARTMENT OF GENETICS—The … (February 19).

…ith a degree in chemistry) IN THE …ch will include laboratory manage… …y relieving the Professors of much …ity of St. Andrews, Queen's

CHAIR OF BIOCHEMISTRY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 28)

CHAIR OF EDUCATION—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (February 28).

IMPERIAL CHEMICAL INDUSTRIES FELLOWS (below the age of 30, with a Ph.D. degree or equivalent research experience) FOR RESEARCH IN VARIOUS BRANCHES OF CHEMISTRY, PHYSICS, BIOLOGICAL SCIENCES, APPLIED MATHEMATICS, ENGINEERING AND TECHNOLOGY AND ALLIED SUBJECTS—The Registrar, Bradford Institute of Technology, Bradford (February 28).

PROFESSOR (with experience in the petroleum industry) OF PETROLEUM GEOLOGY IN THE DEPARTMENT OF GEOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (February 28).

SECOND CHAIR OF EDUCATION—The Registrar, The University, Hull (February 28).

GRADUATE RESEARCH ASSISTANTS (qualified to register in programmes leading to the M.Sc. and Ph.D. degrees) IN THE DEPARTMENT OF DAIRY AND FOOD SCIENCE—Prof. L. F. L. Clegg, Head, Department of Dairy and Food Science, University of Alberta, Edmonton, Alberta, Canada.

LECTURER or ASSISTANT LECTURER (or exceptionally SENIOR LECTURER) IN MATHEMATICS—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

LECTURER or ASSISTANT LECTURER (with special training in entomology, and preferably a particular interest in ecology, or in genetics) IN BIOLOGY at the University of Malawi—The Inter-University Council, 33 Bedford Place, London, W.C.1.

LECTURER or ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHILOSOPHY—The Dean, Faculty of Arts and Science, University of Manitoba, Winnipeg 19, Manitoba, Canada.

MICROBIOLOGIST (young graduate) to take part in a three-year research project on bacterial spore formation—The Secretary, Biochemistry Department, Imperial College, London, S.W.7.

PHYSICIST (with a Ph.D. in physics or qualifications and scientific research experience which taken together are equivalent thereto) at the Australian Atomic Energy Commission's Research Establishment, Lucas Heights, Near Sydney, to work in a small group undertaking research and development in the field of nuclear radiation interactions and detection—Mr. S. Gray, Atomic Energy Adviser, Australia House, Strand, London, W.C.2.

POSTDOCTORAL FELLOW (preferably with some experience in X-ray crystallography) IN THE DEPARTMENT OF CHEMISTRY, to collaborate with Dr. B. Stevens in a study of the photochemistry and radiation chemistry of crystalline aromatic compounds—The Registrar, The University, Sheffield.

SENIOR LABORATORY TECHNICIAN IN THE DEPARTMENT OF BOTANY AND ZOOLOGY to take charge of day-to-day running of botany and zoology degree course laboratories—Head, Department of Botany and Zoology, Sir John Cass College, Jewry Street, London, E.C.3.

ZOOLOGISTS (M.Sc. or Ph.D. with research experience, and preferably some knowledge of histology and histochemistry) to participate in experimental multidisciplinary programmes of basic research in the field of food additive toxicology—The Director, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Technology. Warren Spring Laboratory. Register of Research in the Human Sciences, 1962–65. Pp. vi+353. (London: H.M. Stationery Office, 1965.) 37s. net [2611

Annual Report of the British Council, 1964–1965. Pp. vii+99+12 plates. (London: The British Council, 1965.) 2s. 6d. [2611

National Research Development Corporation. Sixteenth Annual Report, 1964–65. Pp. 53+8 plates. (London: H.M. Stationery Office, 1965.) 5s. net. [2611

The Natural Rubber Producers' Research Association. List of Publications, 1938–1964. Pp. 58. (London: The Natural Rubber Producers' Research Association, 1965.) [2611

The Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 2, No. 7 (October 27, 1965): Conifers—Keys to the Genera and Species, with Economic Notes. By H. M. FitzPatrick. Pp. 67–129+plates 4–10. (Dublin: The Royal Dublin Society, 1965.) 35s. [2611

The Fifth Report of the Leverhulme Trustees, 1962–1964. Pp. 93. (London: The Leverhulme Trust, 1965.) [2611

Meteorological Office. Weather in Home Fleet Waters. Vol. 1: Northern Seas (Norwegian and Barents Seas and East Atlantic North of 60° N), Part 2. Pp. v+277. (Met. O. 732b.) (London: H.M. Stationery Office, 1965.) 35s. net. [2611

Annual Report of the Ordnance Survey, 1964–1965. Pp. 20+9 plates. (London: H.M. Stationery Office, 1965.) 6s. 6d. net. [2611

The Royal Observatory, Edinburgh. Publications, Vol. 5, No. 1: Rotation of the Silicon and Manganese Stars. By B. N. G. Guth[illegible] Pp. 12. 5s. 6d. net. Publications, Vol. 5, No. 2: Observatio[illegible] Reddening. 2. Results for Region in Para[illegible] 6s. 6d. net. (Edinburgh and London: H[illegible]

The Royal Society. In-Service Train[illegible] Mathematics in England. (Report o[illegible] Society on 16 September 1965.) [illegible] 1965.)

[illegible] Facilities in the Uni[illegible]

Edge Hill (Explanation of Sheet 201, New Series). By E. A. Edmonds, E. G. Poole, and Dr. V. Wilson. With contributions by Dr. W. Bullerwell and B. J. Williams. Pp. x+137+7 plates. (London: H.M. Stationery Office, 1965.) 32s. 6d. net [2911

Council for National Academic Awards. Statement No. 3 (November 1965). Pp. 15. (London: Council for National Academic Awards, 1965.) [2911

Ambassade de France, Service de Presse et d'Information. French Foreign Policy—Statement made by the Minister of Foreign Affairs before the United Nations General Assembly, 29th September 1965. Pp. 14. (London: Ambassade de France, Service de Presse et d'Information, 1965.) [2911

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1199-L. Bauxite Deposits of Tennessee. By John C. Dunlap, Harlan R. Bergquist, Lawrence C. Craig and Elizabeth F. Overstreet. Pp. iv+37+plates 1–5. Water-Supply Paper 1548: Geology and Ground Water in Russian River Valley Areas and in Round, Laytonville and Little Lake Valleys, Sonoma and Mendocino Counties, California. By G. T. Cardwell. Pp. vi+154+plates 1–6. Water-Supply Paper 1759-C: Water Resources in the Vicinity of Municipalities on the West-Central Mesabi Iron Range, Northeastern Minnesota. By R. D. Cotter, H. L. Young, L. R. Petri and C. H. Prior. Pp. iv+21+plate 1. (Washington, D.C.: Government Printing Office, 1965.) [2911

United States Department of the Interior: Geological Survey. Water-Supply Paper 1809-B. Geology and Ground Water Resources of Waushara County, Wisconsin. By W. R. Summers. Pp. iv+32+plates 1–3. Water-Supply Paper 1800-H: Reconnaissance of the Chemical Quality of Surface Waters of the Sabine River Basin, Texas and Louisiana. By Leon S. Hughes and Donald K. Leifeste. Pp. iv+71+plate 1. Professional Paper 420-A: Geology of the Los Angeles Basin, California—an Introduction. By R. F. Yerkes, T. H. McCulloh, J. E. Schoellhamer and J. G. Vedder. Pp. vi+57+plates 1–4. Professional Paper 503-C: Early Permian Vertebrates from the Cutler Formation of the Placerville Area, Colorado. By George Edward Lewis and Peter Paul Vaughn. Pp. iii+50. (Washington, D.C.: Government Printing Office, 1965.) [2911

Conseil International des Unions Scientifiques—International Council of Scientific Unions, Paris. Tentative List of Publications of I.C.S.U., Scientific Unions, Special and Scientific Committees and Commissions of I.C.S.U. between 1963 and 1963. Pp. 121. Tentative List of Publications of I.C.S.U., Scientific Unions, Special and Scientific Committees and Commissions of I.C.S.U., Year 1964, and corrections and additions to the 1953–1963 List. Pp. 81. (Paris: I.C.S.U. Abstracting Board, 17 rue Mirabeau, 1964 and 1965.) [2911

United States Naval Observatory. Circular No. 108: Observations of the Sun, Moon, and Planets—Six-Inch Transit Circle Results. By A. N. Adams and D. K. Scott. Pp. 9. Circular No. 109. Annular Solar Eclipse of 20 May, 1966. By J. S. Duncombe. Pp. 23. Circular No. 110: Total Solar Eclipse of 12 November 1966. By J. S. Duncombe. Pp. 19. (Washington, D.C.: United States Naval Observatory, 1965.) [2911

Western Australia. Report of the Geological Survey Branch for the year 1962 (Extract from the Report of the Department of Mines.) Pp. 64+31 plates. (Perth: Geological Survey of Western Australia, 1963.) [2911

Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Chemical Research Laboratories 1964–1965. Pp. 116. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [2911

University of Ibadan. Calendar 1965–1966. Pp. 209. (Ibadan: The University, 1965.) [2911

Contribuicoes Avulsas do Instituto Oceanografico, Universidade de Sao Paulo. No. 6: Redescricao de *Excorallana quadricornis* (Hansen) e Chave de Classificacao para as Especies do Genero. Por A. L. De Castro. Pp. 6. No. 7: *Amphoclia repens* (Lyman 1869) Sinonimo de *Amphiodia pulchella* Verrill 1899—(*Ophiuroidea*). Pp. 5. (Sao Paulo: Instituto Oceanografico, Universidade de Sao Paulo, 1965.) [2911

Quaderni di Fisica, Serie Prima. Pages in Physics, No. 4. Le Modificazioni delle Masse in Moto (The Modifications of Masses in Motion). By Giuseppe Antoni and Enrico Bombieri. Pp. 66. (Turni: Edizioni Thyrus, Casella Postale 52, 1965.) [2911

Skogshögskolan, Stockholm. Studia Forestalia Suecica Nr 26. CO_2 Production as a Measure of Decay Activity in Wood Blocks. By Allan Klingström. Pp. 20. 3 kr. Nr. 27: Om Fröproduktion, Fröträdsval och Plantuppslag i Försök med Naturlig Föryngring. Av Stig Hagner. Pp. 43. 6 kr. (Stockholm: Svenska Skogsvardsföreningen, Box 16816, 1965.) [2911

Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Irrigation Research Laboratory, 1964–1965. Pp. 45. (Griffith, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1965.) [2911

United States Department of Commerce: National Bureau of Standards. Monograph No. 87: Oxidation of Polycyclic Aromatic Hydrocarbons—a Review of the Literature. By R. Stuart Tipson. Pp. iv+52. (Washington, D.C.: Government Printing Office, 1965.) 40 cents. [2911

State of California. Department of Fish and Game. Marine Mammals of California. By Anita E. Daugherty. Pp. 87. Fish Bulletin 130. Ocean Sport-fish Catch and Effort from Oregon to Point Arguello, California, July 1, [illegible]. By Daniel J. Miller and Daniel Gotshall. Pp. 135. [illegible] Structure, Development, Food Relations, Reproduc[illegible] Squid *Loligo opalescens* Berry. By W. Gordon [illegible]. California. Office of Procurement, Docu[illegible] 1965.) [2911

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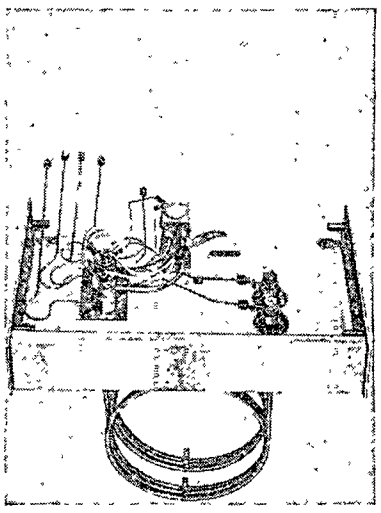
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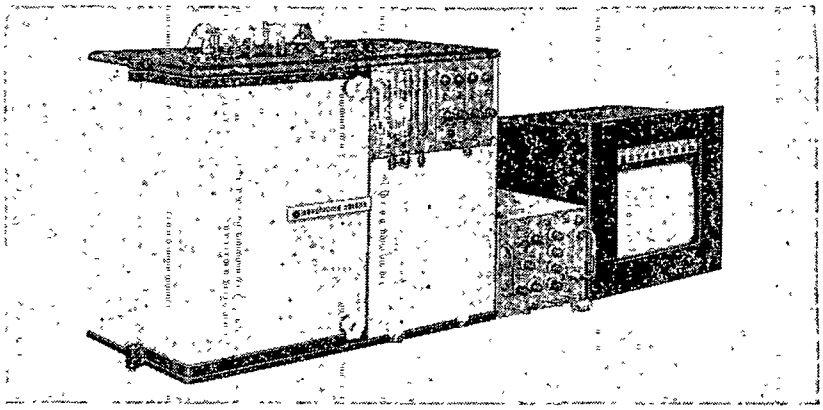
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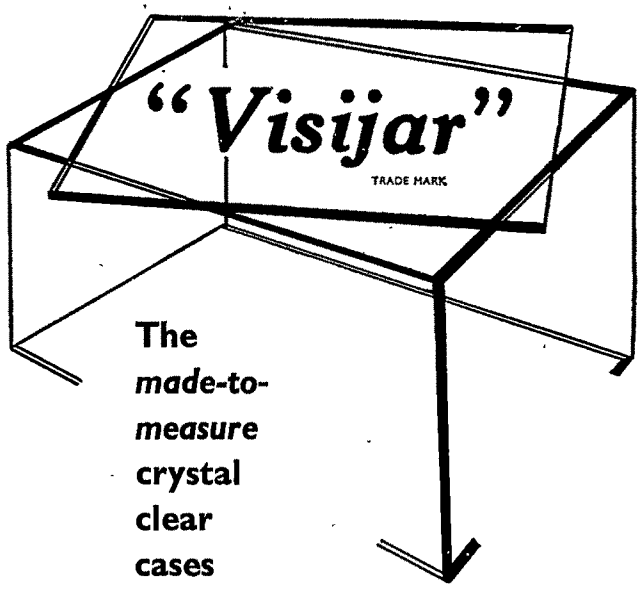
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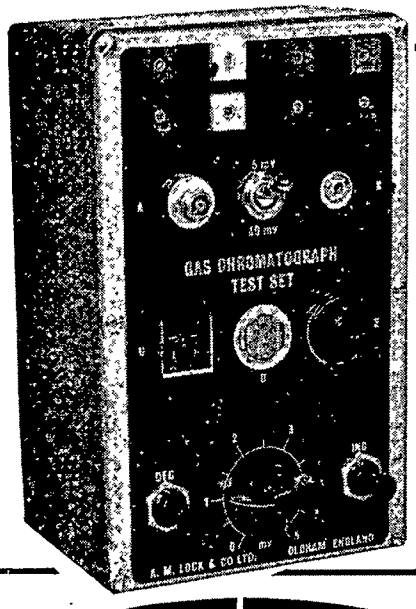
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THE PLIGHT OF BASIC CHEMISTRY RESEARCH

IN the fascinating and well-documented Westheimer Report, *Chemistry: Opportunities and Needs**, American scientists have received some answers to the 64,000-dollar question, "What is the role of basic chemistry research in the United States and is sufficient financial support available to enable this role to be adequately filled?"

The 222-page document is the result of the rapid and remarkable team-work of thirteen leading professors of chemistry and two senior industrial chemical research directors, under the chairmanship of Frank H. Westheimer, of Harvard. This body, termed the Committee for the Survey of Chemistry, was set up in 1964 by the National Academy of Sciences–National Research Council in order to investigate the widespread belief among scientists that American basic research in chemistry has been handicapped by a grave shortage of funds. Financial support for the survey to the extent of 50,000 dollars was provided by the National Science Foundation and the American Chemical Society.

In a comparatively short period of time (two years) the Committee has gone to a great deal of trouble to find out all it can about basic chemistry in the United States and other leading countries. It considered it to be necessary to define from the start what 'chemistry' means; 40 pages of the Report have been devoted to a delightful essay entitled "Basic Research in Chemistry", which deals with current trends in the chemistry of everyday life and its impact on other scientific disciplines. In this the Committee has high-lighted a number of outstanding discoveries, quoting authorship, which naturally brings American scientists to the fore. Among the topics covered are structure, physical properties and characterization, syntheses (particularly of molecules of biological importance), chemical dynamics, reactions in solution, and in biological systems, liquids, solids and surfaces, theoretical chemistry and spectra, and nuclear chemistry. These unquestionably have a great impact on the whole world of science. Some hints are given as to how basic concepts are applied to industrial processes. Quoting from the past, it is cited "that the structural theory of organic chemistry which was originated in Ghent in 1858–65 led promptly to the great German dye industry . . . subsequently the theory has provided the framework for the development of the plastics and pharmaceutical industries". Some will say that this is a far-fetched statement, but the meaning is clear. More precisely it is stated that "the electrochemical industry from chlorine to aluminium is based on the theory of ionization".

We have the statement that "another way of confirming the relationship between basic research and the practical world is by examination of publications from industrial laboratories that announce useful inventions". From a study of these in which citations to previous publications are given in footnotes, it is shown that an overwhelming percentage of the citations refer to publications in fundamental journals and a majority to university research. Figures are provided to show that this particularly applies to the great list of pharmaceuticals and industrial chemicals produced since the end of the Second World War. Basic chemistry and current chemical research point the way to the future. Some complacency is perhaps sensed in the statement that "the data also support the contention that the Chemical Industry, especially in the United States, is remarkable and perhaps unique for the speed and vigour with which it incorporates advances in fundamental science into the body of useful knowledge".

The Report compares chemistry in the United States with chemistry elsewhere. Here the number of papers and patents dealt with by *Chemical Abstracts* provides a means of estimating the share contributed by each nation to the total publications for any particular year, and "as recently as 1960 these indicate clearly that the United States led the World". The Committee could obviously not give a direct assessment of the impact or significance of these papers, but tables have been drawn up showing the citation in publications from other countries to American literature: again the United States leads.

The 'brain drain' (so aptly described as "the free flow of young scientists") has also been used by the Committee as a measure of the quality of basic research. Figures given show that no less than 1,000 European post-doctorals (one for every ten graduate chemistry students) are working in basic chemistry in the United States. (European tax-payers might do well to ponder what this loss, albeit partly temporary, of expensively trained alert minds will mean to their future economy.) The number of Nobel prizewinners in Chemistry is also used as a yardstick, and the United States comes out well, being second only to the British (six winners) with four (R. B. Woodward has since become the fifth) out of the fourteen between 1954 and 1964. Further evidence of American leadership in chemistry is shown by the almost complete dominance of *Chemical Abstracts* in summarizing the chemical literature throughout the world.

Some very striking figures give a good idea of the weight and importance of the chemical industry in the United States. Allowing for lack of precise information from the U.S.S.R., it is claimed that the dollar volume of the chemical industry in the United States is 40 per cent of the world total. In 1962 it produced 32,800 million dollars total value of products compared with 22,900 million dollars produced in Western Europe, and this it did with about half the labour force of Western Europe. Exports in that year were 1,900 million dollars and imports 500 million dollars. European exports were 2,500 million dollars and imports 2,600 million dollars. The growth rate of the European chemical industry is 10 per cent, whereas that in the United States is 6 per cent. All these

* National Academy of Sciences–National Research Council. *Chemistry: Opportunities and Needs* (A Report on Basic Research in U.S. Chemistry by the Committee for the Survey of Chemistry, National Academy of Sciences–National Research Council) Pp. 222 (Publication 1292). (Washington, D.C.: National Academy of Sciences–National Research Council, 1965) 5 dollars.

figures lead to the statement in the Report that the "U.S. Chemical Industry is the largest in the World, and is successful in competing in World Markets".

Attention is directed to the considerable renaissance in chemistry and biochemistry due largely to the development of modern electronic instruments such as mass spectrometers, infra-red, ultra-violet, nuclear magnetic resonance and electron-spin spectrometers, vapour phase chromatographs and other instruments. A survey of several thousand articles in various journals shows that the use of such instruments has increased six-fold in the United States and that this has drastically changed the pattern of chemical research. Attention is directed to the remarkable developments in X-ray crystallography. It is claimed that six of the major instruments are used to about the same extent in the United States, Great Britain, Germany and Japan—all these countries being well ahead of the U.S.S.R. in their use. Great Britain has the lead in X-ray diffraction studies, while the United States is well ahead in the use of computers.

A critical survey is made of the important question of the financing of basic chemical research in the United States and other leading countries. Since the Second World War, the overall pattern of support in the United States has changed from private to Federal funds. In 1964 about 100 million dollars of the Federal support for research went to chemistry. In 1963 the American chemical industry spent 1,250 million dollars on research and development and 140 million dollars on basic research. The total expenditure for 1,300 Ph.D.s in 1964 was between 85 and 95 million dollars, that is, about 70,000 dollars for each Ph.D. awarded, with another 5,000 dollars added for the depreciation of laboratories. Of this, the Government contributes 30,000 dollars per Ph.D. These are surprisingly high figures and are indicative of the high measure of instrumentation.

It is claimed, using figures for chemical research in 1959–60 from *Returns from Universities and University Colleges in Receipt of Treasury Grants*, that the percentage of total research expenditure in Great Britain was 13·6 per cent, whereas chemical research in the United States receives only 5·6 per cent of the Federal total expenditure on research. The Report states: "Chemistry in Great Britain has responded well to the nourishment it has received. The quality of the research in Chemistry is high—note the high proportion of British Scientists among the Nobel laureates".

All the facts and figures collected in the Report, whether strictly accurate or not, are the best available and serve to show that basic chemical research in the United States is insufficiently supported and is greatly in need of increased resources. It is considered that even an immediate increase of 16 per cent in financial support would be inadequate for proper support of chemistry in the United States. The present 'back-log' in needs for chemical instrumentation in universities has been estimated to be 20–30 million dollars and only an additional expenditure of 7 million dollars per annum over and above the normal for the next 3–5 years would allow the universities to catch up. Great emphasis is placed on the growing need of chemists for the use of computers: it is claimed that 15 million dollars would be needed to cover the cost of these. Chemistry also needs funds for specialist projects, particularly those involving techniques of X-ray crystallography. The Committee considers that support for basic chemistry research at universities will need to increase from the 61 million dollars in 1965 to 121 million dollars in 1968. (Some idea of the present rate of increase of financial support can be gained from the fact that in 1964 over-all support was 50 million dollars.)

Some interesting specific recommendations are made. Thus, support of basic chemical research should be extended at the annual rate of 20 per cent for 3 years and 15 per cent for several years further. It is suggested that 5 million dollars should be set aside to meet applications from 'young chemists'. Universities are recommended to modernize their chemistry departments and laboratories. The curricula should be examined and improved and the 'post-doctoral system' should be investigated. It is suggested that the chemical industry could well increase its support of university education and research.

In support of its recommendations, the Committee comments that increments of the order recommended are not "blue sky fancies" or even bold planning; the point is made that the amount at present allocated by the Federal Government to explicit support of basic chemical research in universities in 1 year is equivalent to 5 weeks of Federal expenditure for basic research in the Earth sciences and to only 4 days of the National Aeronautics and Space Administration budget.

We wish our American chemistry friends all success in their aims and ambitions, but we must also look to our own position. In total amount, the figures given in the Report will be quite staggering to British chemists, and Britain's scientific advisers to the Ministry of Education and Science should sit up and take notice. Has anyone in Britain really tried to find up-to-date information regarding the cost of basic chemistry (or indeed any other science) in the universities of Great Britain? At the present time the University Grants Committee is investigating the comparative costs of undergraduate teaching and research. Its method of doing this is not the most efficient and is upsetting a great number of university staff. University research is so closely linked with teaching that it is quite pointless to separate the two.

What Britain needs is a rapid and concise 'Westheimer Report' on the financing of all basic scientific research in the universities of Britain. She needs this to show the Treasury how badly financed basic research is and how very much she needs to keep up with the Americans and the rest of the world in this area. For chemistry, between them, the University Grants Committee and the Department of Scientific and Industrial Research in the past have done their best with the funds at their disposal. In many parts of Britain many fine new chemistry laboratories have been built and when built have been lavishly equipped with the most modern instruments. In other places, however, some considerable chemistry 'slums' still exist; because it is impossible to obtain an equipment grant except for a new building, a great number of nearly obsolete instruments are still in use.

The former Department of Scientific and Industrial Research did its best to fill in some of the gaps, and no doubt the Science Research Council with more than £0·75 million per annum available for basic chemistry research projects will continue to do what it can; however, it is essential that it makes a special point of encouraging some of the younger chemists. There is great need for the University Grants Committee and the Science Research Council to work in close harmony on the financing of basic research: there are some signs that closer co-ordination is already in operation.

Support from the British chemical industry for university research, although very welcome, is relatively small and confined to fellowships or scholarships and the

occasional small piece of apparatus on loan. Possibly the best way that the industry could help would be to place contracts with university departments for some of its more speculative long-range research projects.

Basic research in chemistry and other sciences must be kept in the healthy state it enjoys at present. If Britain is to maintain her present advanced position a considerable increase in financial support must be forthcoming from the Government and industry.

TOWARDS A RATIONAL FUEL POLICY

A MAJOR feature of the meeting of the British Association for the Advancement of Science at Cambridge was the plenary symposium on "World Fuel and Power Resources and Needs" on September 6, 1965. The six papers contributed were published almost simultaneously, with an introduction by Sir Cyril Hinshelwood, in a special issue of *The Advancement of Science*. They provide an appropriate background against which to consider the White Paper on *Fuel Policy** which came from the Ministry of Power in October. This is especially so as, in the final contribution to the symposium, Prof. A. R. J. P. Ubbelohde called in question the whole basis of British policy for energy even more unequivocally than did Political and Economic Planning in its recent Broadsheet, *Questions of Fuel Policy* (see *Nature*, 208, 24; 1965). The symposium itself was indeed intended to stimulate answers to such questions as the magnitude of world needs for fuel and power and the potential contributions and limitations of coal and oil and natural gas, as well as of nuclear energy and hydro-electric power. It sought also to assess the significance of new inventions such as the fuel cell and magneto-hydrodynamics, together with the implications of the answers to the foregoing questions for the less-developed countries.

It was appropriate that the symposium should have been opened by a paper on the world's needs from Sir Harold Hartley, who from 1950 until 1956 was president of the World Power Conference and who had devoted his presidential address to the British Association in 1950 to "Man's Use of Energy" (*Nature*, 166, 368, 636; 1950). Sir Harold concluded his address with a quotation from the final words of his presidential address, from which he saw no reason now to dissent: "On man's wise use of energy depends so much the future of this troubled world", and the ovation which Sir Harold received at its close, as much for his courage as for the sweep of the address itself, is unlikely to be forgotten by any of those present.

Lord Robens discussed the contribution of coal with vigour and confidence, emphasizing more particularly the significance of recent developments in mechanization and organization. Mr. J. P. Berkin was equally confident as to the potential contribution of oil, and he too emphasized the technical advances in oil operations, and the scope for yet further technical advance, flexibility and adaptability. Prof. H. J. Kruisinga was confident that future developments in gas technology and the greater availability of natural gas would make for an increasing contribution to the world's needs for energy. Lord Hinton questioned, however, whether existing knowledge permitted quantitative forecasts of the contribution of hydro-electric power or nuclear power. He thought the development of hydro-electric power was likely to continue for several decades but that, in the long-term, its exploitation would diminish under the competition from nuclear energy, which he expected to lower progressively the limit of acceptable cost.

In this context, it is worth noting the lecture on nuclear power development in the United Kingdom, which Sir William Penney gave to the Japanese Atomic Industrial Forum in Tokyo and which has been published in *Atom*, the monthly information bulletin of the Atomic Energy Authority (108, 198; October 1965). Sir William's outline of British progress in the industrial application of nuclear power, showing the trend from the first magnox station to the advanced gas-cooled reactor at the Dungeness *B* station, indicated that, with a 30-year life and an average load factor of 75 per cent, the latter station would give a generating cost of just more than 0·41*d.*/kWh, or just less than 0·38*d.*/kWh with an 85 per cent load factor. He was confident that the fast reactor offered promise of lower power costs as well as of an immense improvement in the use of natural uranium. Sir William predicted that with a large programme of fast reactors, the cost of the fuel cycle would be less than 0·1*d* per unit, and in the longer term could be about half this amount. The capital costs should be similar to those of advanced thermal neutron stations built at the same time.

The most imaginative contribution to the Cambridge symposium was undoubtedly that of Prof. Ubbelohde, which nevertheless provided a wholesome corrective to some of the earlier optimism. He emphasized the extent to which the need to plan supplies of energy had outstripped the development of wholly reliable techniques of forecasting and also the importance of giving special consideration to the cost and timing of switches between different forms of energy. He reminded his audience that almost all technologically advanced communities are now net importers of energy and that hindrances to free access to energy resources outside national boundaries could become much more obstructive, with increasing danger of famine conditions to countries importing energy on a large scale. He also gave warning of the high quality of political judgment demanded in deciding the extent to which a policy of protection for indigenous production of energy is wise or desirable. He emphasized the importance of ensuring that the methods used to modernize an old industry do not unduly distort invention and design in technological innovation in the newer industries.

Prof. Ubbelohde also observed that until recently the spontaneous urge towards technological advance had been most marked in communities living in climates in which subsistence is neither very easy nor very difficult. He suggested that we might well ask if any of the more advanced nations as yet showed any signs of levelling off in the rapid growth of their *per capita* consumption

* Ministry of Power. *Fuel Policy*. Pp. iii+35. (Cmnd. 2798.) (London: H.M.S.O., 1965.) 3*s*. net.

of energy. If restraining factors were beginning to be felt, it was important to try to describe their nature. Such a question might well be raised about Britain, whose earlier start in technological growth could now be a handicap.

Prof. Ubbelohde's reference to the power technology of the future, including the direct conversion of nuclear energy into electric power, the use of fuel cells, the generation of electricity by magneto-hydrodynamics and the possibility of approaching more closely the vastly more efficient biological power mechanisms, is obviously too visionary to have any practical bearing on the White Paper. This does not apply, however, to what he said about the process of policy-making. The whole implication of his address is that much more needs to be done to improve the process of decision-making and enabling decisions to be based quickly on all the relevant facts. This criticism is implicit in the P.E.P. Broadsheet. The White Paper on *Fuel Policy* does nothing to remove the gravity of the charge in spite of the claim at the outset that the Government has improved and extended the arrangements for securing the effective and co-ordinated development of coal, petroleum and other sources of fuel and power in Great Britain, and of promoting economy and efficiency in their supply, distribution and consumption.

The Minister of Power describes the arrangements for consultation and co-ordination between these industries, particularly the Co-ordinating Committee and the Energy Advisory Council established in January 1965. His White Paper includes a paragraph on research, in which he repeats his statement in opening an exhibition at Cambridge, sponsored by the British Association for the Advancement of Science, that the nationalized industries of coal, gas and electricity are now spending more than £14 million a year on research and development, compared with £6 million in 1958–59. Their research programmes are reviewed by the Minister's Advisory Council on Research and Development, which considers: (1) whether the programmes of research are adequate to the needs of the fuel industries and of consumers; (2) whether there is proper balance between the different lines of research; (3) whether there are any gaps, undue overlapping or lack of co-ordination between the programmes of the different industries and between them and other programmes, including that of the Atomic Energy Authority, in the field of fuel and power. The Minister of Power has also a special responsibility for the research on problems affecting the safety and health of mine-workers which is carried out by the National Coal Board and by the Safety in Mines Research Establishment, under the supervision of the Safety in Mines Research Advisory Board. There is also informal co-operation in research generally between the nationalized fuel industries at all levels. In addition, on the Government's initiative, the Research Liaison Committee of the National Coal Board and the Central Electricity Generating Board has been expanded to include the gas industry.

Even the most appropriate structure or efficient organization, however, does not amount to a policy, nor does the Minister's frank recapitulation of the difficulties which confront the reconciliation of the conflicting claims of gas, coal, electricity and oil indicate what action he could take, still less proposes to take, to rationalize the fuel situation. Without decisions, however, policy is impossible. A disappointing feature of the White Paper is the Minister's evident reluctance to use his powers even in minor matters where friction arises between the gas and electricity industries. If the Government believes that the two industries should reach an acceptable agreement, policy should encourage if not enforce such agreement. Nor is the paragraph on amenity considerations reassuring, in spite of the statement that the Government is encouraging the research efforts of the electricity industry and the cable manufacturers to reduce the cost of underground transmission. It is recognized that reduction of pollution from the emission of sulphur involves greater expenditure, as does the use of underground cables instead of overhead lines. It is not recognized that this expense should be a national and not a regional or local charge, but such a step is essential in any really national policy.

The failure to deal with the investment problem is more serious, and the magnitude of the economic implications is no excuse for ignoring the issues. They are emphasized as much by the possibilities in the production of nuclear power to which Lord Hinton and Sir William Penney referred as by the new prospects of oil and gas from the North Sea. Decisions on taxation policy are involved to which the P.E.P. Broadsheet referred, but the Minister o Power comes no nearer to considering the possibility that there might be sound reasons for encouraging the use of gas where it can be a substitute for electricity than the admission that it may be desirable to curb the demand for space-heating by direct-acting appliances because of the high costs to the economy which are generally involved compared with other forms of space-heating. Nevertheless, Mr. Lee omits to notice either that the demand he thinks desirable to curb involves an investment in power stations of £2 million a day, or the bearing of cheaper energy on Britain's economic development.

To read the White Paper alone is to appreciate that as large a share of the responsibility for the position which the early onset of wintry weather precipitated rests fairly on the Minister of Fuel, including his predecessor in office, as on the fuel industries themselves. Admittedly, as the White Paper acknowledges, while there is little change from year to year in some basic characteristics of the fuel industries, yet their research and development effort continually opens up new opportunities and presents new problems. New sources of fuel supplies are being discovered, and the balance of costs between different fuels changes continually. This, however, only enhances the need for sound judgment, especially where social as well as economic costs are concerned. Nor is it easy to take decisions when, as so often happens, the interests of particular sectors are pressed with complete disregard of the wider perspectives.

The objectives of fuel policy as set forth in the White Paper are reasonable and are consistent with much in the P.E.P. Broadsheet. It is recognized further that some of the objectives desiderated are mutually inconsistent and that the single-minded pursuit of the supply of every fuel at the lowest immediate price would not necessarily be to the best advantage of the nation. As to how this dilemma is to be resolved or what should be the choice of priorities, there is no hint. Similarly, there is no indication of the resolution which will be required to pursue any policy that may be chosen. It may be that, as P.E.P. suggests, the right policy for Britain is to attempt to achieve the chosen degree of support for British coal at the lowest possible cost and with the least distortion of other resources throughout the economy. Nevertheless, the Minister and his Advisory Council must, at the same time, make the closest possible investigation not merely of the reliability of the latest tenders for

nuclear power stations but also of the implications of the developments in fast reactors emphasized by Sir William Penney. Only in this way can full advantage be taken of what is now one of Britain's foremost assets—her trained manpower and the technology it provides.

ON HUMAN BONDAGE

The Technological Society

By Jacques Ellul. Translated from the French by John Wilkinson. Pp. xxxi+449+xiv. (London: Jonathan Cape, 1965.) 63*s*. net.

IN his foreword, R. K. Merton claims that in *The Technological Society* Ellul formulates a comprehensive and forceful social philosophy of our technical civilization. The book is much more accurately described as a rigorous, detailed and persuasive analysis of contemporary civilization. Philosophically it adds nothing to Bavink's discussion of the philosophy of technology more than a generation ago in *The Anatomy of Science*, and virtually the problem of values and judgments of value is ignored by Ellul. Its counterpart is rather Mannheim's *Diagnosis of our Time*, from which, however, it is poles asunder, and it is for its searching, incisive, ruthless analysis of the impact of technology on society and of the changes that are in consequence taking place not merely in industry but in society, in government and in science itself, that this picture of what might perhaps be described as the decline of man has certain claims to be considered a major book of our times. Its prose is as lucid as its thought; the freedom from jargon matches its honesty of thought, and the translator has admirably reflected the incisiveness of the original. Whether we accept entirely M. Ellul's argument, whether or not he is right in his main contention, this is a challenging book which compels us to look below the surface and see where we are going. Any fallacies are to be detected only by hard thinking, and i he is in fact too pessimistic, he stimulates the constructive thought that is required to provide the answers.

M. Ellul's use of the term 'technique' does not make for easy reading, and is the outstanding exception in a book that can otherwise be read with ease and pleasure, due also to the high standard of production; unlike so many expensive books to-day, it is good value. By 'technique' M. Ellul does not mean simply this or that procedure for attaining an end: it is rather the sum o methods rationally reached and efficient (at a given stage of development) in every field of human activity. Accordingly, it is continually new and has no common concern with techniques of the past. Such a definition is sufficiently at variance with the ordinary more restricted concept to be liable to cause confusion, particularly when considering its effects on social relations, political structures or economic phenomena, unless the distinction is kept carefully in mind.

In the first of his five main subjects, M. Ellul elaborates this conception and traces briefly the historical development of techniques, though to a less extent than Mumford in his great trilogy, and with less assurance. In the second chapter he reviews the way in which technology functions in society to-day and the tendency of techniques to dominate the situation and of ends to determine means. In the third chapter, he carries this analysis into the economic sphere and shows how this same process holds there, thus agreeing with Galbraith's analysis in *The Affluent Society*, but not pursuing Galbraith's criticism. The fourth chapter, on technique and the State, examines ruthlessly the implications of this process in the sphere of government, particularly for democratic systems and for the relations between science and public policy, while finally in his fifth chapter, on human techniques, he extends his analysis to the implications of technique not simply for human relations in industry or problems of leisure, but to the long-term effects of television, radio and the cinema on the outlook and the very nature of man. Only in the eight pages of his final chapter does he permit himself a brief look at the future.

It is unfair to an outstanding and cogent diagnosis merely to describe it, though quite accurately, as depicting man as dominated by the machines and the organization he has created, losing more and more his power to control events, even his individuality and his creative powers, until ultimately the great machine and organization run down to a halt, unless some chance first sparks off the total destruction of a nuclear war. Admittedly also he has no place for moral values or judgments: civilization is seen first and foremost as a civilization of means which are in reality more important than ends. Nor does he offset his pessimism by offering solutions to the problems he raises, beyond insisting that correct diagnosis is the essential prelude to treatment.

The calibre of the book is illustrated much more fairly by indicating the particular challenges it presents, though obviously only a few can be mentioned as representative. For example, in this picture of technique, the choice of techniques is increasingly automatic and man is stripped of his faculty of choice, while every technical activity is superior to any non-technical activity. That is of a piece with the opposition which Ellul sees between technique and Nature, but rejection of that view and its implications calls for some deep thinking and not mere superficiality. Again, technique is seen as inconsistent with the freedom of man and no democracy is possible in the face of a perfected economic technique. He writes that technique cannot tolerate the gropings and slow tempo of science, and that it seems impossible that independent research can survive in such a technological society as he describes.

There is a thoroughly realistic passage in which the implications of this theory for scientific policy are clearly indicated and the anti-democratic character of economic technique, though he never mentions either 'scientific adviser' or 'scientific policy'. The emphasis is on the loss of liberty involved in the ascendancy of technique. He discusses the implications of human techniques and suggests that it is impossible to speak of a technical humanism, and he brings before us much that every one of us can recognize as happening. The question is inescapable: Is this where we are really going?

But not everyone will accept without further enquiry that there is no alternative, nor is M. Ellul explicit on the point: this is what could or will be, he writes, not this is what must be. His diagnosis is likely to provoke fresh battle over issues in which von Hayek and Polanyi and others have contended in the past and it would seem to be part of Ellul's purpose to stir fresh debate and enquiry. In this long book there is not a single reference—only a considerable bibliography. The reader is left, for example, to search through Mumford's trilogy for the passages to which Ellul refers, nor is the third and most relevant volume of the trilogy cited in the bibliography. There are some other surprising omissions—the work of Bavink, already noted; and the absence of references in the text is scarcely to be excused by the naïve remark that the books cited are to be read and not consulted.

That is a major blot on an important if not a great book. It is stimulating and suggestive, as provocative as Skew at his best, especially in the opening chapter, but even Mr. Wilkinson's limpid prose scarcely makes it a refreshing book, except perhaps in contrast to the stilted jargon in which so many technological and sociological books are written. This is, in truth, evidence, which Ellul has neglected, that men and women are seeking—and need—a worth-while view of life, that the spirit of man is not yet quenched, that the scientist will not be content to see the very conditions in which scientific work is possible disappear without a struggle, that mankind is beginning to become aware of its peril. To rouse men to

their danger was indeed Ellul's purpose in writing the book: had he considered more deeply Bavink's emphasis on the element in technology of fitness for a purpose he might have written a less pessimistic and a more constructive book. R. Brightman

INTERNATIONAL ENCYCLOPEDIA OF SCIENCE

The International Encyclopedia of Science

Edited by James R. Newman. Vol. 1: A to D. Pp. ix + 1–342 + plates 1–26. Vol. 2: E to L. Pp. v + 343–688 + plates 27–49. Vol. 3: M to R. Pp. v + 689–1034 + plates 50–70. Vol. 4: S to Z. Pp. v + 1035–1379 + plates 71–84. (Edinburgh and London: Thomas Nelson and Sons, Ltd., 1965.) 252*s*. the set.

The International Encyclopedia of Science is a handsome work of four large volumes, totalling nearly 1,400 pages. It is produced in first-rate American style with excellent printing, colour diagrams and photographs and many other illustrations in colour. It was first published in the United States as *The Harper Encyclopedia of Science* in 1963, and now appears, with some additional material, printed in Edinburgh with the original American spelling. At 3 guineas per volume, the price of this valuable work cannot be considered excessive.

Four hundred and fifty scientists and engineers have contributed nearly 4,000 articles which appear in the work. An encyclopaedia, almost like an anthology, is a work of compromise, selection, preference and taste. There are bound to be space limitations no matter how large the project. One may say of the work, like Samuel Johnson of his *Dictionary*, "In this work when it shall be found that much is omitted, let it not be forgotten that much likewise is performed".

The scope of the work is wide and includes all the so-called pure and applied sciences. The mathematics sections are particularly full, even to the extent of some overlapping with one another, when separate authorities write the articles in which there is some common material.

The standard of difficulty of the *Encyclopedia* varies from subject to subject: generally it is of undergraduate level or less, and much of it would appeal to the sixth-former. The intelligent layman and woman will find these volumes admirable as bedside books, although some of the mathematics, albeit logically set out, is not easy and often is of greater difficulty than the rest.

In so large and comprehensive a work, it would be easy and priggish to make criticisms in detail. There is a sizeable article on quaternions, but, of a class of thirty mathematics graduates in one British university, only one knew what they were, and even he could not suggest any applications of them—nor indeed does the *Encyclopedia*.

There is no article on the laser and even the paragraph on the maser does not give a worthy explanation of its action.

In the statistics section, average family income is given as that of the median family in the income hierarchy, and this would be true only if incomes were distributed normally or symmetrically.

More might have been said about entropy and degrees of freedom, both of which have wide applications in the sciences.

Acoustics is dealt with as the physics of sound and not the science of hearing, although there is a separate article on sound. Some of this is rather old-fashioned in conception; for example, the idea that harmonics define a tone quality.

There is an article on π which mentions its irrational but not its transcendental nature, though this is not forgotten in the article on '*e*'. Chemical research is the subject of an article which overlaps with other chemical entries, but there is no article on physical research. In an American publication, it is delightful to find that the American gentleman of fortune, "Rumford, Benjamin Thompson, Count", is described as a British physicist, as indeed he was, among other things! The pituitary body (an endocrine gland) is given an article, but there is no mention of its other name, 'hypophysis'. Lindberg is incorrectly given as the first man to fly the Atlantic.

Although one may feel sorry for writers and compilers of scientific encylopaedias, since much of their work becomes of historic interest only, because of the rapid progress of the subject, there is much in these volumes which will not quickly become effete. Moreover, it can be expected that another edition will be called for very quickly, because of the scope and worthiness of the present work. In this case, a 'tidying up' of the *Encyclopedia* will produce an even better set of books. Large as are these four volumes, 1,400 pages are a not great amount of space to encompass, even briefly, the whole of scientific knowledge. Some of the articles, such as those which deal with pure mathematics, statistics and quantum theories, should be read by future editors, so that common material between the parts of these (and other) subjects respectively might be eliminated. Some of the smaller articles could be combined into larger treatments of the subjects and references made thereto under the present headings. This would save space for more comprehensive essays on some important topics about which little appears in the present volumes.

The many pages of tabulated information, the bibliographies and the index are excellent. The whole is generally a splendid piece of work. W. L. Sumner

INSECT NERVOUS SYSTEM

The Physiology of the Insect Central Nervous System

Edited by J. E. Treherne and J. W. L. Beament. (Papers from the 12th International Congress of Entomology held in London, 1964.) Pp. ix + 277. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 60*s*.

The Physiology of the Insect Central Nervous System is a collection of fifteen articles based on papers presented at the twelfth International Congress of Entomology, and it is not surprising that it is a mixed bag, ranging from original contributions of interest mainly to the neurophysiologist, to reviews of recent work which are of interest to the general reader. It includes the following topics: properties of the axon (Narahashi); composition of the fluid bathing the neurones (Treherne); ultrastructure of synapses (Smith); free amino-acids of the central nervous system (Ray); neurophysiological mapping and pharmacological study of a ganglion (Bernard, Gahery, Callec and Boistel); co-ordination of reflex activities (Weevers, Wilson, Miller and Rowell); neuronal pathways (Hughes); recordings from single neurones of optic lobe and brain (Horridge, Scholes, Shaw and Tinstall); learning in headless insects (Hoyle); and stimulation of areas in the brain concerned with control of sound production (Huber).

The articles by Narahashi and Treherne review their own recent work on the nerve impulse and show that the insect has fluid of an ionic composition differing from that of haemolymph bathing the axon and that the nerve-impulse follows the classical pattern. Smith points out that axons which are closely apposed may have no glial material between their membranes, and he regards this situation as a pre-requisite for a synapse. It is of interest to speculate on the possible function of such presumptive synapses which are found in both central and peripheral systems. In his review of neuronal pathways, Hughes gives in a concise form an excellent account of method-

ology and discovery in this field. For the reader who is interested in locomotory mechanisms the articles by Weevers, Wilson and Rowell give an up-to-date account of recent developments. Miller's account of the control of spiracular movement is particularly lucid and stimulating.

Although Hoyle's paper is not a review but a substantial original contribution, his conclusion that Horridge was justified in using the term 'learning' to describe behaviour in headless insects is of wide general interest. Hoyle demonstrates at the neurophysiological level the phenomenon of 'reinforcement' at the behavioural level. His preparation is the simplest at present available for the study of the physiology of learning. The suitability of insect preparations for neurophysiological work depends partly on the separation of constituent parts of the nervous system into ganglia and peripheral neurones, but particularly on the small number of central neurones involved. The insect achieves with a few cells what the vertebrate uses many cells to accomplish. The spiracular muscle preparation of Miller is another with a considerable future in the analysis of central mechanisms, particularly the role of the median ('autonomic') system. The preparations of Rowell, in which he progressively isolates the prothoracic ganglion, illuminate the apparently widespread phenomenon of non-specific inhibition. The classical type of localization experiment is well exemplified by the work of Huber in locating by stimulation those 'centres' of the brain concerned with sound production. The caterpillar stretch-receptor preparation of Weevers with its single sensory neurone also provides an easily recognizable input to the central nervous system.

This book shows clearly that a variety of techniques is necessary for studying the physiology of the nervous system, but above all it shows the necessity for a sound anatomical basis in physiological work. As Roeder points out in his epilogue, the first gap is in 'old-fashioned' neuroanatomy. It is good that physiologists realize that anatomical studies are indispensable to future progress. Let us hope for a rise in the status of anatomists and histologists, to attract research workers of the necessary calibre.

L. H. FINLAYSON

LEVIATHAN AND CO.

Marine Mammals

By R. J. Harrison and J. E. King. (Hutchinson University Library.) Pp. 192. (London: Hutchinson University Library, 1965.) 15*s*.

MARINE Mammals packs a remarkably complete account of the cetaceans, pinnipeds and sirenians into 200 pages. The very inaccessibility of these creatures, which differ in so many ways from their terrestrial relations, enhances their interest to the zoologist and throws out a challenge to investigate the manner of their lives.

Until about 25 years ago the only methods that could be used for learning about them involved observing living animals in their native habitats, often difficult of access, and dissecting their dead bodies either fortuitously washed ashore or obtained in the field and transported in a more or less mutilated condition to the laboratory. Now the invention of aqualung diving allows the zoologist to approach them in their own element; developments in electronics permit a study of their remarkable underwater powers of echo-location; and the building of the great marine aquaria, the 'oceanariums', has brought the cetacea as well as the pinnipeds and sirenians into captivity where they can be kept under close observation and be made the subject of experiment.

So much has been discovered by these means about the marine mammals that Man has basely tried to enlist their help in his futile quarrels—attempts have been made, misusing the newly acquired knowledge, to train dolphins to find out and advertise the presence of enemy frog-men spying on harbour installations and perhaps trying to destroy them, and so act as unwitting suicide 'heroes' by homing on enemy underwater craft while carrying charges of explosives to sink them.

The authors give a concise account of the systematics of the three orders of marine mammals, and enumerate the species with notes on their appearance, habitats, geographical distribution, and frequency of coming under the notice of Man. Under the modest headings of "Some Characteristics of Cetacea" and "of Pinnipedia" a very good account is given of the biology of the creatures in all its aspects, anatomical and physiological, together with notes on their general natural history. Little that is known about these fascinating creatures escapes notice, although it is necessarily summarized and the information is compressed.

The book is well written and can be read from cover to cover with enjoyment and profit, but it is also a valuable work of reference, for an extensive bibliography cites the more detailed sources from which much of the information is culled. There is a good index, and the pages are embellished with a series of drawings by Mr. Frank Price of the animals and their anatomical details; but what has happened to the eye of the sperm whale, which seems to have migrated to a very unusual position—perhaps it is the roving eye of Moby Dick? This book is a distinguished addition to an already distinguished series.

L. HARRISON MATTHEWS

PROPHYLAXIS AGAINST RADIATION INJURY

Chemical Protection Against Ionizing Radiation

by Prof. Zenon M. Bacq. (A Monograph in The Bannerstone Division of American Lectures in Living Chemistry.) Pp. xvi+328. (Springfield, Illinois: Charles C. Thomas, 1965.) 14 dollars.

THE search for prophylactics against radiation lethality in mammals, begun in the late nineteen forties, has proved unrewarding in that no agent having practical utility has emerged. Nevertheless, as Prof. Bacq reminds us early in *Chemical Protection Against Ionizing Radiation*, it is true that: "Chemical protection has won an enviable place in the vast ensemble of radiation research; it occupies about ten per cent of the time and the publications in congresses, meetings and periodicals dedicated to radiation research". For most, interest now lies chiefly in the properties of existing agents, rather than in the search for new; in their pharmacology, biochemistry and mechanisms of action at one or more levels of biological organization. With its ramifications into many disciplines, there is no disputing with the author that: "chemical protection . . . has become a very complicated subject in recent years". He has produced this book "because a guide written by somebody who has actively lived through its capricious evolution might be useful to those who are engaged in or want to enter one sector of this research field".

Prof. Bacq is to be congratulated for this attempt to bring the mass of published observations on this subject into order within the covers of a single volume, a task for which his long association with the subject fits him well. In this task he has been, in the main, successful. The literature has been covered largely up to 1964. Where obscurity occurs, one feels it is mainly due to the contradictory nature of the material.

In this book, the field has been narrowed down by exclusion of all but passing mention of curative, post-irradiation treatments; prophylaxis alone is considered. However, attention has not been confined to the protection of mammals. There are twenty chapters, the first

three being brief and introductory. Chapter 4 presents the major classes of radioprotectors and should be of use to beginners; it is based on a list of familiar agents impartially selected, "by an anonymous scientific secretary", for the second report of the United Nations Scientific Committee on the Effects of Atomic Radiation (1962). The remainder cover all aspects of the subject but some may be selected for comment. In Chapter 5, on pharmacology, the review of the diverse circulatory effects of thiol protectors in different species seemed of particular interest. In Chapters 6 and 7 are collated a mass of data on the metabolic effects and metabolism of radioprotectors: it is not the fault of Prof. Bacq that any definite relationship between protection by cysteamine and its tissue distribution is still difficult to discern. Chapter 9, which deals with protection against effects other than lethality, gives a useful literature survey. Examples of protection against genetic damage, in micro-organisms, are brought together in Chapter 12. In Chapter 14, possible applications of thiols in connexion with radiotherapy are mentioned and, it is hoped, will be noted. Chapter 19, on protective mechanisms, is impressive: the section on the role of molecular oxygen is, to me, a highly illuminating interpretation of an extensive literature. The final chapter is largely of a speculative nature. Some useful practical information is included, as that on dosage in Chapter 5. There is a large bibliography, and the index is adequate.

In a work of this type, some omissions and errors of detail inevitably escape correction. There are, in fact, numerous minor errors. Eccentricities in spelling such as "gasses" (page 3), "cristallized" (page 9), "sensitizors" (page 32), "exsudate" (page 65) or such forms as "methemoglobinizing" (page 44) and "aminoisothioureae" (page 67) probably do no material harm. The term "physiochemical" appears on page 79 and again on page 221. In chemical nomenclature, in Table 2 (page 17) "N-dimethylcysteamine" might disturb the chemist: N,N-dimethylcysteamine is the correct (American) nomenclature. On page 168, some units are omitted: "Just as in the intact mouse, the active concentration for MEA and AET is around 10^{-4}". If these are grams per ml., this concentration would correspond with a mouse dose of 100 mg/kg.

There can be little doubt that Prof. Bacq has accomplished his object, and that in bold, Gallic style: this work will inevitably be used by those already engaged in this field of research and by the novice. The book is handsomely produced but the price is very high, even for a modern scientific monograph, so that the private purchaser is virtually excluded. J. H. Barnes

PLANS AND AFRICA

Man and Africa

Edited by Gordon Wolstenholme and Maeve O'Connor. (A Ciba Foundation Symposium jointly with The Haile Selassie I Prize Trust.) Pp. xix + 400. (London: J. and A. Churchill, Ltd., 1965.) Cloth 36*s.*; limp 25*s.*

THE Ciba Foundation organized a conference in 1964, at Addis Ababa, on the climate, resources, development and welfare of Africa; *Man and Africa* embodies the papers and the discussions. As at every conference, the papers vary in quality; some embody the same old clichés which have done duty at conferences for the past twenty years, but most of them are factual and fresh. The participants were of many races and of many disciplines.

The geographical introduction by Last is good, in spite of the confident assertion, "We must tell them in 1964 what they must do in 1984" (p. 22). Dekker shows clearly the instability of African climate and the fact that water supply is a limiting factor over much of the continent. Goody shows how the imposition of several European languages on a variety of African dialects may hinder the unification of Africa, but there is little reference to the great ability of Africans in learning languages.

Lambo emphasized the bad effects of migration and urbanization on mental health. But can migration be stopped, when Africans love to travel and to see strange places? "Before major developments in the socio-economic sphere took place, very little was heard of addiction or abuse of drugs as a national or continental medico-social problem" (p. 135). Little may have been heard of addiction to hemp and it was illegal; but there was much of it and one often saw in the countryside the blank, bewildered faces which, alas, one sometimes sees in London. Why do people smoke hemp? The only time that I tried it, I had no erotic dreams, only heavy sleep and a bad hangover.

The paper by Sutton and the discussion on "African Educational Needs" embodies many ideas applicable far beyond that continent: "the now painfully familiar problem of primary-school leavers increasingly hopelessly seeking jobs in crowded towns" (p. 196); "African leaders now denounce the education of 'pen-pushing' left to them by the colonial powers" (p. 197); "there is, I believe, no African country which has shown in recent years a decline in the numbers of children in school. But there are several, including ones with impressive rates of economic growth, where total employment has declined" (p. 198); "Education for most Africans cannot, in the short run, be a preparation for employment. It will either be a preparation for unemployment or for some form of self-employment, mostly agricultural" (p. 200); "it is not easy for young graduates to regard their talents humbly or to appreciate that they may not yet be of much use to society" (p. 205); "Some of those returning from abroad are even unable to work for two or three years afterwards" (p. 216); "Often more than half the high cost of teaching university graduates goes on almost palatial dormitories" (p. 217); "an elite group which may be irresponsive to the national needs and insensitive to the general feelings of society" (p. 218); "Very often in a village one sees schoolboys on holiday sitting around in apathetic idleness" (p. 221).

Haldane's observations on biological research, founded on experience in India, apply closely to Africa; research can be cheap, for all that is needed is power of observation, a truthful mind and freedom from the 'there-is-no-knowledge-but-I-know-it' attitude of some graduates. Good research can be carried out by people with little education; many years ago I taught an African schoolboy of sixteen to weigh coffee samples, and it took him five days to find why his records did not always correspond exactly with my checks—that if he weighed the coffee beans early in the morning, they weighed more than in the afternoon; he did not know the word hygroscopy, but he discovered it.

"Perspectives for Industrialisation in Africa" by Ewing and Patel reads like a piece of science fiction; because production in developed countries increased 44 times in 110 years, that of Africa will increase 25 times by A.D. 2000 (p. 308); steel production will be 100 million tons, "There is no reason why African production should not attain this level" (p. 313). Some of those born in Africa had a more realistic outlook, "The demands of the African family system sometimes make it difficult for ministers and other responsible persons in government to appoint the best men to positions of managerial control, even if they are available" (p. 322); "yet, when I listen to my economic colleagues talking, I feel that the human element is really lacking" (p. 336).

Dr. Wolstenholme was obviously delighted with the great success of the conference, and he is to be warmly congratulated on it, as are the printers, for the book has good paper and the print is extremely clear and well spaced. This book is to be recommended to all those interested in African development. A. S. Thomas

POSITIONS OF RADIO SOURCES DETERMINED BY THE INTERFEROMETER AT THE ROYAL RADAR ESTABLISHMENT

By R. L. ADGIE and H. GENT

Royal Radar Establishment, Great Malvern, Worcestershire

THE determination of the position of radio sources with very high precision is one of the most pressing needs in radio astronomy: accurate positions provide the essential bases for the identifications of radio with optical sources. The calculation of the distance of a source is dependent on the optical determination of its red shift, and the whole knowledge of the radio power emitted by sources, as well as the relation between optical and radio counterparts, depends on reliable identifications. The deduction of properties bearing on the evolution of galaxies and cosmology, and of the energy of radio sources which for certain quasars and strong radio galaxies has so far proved inexplicable in terms of known physical processes, emphasizes the vital importance of identifications, and this demands extreme accuracy in determining radio positions. The interferometer at the Royal Radar Establishment (R.R.E.) has been applied to this task for the past 2 years, and we now present accurate positions of 137 small-diameter radio sources measured with this instrument.

The R.R.E. Interferometer[1] consists of two steerable paraboloids, 25 m in diameter, mounted on movable carriages. To obtain the greatest resolving power for these measurements the aerials were placed at the maximum separation of 750 m. Two separate sets of observations were made using a base-line oriented approximately east–west to measure the right ascension and near north–south to measure the declination, each source being observed for a few minutes near meridian transit. The receiver was tuned to 610 Mc/s giving a lobe-width of 140 sec of arc at this spacing (with the north–south orientation the base-line is foreshortened for directions away from the zenith, increasing the lobe-width up to 280 sec of arc for a source at the lowest observed declination of $-5°$).

Sources with a small angular diameter were selected mainly from the *Revised 3C Catalogue*[2], a few additional ones being taken from the original 3C (ref. 3) and the *CTA Catalogues*[4]. The sources were also observed with a much smaller spacing of about 150 m to resolve the lobe-ambiguities that can arise with the wider spacing where the published position is not sufficiently accurate. In addition, these observations gave a value for the unresolved intensities, which were then compared with the values obtained at the wider spacing to check the angular diameters. The position of the 'centre of gravity' of a source should be obtained with reasonable accuracy provided the amplitude at the wider spacing is greater than half the unresolved amplitude; with a lobe-width of 140 sec of arc this imposes a limit of 60 sec of arc diameter for a single gaussian source or 40 sec of arc separation for a double source. If the amplitude is less than half, the position is still valid if the source is symmetrical about the centre of gravity, but without a detailed picture of the structure the result is obviously less significant.

The interferometer was calibrated by observing sources that had been identified with optical objects having accurately known positions. This problem has already been dealt with in a previous article[5] presenting the results for eighteen calibrators. A few of these sources showed a significant difference between the radio and optical positions, in particular 3C 286, but in this case the discrepancy has now been removed by the discovery of an error in the published optical position[6]. In two cases, 3C 254 and 3C 298, new identifications as quasi-stellar sources were suggested on the basis of the agreement between the radio positions and stellar objects[7]; these identifications have since been confirmed by examination of the optical spectra of these objects[8,9]. The differences for the other sources were explicable in terms of their large angular diameter and complexity.

With many more sources now identified[6,10], a fresh calibration of the interferometer has been made. In addition, more accurate ionospheric data on the total electron content[11] have given slightly modified refraction corrections, and a re-measurement of the delay cable has meant some small changes in the declination results. The revised positions for the original eighteen calibrators differ slightly from the published results[5], but the changes are within the stated errors of measurement and in no way modify the conclusions concerning the identifications.

The measured positions of 137 sources are given in Table 1. The root mean square errors have been compounded from errors due to phase instability in the equipment, calibration, and the confusion produced by other weaker sources in the aerial beam. Errors due to random noise on the records are negligible. By careful design and temperature control of the equipment and cables, a phase stability corresponding to less than 1 per cent of the lobe-width has been achieved over a period of a few days. The root mean square value of the confusion in directions away from the galactic plane was measured and found to be 0·5 flux units, corresponding to a positional error of 2·6 per cent of a lobe-width for a source of 3 flux units (1 flux unit equals 10^{-26} W.m^{-2} (c/s)$^{-1}$).

The last column in Table 1 gives the flux densities obtained at the closer spacing scaled to the absolute values given by Conway, Kellerman and Long[12], the root mean square errors being a combination of stability, calibration and confusion errors. The observed values have not been extrapolated to zero spacing and so will not include the contribution from any large diameter halo (as, for example, in the case of 3C 84). Finally, those sources are noted which give an indication of being resolved at the wide spacing, as these positions may not represent the true centres of gravity.

Accurate positions in both co-ordinates were determined for 122 of the sources. Of these, 72 have been identified and the optical positions measured to an accuracy of a sec of arc or better[7,9,10]. The differences between the radio and optical positions for these identifications are given in Table 2, the sources being divided into two groups, thirty quasi-stellar sources and forty-two radio galaxies. Each difference (expressed in sec of arc) is the total separation between the two positions; the associated error (root mean square) is the resultant error in total separation calculated from the radio right ascension and declination errors alone. All values have been rounded off to the nearest sec of arc for the sake of clarity.

Of the two groups, the agreement is least satisfactory for the radio galaxies, but this is doubtless due to their

Table 1. Measured Positions
1950·0

3C	R.A. h	min	sec	± sec	Dec °	′	″	± ″	Flux§	±
2	00	03	49·00	0·12	−00	21	10 2	3 0	6·6	0·6
9	00	17	49 95	0 18	15	24	19·4	3·2	4·5	0·6
13	00	31	32 68	0 26	39	07	43·8	3·1	3·9	0 6
19	00	38	13·79	0·17	32	53	39·5	2·2	5·3	0·4
20	00	40	19·70	0·13	51	47	07·2	1·1	19·3	0·5
22	00	48	04·70	0·41	50	55	31*	18	4·1	0·6
28	00	53	09·14	0 22	26	08	20 6	2·8	4·3	0 4
42	01	25	42·86	0·18	28	47	29 3	2·4	5·4	0·4
43	01	27	15·00	0·17	23	22	55·5	2·2	5·4	0·6
48	01	34	49 88	0 08	32	54	20·9	1·2	28 8	0·5
49	01	38	28·74	0 18	13	38	23 3	2·9	4 8	0·4
54	01	52	26·12	0·36	43	31	13·7	4·0	3·4	0 6
55	01	54	19·44†	0·62	28	37	03·6	2 4	5·9	0·6
63	02	18	22·17	0·13	−02	10	35·6	2·9	7·2	0·4
65	02	20	37·06	0·19	39	47	17·4	2·1	8·2	0·5
71	02	40	07·22	0·11	−00	13	31·7	3·0	7·9	0·5
78	03	05	48·92†	0·25	03	55	18 0	2·6	10·2	0·6
CTA21	03	16	09·15	0·14	16	17	39 8	2 3	9 3	0·7
84	03	16	29·66	0·11	41	19	51·6	1·1	14·2	0·6
86	03	23	30·49	0·35	55	09	57·7	2·0	12 2	1·0
89	03	31	42·57†	0·24	−01	21	29 0	4·2	6·6	0·5
91	03	34	03·83	0·23	50	36	02 7	2·0	6 2	0·4
93	03	40	51·38	0·19	04	48	23·2	3·4	5·7	0·6
103	04	04	35·20	0 20	42	52	00*	13	10·7	0·5
105	04	04	48·08	0·20	03	32	53 4	3·2	7 2	0·6
107	04	09	49 78	0·28	−01	07	14 6	5·4	3 6	0·6
109	04	10	55·08†	0 20	11	04	47·3†	4 2	8·0	0·7
119	04	29	07·78	0 12	41	32	08·2	1·2	13·0	0 7
123	04	33	55·38	0·08	29	34	13 4	1·0	89·2	1·4
131	04	50	10 53	0 20	31	24	33·4	2 1	5·9	0·6
132	04	53	42·72	0·16	22	44	49·0	2·4	5·8	0 6
133	04	59	54·36	0 11	25	12	11·3	1·7	10 6	0 5
134	05	01	17·97	0·10	38	02	04*	11	22·4	0·4
137	05	15	37·12	0·72	50	51	41*	22	3·2	0 6
138	05	18	16 46	0·11	16	35	27·1	1·9	12·9	0·5
141	—	—	—	—	32	47	30·0	2·7	5·2	0·6
147	05	38	43·62	0·10	49	49	42·4	1 0	36·0	0 6
152	06	01	30 12	0 20	20	21	41·4	3 0	4·7	0 4
158	06	05	44·41	0·19	48	04	49·6	1·6	7·6	0·6
154	06	10	43·17	0·14	26	05	29·7	1·7	10 0	0·5
158	06	18	50·28	0·19	14	33	38 3	3·1	6·3	0·6
161	06	24	43·13	0·07	−05	51	16 5	2·1	30 8	0·5
166	06	42	24·83	0·21	21	24	57 6	2·8	4·5	0 6
171	06	51	10 88	0·20	54	12	49·9	1 8	6 6	0·6
175	07	10	15 61	0·20	11	51	24·7	2 8	6·0	0 5
180	07	24	33·20	0 20	−01	58	32·5	6·0	5 3	0·6
181	07	25	20·35	0·26	14	43	20*	24	4 0	0 4
186	07	40	56·41	0 34	38	00	32·3	3·3	3·5	0 4
190	07	58	44·98	0 20	14	23	06·0	3·1	5·0	0 6
191	08	02	04·00	0 20	10	23	55 4	3·6	4·8	0·4
194	—	—	—	—	42	36	59·7	2·8	4·3	0 6
196	08	09	59 43	0·10	48	22	08·0	1 0	27·0	0·5
200	08	24	21·58	0 22	29	28	41·9	3·1	4·2	0 4
205	08	35	10 36	0·54	58	04	59*	20	3·5	0·6
210	08	55	11·22	0·28	28	02	30·2	3 4	3·9	0 6
212	08	55	55·91	0 17	14	21	28·1	3·4	5·3	0·6
215	09	03	44·12	0 30	16	58	18 8	4·3	3 9	0·6
216	09	06	17·55	0·16	43	06	00·2	1·8	8 6	0 5
222	09	33	55·09	0 32	04	35	49 9	5·4	2·1	0 6
228	09	47	27·73	0·14	14	34	02 0	3·1	6 2	0·6
230	09	49	25·33	0 12	00	12	35·4	3·6	7·6	0·6
234	09	58	57·1*	0 6	29	01	38 5	1·8	11·3	0·4
237	10	05	22·09	0 09	07	45	00·2	2·1	11·6	0·4
238	10	08	23 10	0·13	06	39	31·0	2·9	6·9	0 6
239	10	08	39 02	0·42	46	42	52*	20	3 7	0 6
241	10	19	09 37	0·21	22	14	40 4	3·4	3·6	0 4
244·1	10	30	19·43	0·38	58	30	16*	12	6·8	0·6
245	10	40	06·07	0·13	12	19	16·2	2·4	5·7	0 6
247	10	56	08·25	0·25	43	17	31·6	2·4	5·0	0·5
249	10	59	30·58	0·15	−01	00	08·4	3·9	5 7	0 5
254	11	11	53·05	0·18	40	53	43 0	1·9	6·2	0 4
255	11	16	52 61	0·22	−02	46	23 5	5·2	3·0	0 6
256	11	18	04·35	0·29	23	44	22·9	3 8	3·2	0·4
264	11	42	30·95†	0 29	19	53	27 9†	3 0	10 0	0·5
265	11	42	54·32†	0 47	31	50	28 1	2 3	6·6	0 5
267	11	47	22·16	0 29	13	04	01·3	3 7	4·8	0 6
268·4	12	06	42·28	0·29	43	56	00·6	3·1	4 3	0 4
273	12	26	32·69	0·07	02	19	34·0	1·9	49 0	1·5
275	12	39	44·07	0 14	−04	29	52 5	4 1	6 3	0·6
275·1	12	41	27·45	0·19	16	39	20 9	3 0	5 5	0 6
277·3	12	51	46·03	0 24	27	53	50·7	3·1	4·7	0 6
279	12	53	35·85	0·11	−05	31	06·1	2·9	9 7	0 5
280	12	54	41·37	0·16	47	36	32·7	1·4	9 7	0 4
286	13	28	49·65	0·09	30	45	58·5	1 2	20·4	0 5
287	13	28	16 19	0 10	25	24	36·6	1·5	10 1	0·5
288	13	36	38·17	0 21	39	06	26 9	2 0	7 2	0 6
293	13	50	02·91	0 22	31	41	42 5	2 5	7 0	0 6
294	14	04	34·75	0 29	34	25	43 8	3·8	2·8	0·6
295	14	09	33·34	0·11	52	26	12·9	1·0	39 6	0·7
298	14	16	38 85	0 07	06	42	22·9	1·8	14·7	0·4
299	14	19	06 36	0·21	41	58	33·2	2·1	5·9	0 4
300	14	20	40 83	0·16	19	49	08 5‡	2·3	—	—
303	14	41	23 51	0·34	52	14	19·4	2·4	5·0	0 4
315	15	11	30 89	0 19	26	18	34 8†	6 6	7·7	0·6
317	15	14	17·05	0 07	07	12	17 7	1 7	15·4	0 5
318	15	17	50·54	0 16	20	26	55·2	2·8	5·6	0·4
320	15	29	29·34	0·26	35	43	45·3	3 0	4 5	0 4
321	15	29	40 72	0 18	24	12	52 0	2·7	5·4	0 4
324	15	47	37·66	0 16	21	34	44 2	2 6	6 2	0 5
334	16	18	06·77	0·30	17	43	41 5	3·8	4·1	0 6
336	16	22	32·84	0 20	23	52	06 7	2·7	5·0	0 4
338	16	26	55·79†	0 32	39	39	33 5	1 4	11·1	0 4
340	16	27	29 74	0·30	23	26	51 0	2 9	5 4	0 6
345	16	41	17·60	0·17	39	54	10·6	2·0	7 1	0·4
346	16	41	34·79	0 15	17	21	24·6	2·4	6 6	0 4
349	16	58	05·74	0·29	47	07	09·1†	4 0	5 7	0 4
351	17	04	04·42	0 50	60	48	55*	16	4 7	0 6
352	17	09	18·19	0·27	46	05	11 0	2·7	4 3	0 4
356	17	23	07·38	0·40	51	00	38*	20	3·6	0·4
357	—	—	—	—	31	48	26·0	3 3	4·1	0·6
368	18	02	45 70	0·34	11	01	14·1	6·0	3·8	0·5
380	18	28	13·55	0·11	48	42	43 7	1 1	25·3	0 6
381	18	32	24·35	0 22	47	24	56 5†	5·0	6 8	0·4
388	18	42	34·59	0·15	45	30	27 8	1·5	11·0	0·4
390	18	43	15 28	0·18	09	50	34·1	3·9	10·2	0·7
394	18	57	04·54	0·34	12	55	05·8	4·4	6 1	0·6
399·1	19	13	59·82	0·33	30	14	31·2	3·9	4·6	0 6
401	19	39	38·84	0 22	60	34	33·0	1·6	9 0	0 5
409	20	12	18·01	0·07	23	25	45·0	1·2	30·4	0·8
410	20	18	03·88	0 15	29	32	49 0	3·0	17 8	0·6
411	20	19	44·16	0 14	09	51	33·7	3·1	6 2	0 5
424	20	45	44·59	0·16	06	50	10·1	3·3	5 0	0·4
430	21	17	01·74	0·30	60	35	29*	8	14·2	0 7
431	21	17	08·95	0 51	49	−24	18 5	3 0	8·4	0 6
432	21	20	25 84	0·21	16	51	48·3	3·2	4 6	0·4
433	21	21	30 69	0·08	24	51	22·7	1·3	23·6	0 5
435	21	26	37·59	0·25	07	19	50·1	4·1	5·0	0 6
436	21	41	58·34	0·17	27	56	32·2†	4·3	6·8	0·4
438	21	53	45·52	0 10	37	46	13 9	1·2	15 4	0·6
441	22	03	49 54	0 20	29	14	45·6	2·5	5·3	0 6
446	22	23	10·94	0·12	−05	12	21·4	3·6	—	—
CTA102	22	30	07·71	0·14	11	28	22·6	2·5	8·1	0·7
454	22	49	07 90	0 29	18	32	45·5	3·7	4·4	0 6
456	23	09	56·71	0·17	09	03	12 8	2·9	4·5	0·6
459	23	14	02·33	0·11	03	48	54·0	2·4	9 0	0 5
460	23	18	59·44	0·30	23	30	25·4	3·8	3·7	0 6
470	23	56	02·60	0 28	43	47	54 6	3·1	3 6	0 4

Notes: * Position measured at 300λ spacing, † position may be influenced by partial resolution; ‡ possible lobe ambiguity; § flux values are in units of 10^{-26} W.m^{-2} (c/s)$^{-1}$.

larger radio angular size and not to incorrect identifications.

Considering the quasi-stellar sources alone and excluding 3C 273 (known to be a double radio source with 20 sec of arc separation[13]), 25 out of 29 sources (86 per cent) have differences less than twice the quoted root mean square errors. It is possible that the differences for the four remaining sources are real, as in the case of 3C 273. The mean difference is only 3 sec of arc, which substantiates the accuracy of the measurements and confirms the estimate of the errors involved in the radio and optical determinations.

The other conclusion that can be drawn from Table 2 is, of course, the confirmation of the validity of these

Table 2. Agreement between Radio and Optical Positions

Quasi-stellar sources 3C	Differences ″	Error ″	Radio galaxies 3C	Differences ″	Error ″
9	3	3	2	6	2
43	4	2	28	3	3
48	1	1	63	5	2
93	2	3	71	2	2
119	1	1	78	5*	3
138	1	2	84	3	1
147	1	1	89	12*	4
186	3	4	109	7*	4
191	4	3	132	6	2
196	1	1	153	3	2
215	3	4	161	5	2
216	3	2	166	5	3
245	1	2	171	2	2
247	8	3	175	3	3
254	4	2	180	8*	6
273	13*	2	200	1	3
275·1	4	3	212	6	3
279	2	2	237	1	2
286	1	1	264	31*	3
287	1	1	265	12*	6
298	1	2	267	25*	4
303	6	3	277·3	4	3
334	12	4	288	7	2
345	1	2	293	12	3
380	5	1	295	1	1
432	4	3	300	11	2
435	4	4	315†	1	4
446	5	4	317	1	2
CTA102	0	2	318	3	2
454	1	4	320	5	3
			338	6*	3
			346	5	2
			349	11*	3
			381	18*	5
			388†	9*	2
			401	2	2
			424	3	2
			433†	13*	1
			436	6*	3
			441	7	3
			456	5	3
			459	2	2

Notes * Position differences explicable in terms of the large radio angular size; † optical position has been taken as the mean of the two galaxies.

identifications, since the chance coincidence of two unrelated objects to within a few sec of arc is very small.

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WHY IS AN ODOUR?*

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A SMELL is a sensation we perceive when odorous molecules impinge on sensitive nerve endings in our noses. There are three important things about smells. The first is that the nose is the most sensitive chemical detector known: at its best, its threshold of perception is more than one hundred times lower than the best of our gas chromatographs. Secondly, smell sensations are highly specific: most of us can easily distinguish cigarette smoke from cigar smoke or a rose perfume from a violet scent, but an experienced perfumer can do much better, so that, for example, there are men who can not only name the country of origin of a sample of lavender oil, but can even name the farm from which it came. The third important thing is the emotional impact of a smell. The trade in perfumes is based on this, and even such unromantic objects as second-hand cars can be given an added market value by an artfully applied 'new car smell'.

From a scientific point of view, the sensitivity and specificity are the most interesting, though I will have something to say about the emotional import later on.

Prof. J. T. Davies, in the University of Birmingham, has developed a theory of the olfactory threshold that allows him to calculate the approximate threshold concentration of many odorous substances from two basic parameters[1]. One is the partition coefficient of the substance between air and a water–oil interface. The other is the cross-sectional area of the odorous molecules. According to his theory, the partition ratio determines how many odorous molecules will collect on the receptor surface, and the contact area in some way (which he does not try to explain) determines their ability to 'puncture' the nerve cells and initiate action potentials which travel to the brain.

Davies's theory gives very good quantitative threshold values, but it says nothing about the quality of the sensation. It does not tell us what makes one smell different from another.

Thus, we know some of the properties of substances that relate to their odours but not others. For example, a substance that is completely non-volatile can have no odour, but volatility alone is not enough—some degree of lipid solubility is also needed. Moreover, a substance that is very acidic or very basic, and one that is strongly oxidizing or reducing, is likely to generate a sensation of pain because of its chemical reactivity, and this will usually hide any true odour it may possess.

The properties of a substance that are related to its smell are called its 'osmic properties', and the position to-day is that the osmic properties that have been identified are the non-specific ones. They do not correlate with particular odours such as those of rose, or musk, or peppermint. The fact is that the osmically specific properties of odorous substances have still not been securely identified, and until they have been, we will not be able to give a really precise answer to the question "Why is a smell ?".

To identify the osmically specific properties of odorous molecules, we must look for secure correlations between certain molecular properties and the olfactory sensations that go with them. A great many correlations are conceivable, but most of them have not stood the test of experiment. At present, two, or possibly three, lines are receiving the most attention.

I would like to mention first the 'Profile—Functional Group' or 'PFG' theory put forward some years ago by Dr. M. G. J. Beets in Holland[2]. He supposes that the particular kind of sensation a molecule evokes is determined by the interplay of two factors. These are: the overall shape or 'profile' of the molecule, and the 'functional group' that acts as a point of attachment and so determines which of its various profiles the molecule presents to the receptor surface (Fig. 1). This is evidently a refinement of Davies's ideas since the cross-sectional area of many molecules would vary according to the direction from which they were viewed.

Dr. J. E. Amoore in California has carried this idea still further in his stereochemical theory. Originally, he supposed that the sensitive receptor surface was provided with 'pits' or 'sockets' of several rather rigidly defined shapes that only certain molecules could fit into[3]. More recently, he has abandoned this idea in favour of a specific 'silhouette' very much like Beets's 'profile' but much more clearly expressed[4]. Instead of constructing a solid model of the receptor site into which molecules with a 'floral' or 'minty' odour will fit more or less well, he now selects one molecule as typifying each odour class (floral', 'camphoraceous', 'musky', 'minty', and so on). He then uses a system of shadow matching to measure the similarity in shape between the standard molecules and other

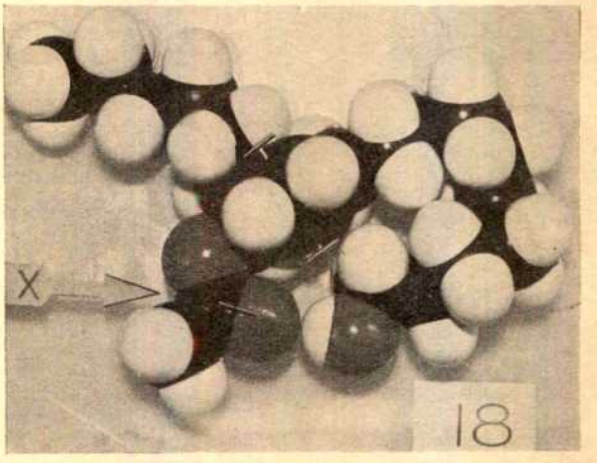


Fig. 1. Model of an analogue of the sex attractant scent molecule of the gypsy moth. The 'profile' it presents to the receptor surface is highly dependent on which 'functional group' governs the orientation

* Substance of an address to the annual meeting of the Pacific Northwest International Section of the Air Pollution Control Association, November 3, 1965.

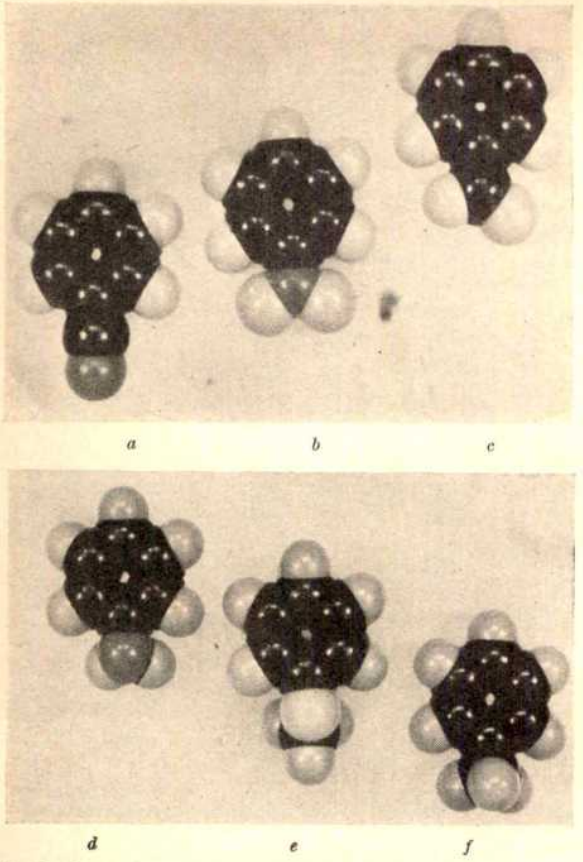


Fig. 2. Benzonitrile (*a*), nitrobenzene (*b*) and benzaldehyde (*c*) have quite similar odours, but their profiles are no more similar than those of aniline (*d*), anisole (*e*) and toluene (*f*) which have distinctly different odours. Vibrational blurring of the edges would still further reduce the differences in shape

molecules when the silhouettes are superimposed. He uses photographs of three-dimensional models taken from three different directions to make the silhouettes, and he finds some degree of correlation between the profiles of substances the odours of which are in a general way similar to the various standard odorants.

The stereochemical approach has received much very favourable comment, and is at present regarded by many people as the most promising line of investigation. The principal objection to it is its inability to account for the remarkable specificity of odour sensations. Thus, Amoore lumps together under the single heading of 'floral' such disparate odours as geraniol (rose-like), anisole ('aniseed'), ionone (violet) and terpineol (lilac-like). The more or less substantial differences in smell within his group of generally 'floral' odours would indicate that the molecular profile is osmically specific only to a very rough approximation (Fig. 2).

The third of the present lines of attack aims at pinpointing the osmically specific properties with precision by identifying them with particular vibrational movements of the odorous molecules.

From a vague beginning more than a hundred years ago, when Faraday and later Tyndall speculated that odour might be associated in some way with molecular vibrations, the theory has become more and more specific with the passing years. The first major advance occurred 30 years ago when Sir Malcolm Dyson suggested that the then recently discovered Raman effect made it possible to give numerical values to the vibration frequencies of molecules, and so, for the first time, opened the way to an exact correlation of particular odours with particular patterns of vibration frequencies[5]. Very little development occurred until about 10 years ago, when I pointed out that the osmic vibrations must necessarily be low-frequency ones[6]. This is because they alone can acquire the necessary quanta of energy from the rather soft collisions which molecules make with each other at room temperature (which is only 300° C away from the absolute zero). Low-frequency oscillations are, however, the most difficult ones to study experimentally, and then only rather simple molecules of about the degree of complexity of nitrobenzene can be studied (Fig. 3).

Quite recently, and on the basis of some of the latest information to be published, I have found indications that not all the low-frequency vibrations of a molecule are necessarily osmically active: the active ones are probably those which involve motions that are perpendicular to the surface of the olfactory receptor organ on which the odorous molecule is lying[7].

When a molecule vibrates, each atom in it oscillates about a mean position in a very complicated way, but its motion can be resolved into components along three axes at right angles to each other. We can choose these axes any way we like, and so I like to think of two of them as parallel to the surface of the receptor organ in the nose, and the third as perpendicular to it. Then, it is only the oscillations along that third axis that appear to be osmically active and specific (Fig. 4).

This seems to be a rather important addition to the theory, because its effect is to draw together into a single focus all the various approaches I have outlined.

Thus, the role of Beets's 'functional group' is to orient the molecule in a certain way with respect to the surface and hence to determine which of its oscillations will be osmically active.

The amplitude of these oscillations is considerable. For example, calculations show that in their out-of-plane

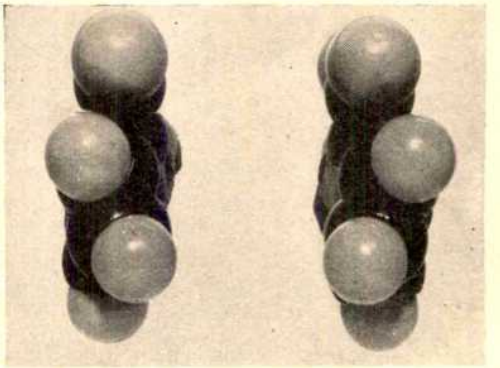


Fig. 3. Models showing approximately the amplitude of movement in one of the out-of-plane modes of nitrobenzene. Oscillations of this magnitude limit the precision with which the shapes or 'profiles' of molecules can be specified

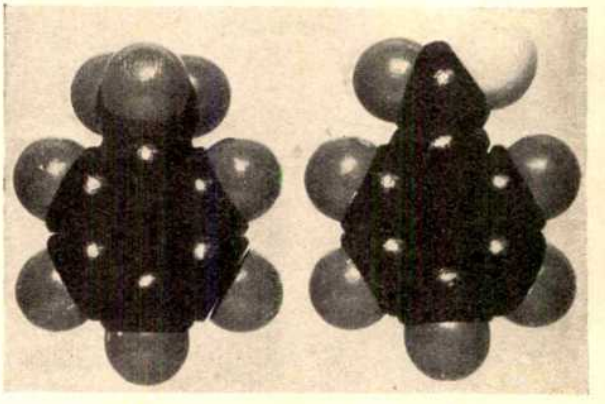


Fig. 4. Toluene (*a*) and benzaldehyde (*b*) have closely similar shapes or 'profiles' especially when the edges are blurred by vibration, but the distinct differences in their odours are paralleled by correspondingly distinct differences in the low-frequency, out-of-plane movements of the atoms in the ring

movements, carbon atoms in benzaldehyde may be displaced by as much as a quarter or a third of their diameter (that is, by as much as 0·3 Å) from the equilibrium position. This means that the molecular shape is only approximately described by Amoore's molecular silhouettes, and in reality their edges should be rather fuzzy because of the vibrations. At the same time, his basic idea gains added force if we picture the out-of-plane movements of the odorous molecule as conforming with corresponding out-of-plane movements of the sensitive surface. To conform in this way, the two molecules must have nearly the same frequencies in nearly the same places, so that the net effect depends both on shape and on frequency.

Furthermore, the interaction would be stronger the greater the area over which the motions of the odorant and the receptor coincided—which brings us right back to Davies' calculations which seem to show that (other things being equal) a very low threshold is associated with a large area of contact. The restriction of the contact to areas that fit sterically because they mesh vibrationally gives to Davies's theory the specificity it has lacked up to now.

The synchronous 'throbbing' of the odorous molecules and the receptor surface over a considerable area would permit of a much closer approach than would otherwise be possible, and hence allow a closer than usual electronic (van der Waals) bonding between them. This may be an important hint as to the kind of primary event that initiates an olfactory sensation.

One of the common objections to the vibrational theory is that the quanta of energy in a low-frequency mode are too small to be in any way effective. In other words, if it is the vibrational energy that is the important thing, there is too little of it in a low-frequency mode to be significant. This objection disappears if the energy is not the prime mover in olfactory stimulation.

Another objection to the vibrational theory is that it has appeared to presuppose a type of dipole-to-dipole coupling between the receptor and the odorous molecule that is inconsistent with quantum-mechanical principles and with some of the spectroscopic data. This objection also loses its force if we suppose that the coupling does not take place between oscillating dipoles, but is an ordinary van der Waals interaction made stronger than usual by an unusually close approach over an unusually large area of contact.

Quantum mechanical theory is concerned with transitions between different levels of vibrational energy, and is only vaguely conscious of what the molecule is doing between the transitions. In contrast, the vibrational movements that go on between transitions are the ones that may be giving various substances their specific odours. The fields of interest of the molecular spectroscopist and the odour chemist are therefore not the same. The osmically significant fact is that a molecule is vibrating, whereas the spectroscopically significant fact is that it can change from one level of vibrational excitation to another with the absorption or emission of radiation.

The complex oscillations of a molecule can be analysed into certain independently excitable movements which are called its 'normal modes' of vibration. The spectra sometimes show indications that an energy transition takes place into or out of two modes simultaneously, but spectroscopically the resulting bands are usually weak. The spectroscopists regard them as rather exceptional happenings which result from the fact that the two normal modes are not entirely independent, but are 'coupled' through what is termed their 'anharmonicity'. There is nothing to prevent two modes from becoming excited simultaneously given enough energy in the collision to do it, and this can happen whether or not a corresponding combination frequency appears in the spectra. The physical conditions during excitation by collision are completely different from those during a radiative excitation.

When a single mode is excited, the period of the vibration is the interval between successive passages through the equilibrium configuration. When two normal modes are excited simultaneously, the period of the complex oscillation is defined in exactly the same way, and its frequency is the difference between the frequencies of the two normal modes. Osmically, such a difference frequency should be just as significant as a pure normal mode of comparable population. Whether or not a corresponding 'difference band' appears in the spectra is irrelevant, because the spectroscopic transition into or out of two modes simultaneously depends on entirely different considerations.

Previous searches for 'osmic frequencies' have not taken into account the possible role of the difference frequencies that must arise when two normal modes are excited in the same molecule at the same time. When this possibility is included (something that is only now becoming possible, thanks to the advance of molecular spectroscopy), it serves to fill what would otherwise be awkward gaps in the correlations.

The vibration theory—at least since Dyson's contributions—has been unique among odour theories in being capable of precise verification, at least in principle. Whether it would be so verified in practice depends on developments in spectroscopy which are only now becoming adequate for the purpose. So far as matters have gone, the effect has been a progressive refining and sharpening of our picture of the part played by vibration in giving molecules their osmic specificity. At the same time, there begins to emerge a picture of the actual triggering mechanism that incorporates several seemingly distinct approaches to the problem. Thus, as has happened so often in the past, what appeared at first to be competing alternatives may prove to be complementary parts of a single whole. The next few years should see interesting developments in the whole field.

Interest in the matter is not merely scientific or academic. So far, I have been trying to answer the question, "Why is a smell a smell?" Now I must answer the question, "Why is a smell important?" To do this, let me first recall something that has nothing to do with smells.

Forty years ago, the populations of most of our cities were much smaller than they are now, but air pollution was much worse then than now. In those days, every house had a coal-burning kitchen range, and those with central heating had a coal-burning furnace as well, and they all poured out immense amounts of fly ash and soot. Soot was the main and, indeed, almost the only serious air pollutant. Forty years before that, dried, wind-blown horse manure matched it in importance if not in public mention. Now, what was it that has eliminated these two forms of air pollution? Was it restrictive legislation? Was it ingenious scrubbing devices? Or was it the automobile which took the horses off our streets and the gas and electric stoves which took the coal bins out of our houses?

The moral of this story is that it is better to make air pollution uneconomic and obsolete than it is to make it illegal, though this is not to say that legal restrictions are not a useful prod in the right direction.

To-day, automobile exhaust gases are the main source of air pollution in large cities, but there is another kind of pollution that affects country and city alike and is, indeed, a world problem. This is the danger from pesticide residues. Rachel Carson in her book, *Silent Spring*, pointed to a danger, but was not able to suggest a realistic solution.

At the beginning, I mentioned the emotional impact of smells. At the human level, this reflects the fact that smell is our most primitive sense. It is no longer of primary importance to us in comparison with sight and hearing, but, as we go down the ladder of living things, we find that smell plays a more and more important part in the lives of most creatures.

A very great many insects (though perhaps not all) regulate such vital activities as mating, eating and egg-laying by chemical signals that are nothing more nor less than smells. When we can understand better what scents are and how they are registered by the insects' antennae, we will be in a position to control insects by misleading them, by confusing the signals, and by exhausting their ability to respond; and the chemicals needed to do this will not be poisons. Instead, they will be smells that affect one kind of insect only, and in one special way only, and are harmless to everything else. We will be able to control one pest at a time and leave the balance of Nature undisturbed, or disturbed as little as possible.

When that stage is reached, the contamination and pollution of our environment by pesticides and pesticide residues will be as uneconomic and as obsolete as pollution by coal smoke and horse manure are now. This is one practical answer to the question, "Why is a smell important ?"

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PROGRESS OF CANCER RESEARCH

THE forty-second annual report (for 1964) of the British Empire Cancer Campaign for Research*, like its predecessors, is a weighty tome of 700 pages describing hundreds of investigations carried out at the centres financed by the Campaign or working under its aegis; these researches, most of them having a direct bearing on cancer, can be classified into three main groups and will be treated separately by the three reviewers, namely, carcinogenesis and the biology of cancer (I. H.); radiotherapeutics and radiobiology (P. A.); clinicopathology of cancer (A. L. L.).

Carcinogenesis and the Biology of Cancer

In the field of carcinogenesis, Haddow has found that the glycidyl ethers induce sarcomata in rats, and Roe and colleagues, also at the Chester Beatty Institute, are working on tumour-inducing characteristics of a variety of agents such as polyvinyl sponge, contraceptive chemical preparations for use intravaginally, asbestos, hydrazine compounds such as the anti-tuberculosis drug isonicotinic acid hydrazide (INAH), thalidomide, tobacco smoke fractions and nitrosonornicotine. At the same centre Birbeck and Kerawalla have studied the progressive changes in skin-cell ultra-structure, using the electron microscope, after application of carcinogens. Boyland and associates, from a study of urethane and related compounds as carcinogens and antiviral agents, conclude that "the effect of urethane and hydroxylamine derivatives may therefore be exerted through reaction with pyrimidine bases of DNA", which greatly simplifies the situation, but where the two processes under discussion, carcinogenesis and antiviral action, diverge still remains a difficult question.

The introduction of new carcinogens continues—only to make any chemical theory of carcinogenesis less and less capable of explaining transformation from the normal to the malignant.

The excitement first generated by the lung cancer–cigarette smoking discovery has now petered out into a tortuous search for carcinogens in cigarette smoke, which is something of an anticlimax. (Why do cigarette manufacturers' research teams not look for some other way of administering the narcotic to addicts without having to supply at the same time tar, benzpyrene, etc. ? Perhaps that is precisely what the manufacturers are trying to avoid.)

At the Middlesex Hospital a comprehensive study of azo-dye carcinogenesis is under way, using electron microscopy and cytochemical and biochemical methods; the investigators conclude that the question of the binding of dye, or of a metabolite, to protein as a necessary step in carcinogenesis has not yet been settled.

Rees *et al.* at University College Hospital have been investigating intranuclear changes in carcinogenesis; contrary to the oft-quoted, but extremely naïve, statement that carcinogens bind to DNA and therefore carcinogenesis is primarily a chemical change in DNA (which ignores carcinogenic agents like plastic film), Rees concludes that "It is therefore considered that some of the nuclear changes are the result of direct alterations in nuclear metabolism and others are secondary to metabolic changes in the cytoplasm. It was also noted that there were similarities in biochemical changes in the nucleus 2 weeks after feeding different carcinogens, despite differences in histological appearance".

At St. Bartholomew's Hospital, experiments have been in progress to see if pulmonary tumour induction in mice by urethane or by DMBA can be facilitated by irradiation (400–500 r.); it was found, on the contrary, that irradiation was inhibitory to the action of urethane.

At Birmingham, Orr *et al.* have continued their study of carcinogen-induced breast and ovarian tumours in mice; in one particularly interesting experiment "ovaries from mice which were given fortnightly skin paintings of 0·5 c.c. 0·5 per cent DMBA in olive oil were capable of developing into tumours in 50–100 per cent of cases after transplantation into untreated mice whose own ovaries were removed. Even ovaries grafted only 10 days after a single painting developed tumours in three or four cases after 14 months, while ovaries grafted from untreated mice gave no tumours in ten cases 20 months after grafting. . . . It would seem that a single treatment of a mouse with DMBA sets in motion a series of events in its ovary which eventually will lead to tumour production, even when the ovaries are transferred after as little as 20 h to normal hosts. This rapid effect of a single dose of DMBA could provide a useful tool for further investigations of the mechanism of action of this carcinogen".

From the Queen Elizabeth Hospital in Birmingham comes the following cautious statement: "Steroid contraception. In collaboration with the Family Planning Association and the Council for the Investigation of Fertility Control, the Department has been screening patients on steroid therapy as a long-term project. The results of this study are in print, but it can be said that cytologically and histologically there is evidence in a small percentage of the cases of nuclear abnormalities. It is not, however, justified to undertake immediate biopsy but follow-up would seem to be vital for this group of patients. Our total number of biopsies from patients on steroid contraception is so far 15. It is already obvious, however, that within 6 months modern steroid contraceptive therapy leads to a condition of cervical pseudopregnancy. It will require between 5 and 10 years before the significance of these changes is clear and especially with regard to the incidence of pre-cancer in patients on

* British Empire Cancer Campaign for Research. Forty-Second Annual Report covering the year 1964. Part 1: The Chairman's Statement and the Accounts of the Central Organization. Pp. xxxvi. Part 2: The Scientific Report of the Researches Undertaken by the Central Organization and its Autonomous Councils in the United Kingdom, and by some of its Affiliated Organizations Overseas. Pp. xxxvii–lix + 705. (London: British Empire Cancer Campaign for Research, 1965.)

steroid therapy. At the moment we can recommend that all patients who are on the contraceptive pill should submit to regular cytological screening tests and that the 'positives' should be watched closely both by the clinician and the cytologist".

The two branches of cancer research which have attracted most attention in recent years have been the immunological approach and the study of tumour-inducing viruses; Alexander *et al.* find that implantation of heavily irradiated benzpyrene-induced tumours grown in rats confers resistance to a challenge dose of 2×10^6 cells; they conclude that the lymphocytes play an active part in the resistance to tumour cells and that they attack the tumour antigens directly.

At Guy's Hospital, Batchelor has been studying immunization in mice to epithelioma produced by dimethylbenzanthracene; and at King's College Hospital, Tee and Wang in experiments on the antigenic composition of human tumours state: "An antigenic analysis has been made of seventy-two tumours and thirty-one normal tissues using anti-tumour antisera and the agar gel double-diffusion technique. Tumour-specific reactions were observed with some tumours, regardless of whether the tumours were derived from different patients or from different sites. A higher proportion of positive reactions was observed with tumours from the same site as that of the immunizing tumour. Anti-tumour antisera did not react with the normal tissues. . . . It was confirmed that these additional antigens were not due to isoantigenic differences between the cells of genetically different individuals. Loss of antigens was shown in at least a proportion of the tumours. It was not possible to detect antigens serologically identical with the tumour-specific antigen in extracts of foetal tissues and HeLa cells". Tee and Watkins, using the analytical ultracentrifuge and gel-filtration through 'Sephadex' columns of the globulins in mice immunized with a mouse leukaemia, conclude that, "The results may suggest that the immunization has caused stimulation of specialized plasma cell clones producing a particular fraction of the 7*S*γ-globulin. Further experiments are in progress to measure the M^z/M^w ratios of the 7*S*γ-globulins from different strains of mice immunized with other transplantable tumours and to see if similar results can be obtained by immunization with other antigens, in particular transplantation antigens".

Salaman *et al.* at the London Hospital have found evidence of a new mouse sarcoma virus (MSV) in the cell-free plasma of rats with Moloney virus leukaemia (MLV); the MSV virus when inoculated in rats and mice induces solid sarcomata, angiomata and cysts, filled with clear fluid or blood, of the thighs, abdominal or thoracic walls, the flanks, axillae and lymph nodes. The sarcoma is transplantable to mice and hamsters, and the cell-free filtrate produces tumours rapidly, especially in new-born rats and mice. The virus is neutralized by mouse antisera to Friend virus (FV) and also by undiluted monkey and rabbit anti-sera against Rauscher virus. Polyoma virus which has been reported as occurring commonly in mouse colonies is not identical with MSV for a number of reasons, for example, much more rapid tumour induction with MSV; absence of salivary gland tumours with MSV; viraemia in mice infected with MSV but not with polyoma; inactivation of MSV with ether or heating at 56° C (polyoma resists both). Salaman and Flocks have attempted to isolate a leukaemogenic virus from urethane-induced leukaemia in mice; they conclude that the agent belongs to a type isolated in many laboratories from a variety of strains of mice and that its action is probably on haemopoietic stem cells of varying state of differentiation giving rise to erythroleukaemias of various types.

At the Mount Vernon Hospital a group of workers studying the cytogenetics of cancer state that "the clearest evidence for clonal development is the presence of the same marker chromosome in many cells; similarities in an analysis of 23 malignant tumours . . . suggest that all the cells have been derived from a single chromosomally altered cell. . . . Data on DNA content and sex chromatin, taken together with chromosome findings, support the hypothesis that human malignant tumours usually arise from one cell (or perhaps in some instances from two or some other small number of cells) in which chromosome changes have occurred". At the same centre, Powell has used propiolactone to induce malignancy in rat embryo lung cultures *in vitro*; the malignant changes were morphological and behavioural only, for when the cultures were inoculated *in vivo*, no tumours resulted even after six months, which makes the use of the term 'malignant' in this connexion somewhat dubious.

At the Hospital for Sick Children, Great Ormond Street, London, an attempt is being made to grow childhood tumours in neonatally thymectomized mice; this procedure destroys the antigenic mechanisms, but the only success was with a human embryonic sarcoma. At the same laboratory, chromosome studies of untreated neuroblastoma showed that 60 cells out of a total of 76 examined contained fragments or double minutes. In a series of nephroblastomata ranging from undifferentiated (primitive blastoma) to partially differentiated (tubular, mesenchymal and papillary type) the chromosome number suggested that the more differentiated tumours were associated with a higher chromosome number.

At the Cambridge centre the possibility of antigen liberation from tumours is the subject of experiments where a liquid nitrogen probe is inserted in the tumour in the hope of destroying part of it and liberating antigens without denaturing the proteins.

At the Strangeways Laboratory, Daniel and collaborators investigated the loss of antigen which occurs when cells are cultured *in vitro*; primary cultures from skin and lungs of human embryos were found to contain antigens of the *A* and *B* group, but already at the second sub-culture some antigens were missing and subsequent cultures consisted of a mixed population of cells some of which were strongly antigen-positive and others antigen-negative.

At Nottingham, Baldwin and associates in an extensive series of experiments on tumour immunity find that: "These studies show that DMBA-induced liver tumours possess specific tumour antigens, but they appear to be much weaker than those demonstrated in 3-methylcholanthrene-induced sarcomata (Baldwin, 1955). Although further evidence is required, the results so far indicate the absence of any strong cross-antigenicities between liver tumours. These findings contrast with the demonstration by immuno-diffusion studies (1963 Report) of common microsomal and cell-sap antigens". While tumour-specific antigens can be demonstrated in carcinogen- and viral-induced tumours in mice, and to a lesser extent in rats, little success has been achieved in experiments on spontaneous tumours. Baldwin finds that by ligation of the spontaneous tumour and thereby its disintegration, sufficient antigens are liberated to protect against a challenge with $1{\cdot}5 \times 10^6$ tumour cells; the failure of heavily irradiated tumour grafts to induce immunity may be due to sensitivity of tumour antigen to γ-irradiation.

Dawson in Newcastle finds that inoculation of neonatal rats with spleen from mice infected with Friend virus gives rise to lymphatic leukaemia; the thymus and involved lymph nodes show complete loss of normal differentiation between cortex and medulla; masses of lymphocytes infiltrate the sinuses of the red pulp of the spleen, the portal tracts of the liver, the sternal marrow and the spinal cord. In the reverse experiment (inoculation of mice with cells from rats) the cell-free filtrate from spleen and thymus of these rats gave rise to lymphatic leukaemia but not Friend disease, suggesting that the filter-passing agent causing leukaemia in rats is different from mouse Friend virus although it arises by infection with this virus.

Stuart-Harris and collaborators at Sheffield have induced anaplastic-celled tumours, subcutaneous or intrathoracic, by inoculating suckling hamsters with human adenovirus carried in tissue culture of human amnion or embryo cells and originating in aseptic meningitis in a child.

At Edinburgh, Carter *et al.* have been studing osteopetrosis virus infection in the fowl; this virus proliferates in the kidney, muscle and other tissues and causes characteristic bone lesions. In co-operation with the British Museum (Natural History), an examination of a dinosaur fossil bone (70 million years old) shows very similar lesions to contemporary osteopetrosis gallinarum, where tumours appearing in the muscles can be transplanted. At the same centre it was found that injection of cells from a minced 10-day chick embryo gave rise to an unusual sarcoma different from other transmissible tumours in the laboratory, but eighteen subsequent embryos from the same hen failed to produce further tumours.

Leslie *et al.* in Belfast have continued their study of tissue cultures of DMBA-induced rat mammary tumours; at first both epithelial and fibroblastic cell types are present, but the colonies of epithelial cells finally disappear, leaving cultures of fibroblastic cells which have little cell-adhesion and easily give suspensions of single cells. Schild and Dale at University College, London, in studies of tumour antigens find that antigenic material in the serum of tumour-bearing animals is absent in the sera of normal rats and mice, but note that the difference could be either due to the presence of a tumour-specific antigen or that the tumours used were genetically different from the host animal, and that antigenic substances could be produced or released merely because of the presence of rapidly proliferating tissue; anaphylactic tests suggest that the antigen was not tumour-specific.

Stoker *et al.* at Glasgow, in an investigation on neoplastic transformation by DNA-containing viruses, find that early-passage baby hamster kidney-cell cultures when transformed by polyoma virus increase their transplantation efficiency by a factor of 10^4; a new virus-specific cell antigen is demonstrable by rejection of transplanted transformed cells in virus-immunized animals, or by treatment with irradiated and lyophilized transformed cells. Thomsett and Allison at the same centre have found evidence that canine dermal papillomatosis and the oral variety are due to two different viruses and that, while the dermal form has not been transmitted in their experiments, the oral condition is reproducible in puppies.

Weipers *et al.* have successfully transmitted leukaemia in the cat by virus particles which are very similar to mouse leukaemia virus, and the particles can be grown *in vitro* and can be found in electron-micrographs. Weipers refers to reports from European countries that lymphosarcoma in cattle has attained enzootic proportions (Denmark has slaughtered 20,000 cattle in a control programme); in Scotland the morbidity is 250 per million cattle slaughtered.

Woodruff in Edinburgh, using a mouse mammary carcinoma growing in a hybrid strain (*A* × *CBA*), finds that the tumour is inhibited by intravenous injection of spleen cells from *CBA* mice immunized against the tumour; experiments are in progress using thoracic-duct lymphocytes instead of spleen cells and also methylcholanthrene-induced sarcoma (instead of mammary carcinoma) which possesses strong tumour-specific antigens. Woodruff does not state whether the mammary tumour was spontaneous or carcinogen-induced (the former type, unfortunately, is lacking in tumour-specific antigens).

From Ibadan comes a report by Lawson that cancer of the cervix is the commonest malignant tumour seen in Nigeria; since all males in Southern Nigeria are circumcised, some factors other than foreskin or smegma must be involved (age at circumcision is not mentioned). Oettlé in Johannesburg has been working with the kangaroo rat (*Mastomys*); this rat was at one time reputed to be the only rodent with a large incidence of spontaneous gastric carcinoma, but in recent years this incidence has receded, for it is no longer found in *Mastomys* which, however, shows a remarkable tendency to papillomatosis of the skin, perineum and vagina.

The Fahmys have continued their work on transformation versus mutation with DNA in *Drosophila*; in an experiment where seven eye colour mutants were found, the DNA was given to the fathers of the mutant flies although the mutations occurred in the maternal *X*-chromosomes.

Whisson and Connors find that aniline mustard is lethal to a mouse plasma-cell tumour but causes no regression in the rat Walker tumour (possibly the difference in efficiency depends on the state of aggregation of the cells in the two tumours).

Koller and associates, in cytogenetical investigations of neoplastic cells and leukaemogenesis, conclude that in neoplastic tissues from primary leukaemias "no correlation was found between the degree of malignancy, mitotic rate and abnormal chromosome constitution".

At St. George's Hospital, a study is being made of cytogenetics and the assessment of marrow dyscrasia: "With the cardinal exception of chronic granulocytic leukaemia, chromosome morphology in the myeloproliferative syndromes has shown no characteristic or consistent deviation from the normal pattern sufficient to merit separate categorization. The presence of a supernumerary *C* chromosome (6–12) has been noted in a variety of marrow leukaemias (Hungerford and Nowell, 1962) and in leukaemia associated with mongolism (Warkang *et al.*, 1963): in our experience the appearance of this feature bears no relation to any current haematological classification. Many different examples of chromosome aberration, both in number and in morphology, have been described since Ford, Jacobs and Lajtha (1958) first reported an abnormal pattern in 'blastcell' leukaemia. Since not all forms of this and allied marrow diseases show morphological chromosome change, it is tempting to speculate on an association between those that do so; but this would imply a capacity to distinguish between cause and effect which we do not yet possess".

The most arresting development in biochemistry, that dealing with DNA, RNA and *m*-RNA, has been the subject of a number of investigations in cancer; the current ideas in this field are very difficult for outsiders to grasp properly; for example, Kemp at St. George's, in discussing the effects of actinomycin *D* (AMD) on human chromosomes, says: "AMD is known to bind to the guanine moiety of DNA and thereby inhibit DNA-dependent RNA synthesis, possibly by interfering with the pathway and function of the DNA-dependent RNA polymerase operating in the minor groove of the DNA molecule (Reich, 1964). While it is known that AMD binds to those regions of the DNA molecule actively engaged in RNA synthesis, it is not known whether it also binds to the genetically repressed regions of the DNA. If, as has been suggested, this repression results from the close association of the DNA with other molecules, such as histones, it would seem quite possible that the binding of AMD to such repressed DNA would be prevented. Further, if the sites of binding of AMD to the chromosomal DNA could be detected, it would be possible to map the genetically active regions along the chromosome and to compare these regions in differentiated cells with different functions and in malignant cells and their normal counterparts". Elek *et al.* at the same centre have studied the nature of attachment of antibody molecules to infective particles of polyoma virus using electron microscopy and negative staining of flagellar fragments; electron micrographs indicate that the antibody molecules carry the specific sites at the ends of their long axis. The molecule of antibody thus becomes attached to a cylindrical antigen radially surrounding the antigen like the bristles of a

bottle brush. This orientation suggested that the antibody is inserted into the surface of a flagellum in a manner that is directionally fixed, presumably by surrounding molecules. This type of coating provides a physical explanation of the inhibition of the infectivity of viruses in the presence of antibody. At present, experiments are in progress to study the transformation of hamster kidney cells by polyoma virus in a quantitative manner, and in particular in relation to the phase of cell division.

Amoroso and Stein-Werblowsky at the Royal Veterinary College, London, find the semen of boars, bulls and to some extent humans inhibitory for the growth of transplantable tumours in the rat and mouse; the active factors appear to be the phospholipids in the semen.

Currie and associates at Aberdeen have studied the growth and regression of DMBA-induced mammary tumours in rats treated by oral or intravenous administration; 80 per cent of the mammary tumours regress when the ovarian hormones are removed by ovariectomy (the latest techniques were used in this work, tritium-labelled thymidine, electron microscopy, enzyme studies and neonatal thymectomy).

Foulds, in a theoretical discussion of genetic action in normal and neoplastic development, introduces a new terminology—total genome, effective genome and facultative genome, and the neoplastic expression of the effective genome is via "selection, transcription and translation of the RNA transcript in the synthesis of a specific protein".

Radiotherapeutics and Radiobiology

Radiation has been shown in many instances to be a cause of cancer and leukaemia in man, although, in general, the doses that have been received by the irradiated populations were large. The Oxford Survey of childhood cancers, under the direction of Alice Stewart, first revealed that malignant disease can be produced *in utero* by doses of the order of 10 r., and the latent period for leukaemogenesis under these conditions has now been shown to be 4–5 years and this is substantially shorter than is required for the induction of leukaemia in adults. The possibility that radiation may play a part in lung cancer associated with smoking has frequently been considered since the high incidence of this disease among the early uranium miners was believed to be an example of radiation carcinogenesis. So far, however, no evidence for such a hypothesis has been found. Radiation-emitting particles of natural origin are continually inhaled and the lung is the organ most heavily exposed to normal environmental radiation. The finding in New Zealand that cigarettes contained minute quantities of polonium led to the post-mortem measurement of polonium in different organs of smokers and non-smokers. Though the lungs of the former contained three times as much polonium as those in non-smokers, there is no evidence that this is a causal factor nor that the increase is due to the polonium content of the cigarettes. Damage to lungs by smoking may lead to impairment of the normal physiological processes which keep the lungs clean. In Glasgow the natural radiation burden of the lungs of mice is being greatly reduced by maintaining the animals in an atmosphere free from radon, but this does not appear to have abolished the appearance of lung adenoma. A paradoxical finding is that the induction of lung cancers by high doses of radiation only is increased if they are irradiated in atmospheres of nitrogen instead of in air. This is the exact opposite to cell killing or mutation induction, in which anoxia protects against radiation damage.

The complexities of radiation carcinogenesis are illustrated for thyroid carcinoma which has been induced with radioactive iodine in man and in experimental animals. Donniach and his colleagues showed that if the radionucleotide is given to rats at birth the production of thyrotrophic hormone by the pituitary is stimulated and that this is involved in the eventual appearance of tumours.

Accurate dosimetry continues to present problems, especially in connexion with some of the newer very-high-voltage equipment which is now coming into clinical use. A considerable amount of research effort is being devoted —chiefly by the physics departments of the major teaching hospitals—to these and other technical problems and several ingenious new methods have been devised. The use of computers for treatment planning is being tried out at the Medical Automation Centre of University College Hospital, and plans are being made to have a direct *telex* link between the centre and the hospitals. A great deal of clinical research is being devoted to the problems of irradiation under high-pressure oxygen. Radiobiological considerations indicated many years ago that local clearance of large tumours ought to be facilitated by irradiation under high pressures of oxygen, but as yet it is not clear what the benefits are in practice. Good commercial equipment for treatment under hyperbaric oxygen has now been installed at several centres and carefully controlled clinical trials should show within a few years the degree of usefulness of this procedure.

Much experimental work is being devoted to the search for radiosensitizers; if such substances are to find application in practice, then they must show selective action for tumour tissue since the maximum dose that can be given is determined by normal tissue tolerance, and if this is sensitized to the same extent as the tumour no advantage is gained. The radiotherapist is equally concerned with the response of the surrounding tissue as with that of the tumour; yet, until recently, there has been little quantitative experimental work in the relationships between dose and the fraction of cells killed in normal tissues, and most of the information on the radiation response of cells was obtained with tumour cells. This situation is now being remedied; Withers has developed a method for measuring the dose-response curve for skin cells in mice; workers at Guy's and Westminster Hospital are making quantitative studies on the effects of radiation on bone growth, and Denham is investigating the relationship between sensitivity to X-irradiation and metabolism and growth cycle for the developing rat retina. The radiosensitivity of the mouse ovary has been found to depend critically on age, the D_0 being 290 r. at one day of age, falling to 16·5 r. at one week and rising again to 54 rads at one month (St. Bartholomew's Hospital). The radiosensitivity of erythropoietic stem cells in the mouse was found at the Christie Hospital to depend on the level of erythropoietin. From all this work it is very clear that the radiosensitivity of normal tissue is very variable and is influenced by many factors which are not yet understood. A key parameter in determining the fractionation schedule for radiotherapy is the recovery of normal tissue in the intervals between irradiations, and Lamerton and his colleagues have shown remarkable differences in this respect between different organs. Jolles has observed profound changes in connective tissue and believes that circulating factors which increase capillary permeability are released on exposure to X-rays. Both he and the late Dr. Wilson noted that radiation alters the behaviour and turnover rate of the mucopolysaccharides in extracellular tissue. The implications of such changes for radiotherapy remain to be elucidated.

Another line of investigation which emphasizes that radiotherapy cannot be understood—or improved—by concentrating on the tumour cells alone, and that the complex interplay with the host has to be taken into account, is provided by a comparison of the radiosensitivity of cells cultured from different tumours *in vitro* and the response of the tumour *in vivo*. Thus, cells from a fibrosarcoma induced with a plastic film were found to be sensitive to X-rays *in vitro*, while on injection into syngeneic rats they give tumours which are very radioresistant. For a series of primary chemically induced

fibrosarcomata induced in rats it was shown that the host environment exerts a predominant influence on radiosensitivity (Dawson, Field and Madoc-Jones). An observation made at the Chester Beatty Research Institute points to the possibility that radiosensitization with compounds having radiobiological properties similar to desoxybromouridine (BUDR) may be of practical value. BUDR is known to be incorporated into the DNA of dividing cells and this resulted in such cells being more sensitive to X-rays. In a series of leukaemia cells having different radiosensitivities in tissue culture it was found that incorporation of BUDR sensitized the radio-resistant cells to a greater extent than the more radiosensitive cell lines. The differences in radiosensitivity of these cells arise out of differences in their capacity to restore radiation-induced lesions, and BUDR appears to sensitize by interfering with post-irradiation repair processes. BUDR exerts its greatest effect on those cells which have the greatest repair capacity and in this way may provide useful differential radiosensitization.

Clinicopathology of Cancer

Tumours arising from human trophoblast are rare, and incidentally are examples of a tumour arising in the foetus which infiltrates the mother. From Charing Cross Hospital comes a report of forty-four patients with trophoblastic tumours, thirty-nine of them with overt metastatic disease, who were treated with a combination of methotrexate and 6-mercaptopurine during a 6½-year period. Only six patients died, and the remainder are in complete remission as shown by clinical, radiological and hormonal evidence. Six patients had successful pregnancies after chemotherapy, and the infants were normal. Chemotherapy of trophoblastic tumours is now established as a triumph of modern medicine over what was once a fatal disease.

The University of Glasgow Veterinary School continues its researches on lymphosarcoma in domestic animals and includes in its study the curious lymphosarcoma-like, transmissible venereal tumour of dogs. It will be recalled that this was the first tumour (or tumour-like condition—there is no real distinction) shown to be transplantable. No microbiological agent has been demonstrated as a causative factor, transmission depending on the inoculation of viable tumour cells. The disease is almost extinct in England but is common in some parts of the world, East Africa and Japan, for example. The tumour is said to have only two-thirds of the normal chromosome complement. An attempt is still being made to isolate a virus from it.

It is apparent that the concentration of patients and the specialist knowledge available in the few large institutions devoted to the management of cancer is good for both specialist and patient. It is a fact, however, that 'cancer' is not recognized as a speciality in the National Health Service, though one form of cancer therapy, that is, irradiation, is so regarded. Pooling of material for clinical research has the disadvantage that some clinicians must sacrifice familiarity with the conditions selected for study, but this is offset by the rapidity with which unsuspected data may come to light and the adequate testing of new therapeutic measures be carried out. One notes with approval the setting up of a Thymic Tumour Panel; thymic tumours are rare, and so far only in the United States has there been any opportunity of examining a large series of cases. The publication of the researches of the Testicular Tumour Panel has been conspicuously successful.

The early detection of cancer is still a major topic. St. Mark's Hospital reports that only a little more than 3 per cent of all rectal cases seen were at an 'early' stage of development. In a study of early cases no evidence was found over the past 40 years that patients are coming earlier for treatment. With a mounting campaign for the setting up of centres for the early detection of cervical carcinoma in well women, reports from a number of centres corroborate the established value of the present cytological procedure. The final report from Way of the North of England Council states that, between 1947 and 1963, 476 cases of occult cervical carcinoma were discovered and treated, some of the patients later having successful pregnancies. During this same period 2,094 clinical cancers were treated. In 1964 the number of preclinical cancers detected by cytology exceeded the numbers detected by clinical methods for the first time ever. In contrast with this gratifying state of affairs it is fair to say that there are still no comparable methods for the detection of early visceral malignancy nor for the early mammary carcinoma. The value of the prophylactic surgery for preclinical cervical carcinoma will only be confirmed when the death rate from cervical carcinoma is seen to fall.

At University College Hospital, Ross and Marshall-Jones have studied the metabolic disturbances associated with carcinoma of the bronchus. These patients with bronchial carcinoma developed bilateral adrenal hyperplasia with extremely high levels of blood and urine corticosteroids, not elevated by corticotrophin nor depressed by dexamethazone. Two of these showed hyperkalaemic acidosis due to corticotrophin production by the tumour. Another two exhibited dilutional hyponatraemia due to the production of antidiuretic hormone by the tumour, one of them having severe myasthenic neuropathy. Urich, Dayan and Montgomery summarize their published observations on the obscure and uncommon neuromyopathy, the result of bronchial and other carcinomas. Both these reports underline the potentially rich field of investigation which lies in the oblique approach to tumours by studying their effects or concomitants, and again emphasize the advantages to be gained from co-operation among specialists.

P. Alexander
I. Hieger
A. L. Levene

OBITUARIES

Dr. F. J. Wilkins

Dr. F. J. Wilkins, a director of Glaxo Group, Ltd., was killed in the early hours of the morning of October 27, 1965, at London Airport as the result of the crash of a Vanguard aircraft on a flight from Edinburgh to London. The original flight, timed to leave Edinburgh at 5.30 p.m. on October 26, had been cancelled owing to fluctuating fog in London. When another flight became available at a very late hour, Fred Wilkins was one of those who decided to take it instead of travelling by train or remaining in Edinburgh overnight. This decision was entirely characteristic of him, and it was a product of the restless energy which led him to achievements denied to many of his contemporaries who had seemed likely to surpass him when they were younger.

Wilkins was born in Cardiff in 1905, and he obtained all his early education in that city. After obtaining a London B.Sc. degree and a Diploma in Industrial Chemistry in 1924, he decided to engage in research at Cardiff Technical College, and he spent the next three years on investigations relating to nitrosyl sulphuric acid and to the catalysed oxidation of ammonia. For this work he was

awarded a London M.Sc. degree. He then decided to pursue reaction kinetics in greater depth than was possible at Cardiff. To this end, and with the help of a research grant from the British Non-Ferrous Metals Research Association, he joined the Department of Physical Chemistry in the University of Cambridge, where he worked on a number of topics under the direction of E. K. Rideal. The most important of the subjects he studied were the oxidation of copper, and, as an offshoot, the oxidation of hydrogen with copper as the catalyst. He showed that the oxidation of copper could be considered as the sum of four consecutive processes, while the reduction of cuprous oxide by hydrogen consisted of six consecutive reactions. In all, he published, either as sole or as joint author, some six papers and two short notes while he was at Cambridge, and he received a Ph.D. degree in 1927.

In 1930 he obtained a Department of Scientific and Industrial Research senior research award and he moved to University College, London, where F. G. Donnan was the professor of physical chemistry. There Wilkins worked on gaseous adsorption, and he published one paper on the effect of intensive drying on the accommodation coefficient of liquid and solid surfaces for molecules of their own vapours. However, in spite of the fact that his main work allowed him plenty of time to indulge his tastes for music and the visual arts, Wilkins gradually found himself becoming less interested in an academic life, and in 1933 he joined the nitrates and sulphuric acid group in the Billingham Division of Imperial Chemical Industries, Ltd.

The problems and environment there were much to his liking, and he threw himself with great energy into improving the efficiency of the plants. After three very successful years as a plant manager, he moved first into the research department and then into the technical department, in the latter of which, during the first two years of the war, he was in charge of the start-up and proving trials of a number of ammonium nitrate explosive factories.

In 1941, C. S. Robinson, who had been managing director at Billingham and who had been seconded to the Ministry of Supply as director-general of the Royal Ordnance Filling Factories, was faced with the task of expanding the Royal Ordnance Factory organization in order to meet the constantly changing demands of the Services, and he managed to extract a number of 'bright boys', Wilkins among them, from Billingham. Wilkins was located at Bridgend, which was the headquarters of the western regional filling factories of Bridgend, Glascoed and Hereford, and he created and managed a highly efficient development team. Among their achievements were substantial improvements in the operation of a fully mechanized detonator filling plant and of a continuous 25-pounder shell filling plant. The risks involved in these processes were substantially reduced, and both productivity and quality were much improved.

At the end of the war, Wilkins decided to leave Imperial Chemical Industries and stay in Government service, and he became, first, director of chemical research and development, and, later, principal director of scientific research (defence). However, after some time he found that the task of being a head-office administrator was not an entirely satisfactory one, and in 1949 he accepted an invitation to become a local director of Glaxo Laboratories, Ltd., in charge of their northern factories.

All his work up to that time had been connected with well-known inorganic chemical processes involving the same types of reactions as those which he had first studied in 1924. The complex organic chemistry of the corticosteroids and the production of antibiotics by fermentation were new fields of endeavour to him, and they presented him with a major challenge, a situation which always brought out his best qualities. But he did not confine his interests to purely technical matters, and he became intensely interested in labour relations and in the training and proper utilization of graduate staff. Because of the new ideas which he introduced and his forceful personality, his influence throughout the Glaxo organization spread well beyond the realms of his own bailiwick, and in 1956 he joined the main board of the company as deputy managing director. In 1961, when the Glaxo organization was remodelled in the form of a holding company, Glaxo Group, Ltd., and of a number of operating subsidiaries, he was made chairman and managing director of Glaxo Laboratories, Ltd., the largest of the subsidiaries in Britain, as well as being made a member of the board of the Group. Under his direction, Glaxo Laboratories, Ltd., continued to expand and flourish, in spite of some slowing down of innovation in the pharmaceutical industry of Britain as a whole.

Fred Wilkins's interests were extremely wide, and he even found time to go in for farming on quite a large scale, employing always, of course, the most up-to-date methods. He travelled widely on the Continent and in North America, and he made many friends whom he and his wife were fond of entertaining at their home in Burnham Beeches.

Although Wilkins made a very considerable contribution to all the organizations in which he worked, his influence on Glaxo Laboratories was much the most profound. His outlook was partly that of a scientist with a highly analytical mind, and partly that of an artist, for he took a great delight in music, in ballet and in the art of the High Renaissance. He was never at a loss for a new idea, and he was always anxious to give the younger members of the staff the best possible chance of proving themselves. He was warm-hearted and volatile, and, as he liked saying, a true Welshman. For him, technological innovation and artistic creativity were the two aspects of human endeavour which were most worth while.

A. H. Wilson

Prof. Mary Barber

The sudden death of Prof. Mary Barber on September 11, 1965, is an irreparable loss to bacteriology.

Mary Barber was born in 1911. Her father, Dr. Hugh Barber, is a distinguished physician who trained at Guy's Hospital, became a Fellow of the Royal College of Physicians in 1933 and has practised medicine for many years in Derby. She obtained the M.R.C.S., L.R.C.P. qualification from the Royal Free Hospital in 1934, and the M.B., B.S. (London) in 1936. Between 1934 and 1937 she held the posts of house physician, clinical assistant to out-patients, resident pathologist and A. M. Bird Scholar in pathology at the Royal Free Hospital. In 1958 she went to the Archway Group Laboratory in Highgate and in 1940 became assistant in the Department of Pathology at the Postgraduate Medical School of London. She took the London M.D. in pathology in 1940. In 1947 she was appointed lecturer in bacteriology at the Postgraduate Medical School and in 1948 became reader in bacteriology at St. Thomas's Hospital Medical School. While holding this post she spent a few months at the Institut Pasteur in Paris during 1950–51. She stayed at St. Thomas's until 1957, when she returned to the Postgraduate Medical School, this time as reader in clinical bacteriology. In 1964 the title of Professor of Clinical Bacteriology was conferred on her by the University of London.

Mary Barber will always be remembered for her work on hospital infections, particularly those caused by staphylococci: but it should be emphasized that this work in which she established her unique reputation was broadly based on a sound general knowledge of clinical bacteriology and pathology. In her early work she was interested in listerella and erysipelothrix infections, in puerperal sepsis and in the antibacterial activity of 2-aminophenol and some simple derivatives. Later she became increasingly absorbed in the staphylococci and very soon became an authority on almost every aspect of the challenge they present. A clinical bacteriologist by training and ex-

perience, she had a wide knowledge of the clinical and epidemiological effects of staphylococcal infection; when penicillin and other antibiotics became available she was quick to appreciate the great opportunities and the dangers created by their use. She was able to give sound advice to clinicians on the proper use of antibiotics, but without attempting to usurp the responsibility of the physician for the care of patients. She was one of the first to point out the dangers of the development of drug-resistant strains of staphylococci and other bacteria, and saw very clearly that the only rational way to meet this threat was to have a rational policy for the use and, if necessary, the restriction of antibiotics and for the control of cross-infection.

But Mary Barber was more than a clinical bacteriologist. She was intensely interested in bacteriology or microbiology for its own sake, as a branch of biology. She was always experimenting with different laboratory methods for demonstrating the behaviour of staphylococci in laboratory media, and particularly for detecting the penicillinase-producing strains which have become so prevalent in hospitals in almost all parts of the world—though she had great faith in the well-tried and simple method of the ditch plate, a fact familiar to all who heard her give papers at meetings or read her published works. Besides her technical skill as a bacteriologist, she had also a wide knowledge of microbiology, of microbial chemistry, microbial genetics and of the relation of structure to function in antibiotics and chemotherapeutic drugs. Although in these fields she never claimed expert and detailed knowledge, her mastery of the principles, her ability to see the wood for the trees, and even at times her command of detail were remarkable. The semi-synthetic penicillins with their new range of antibacterial activity presented an exciting challenge which she was quick to take up, since it brought together so many different facets of microbiology.

As a speaker she was always interesting and stimulating, often humorous and provocative, never dull. As a person she was unique. Essentially friendly, sociable and humane, she had no use for pomp or pretentiousness. But she was forceful and outspoken in attacking attitudes of mind with which she did not agree, and in defending causes in which she believed. The world of bacteriology, and indeed the bacteriology of the world, has lost far too soon one of its most active and stimulating workers. R. Knox

NEWS and VIEWS

Metallurgy in the University of Cambridge : Prof. R. W. K. Honeycombe

Prof. R. W. K. Honeycombe, whose election to the Goldsmiths' chair of metallurgy in the University of Cambridge was announced recently, was born and educated in Australia. He studied metallurgy in the University of Melbourne under Prof. J. Neill Greenwood. As a research officer in the lubricants and bearings section of the C.S.I.R.O. from 1942 to 1947, he worked on bearing metals and plastic deformation under Dr. F. P. Bowden. His collaboration with Dr. W. Boas during this period led to an appreciation of the role of the anisotropy in the plastic deformation of non-cubic metals when they are thermally cycled. In the Cavendish Laboratory from 1948 until 1951, first as I.C.I. Fellow, and then as a Royal Society Armourers' and Braziers' Fellow, Honeycombe's principal work was concerned with the deformation of single crystals. On his appointment as senior lecturer in physical metallurgy in the University of Sheffield in 1951, he became deeply involved in teaching as well as research and played a prominent part in developing postgraduate courses. In 1955 he was appointed to the chair of physical metallurgy. At Sheffield he has conducted researches into the deformation of alloys and into tempering, intergranular brittleness and precipitation hardening in alloy steels. He holds a substantial Science Research Council research grant in support of his work on alloy steels. Prof. Honeycombe has received the Rosenhain Medal and the Sir George Beilby Medal and Premium; he was awarded a D.Sc. by the University of Melbourne in 1961 and he has held visiting professorships in that University and in Stanford University. He has taken an active part in the life of the University of Sheffield, has served as Dean of the Faculty of Metallurgy and has been a member of the small Senate Committee charged with planning future academic developments in the University. Prof. Honeycombe succeeds Prof. A. H. Cottrell, who has been appointed deputy chief scientific adviser, Ministry of Defence (*Nature*, 208, 19; 1965).

U.S. National Bureau of Standards : Samuel Wesley Stratton Award

Dr. Harry Allen, deputy director of the Institute for Materials Research, and Dr. Deane B. Judd, assistant chief of the Metrology Division, have received the 1965 Samuel Wesley Stratton Award of the National Bureau of Standards, U.S. Department of Commerce. The Award is given each year to recognize outstanding scientific or engineering achievements by a member of the staff of the Bureau. Dr. Allen was cited for "distinguished research contributions to infrared and quadrupole spectra of molecules and for leadership in advancing high resolution analysis of asymmetrical molecular tops". Dr. Allen was chief of the Analytical and Inorganic Chemistry Divisions and of the Inorganic Materials Division of the Bureau before being appointed to his present position in 1965. Dr. Judd's award was for "major basic contributions to the science of color measurement and color vision and the development of color standard for business, science and industry". Dr. Judd has worked in the National Bureau of Standards for 38 years on problems ranging from standards for colour measurement to camouflage and colour blindness.

U.S. National Bureau of Standards : Dr. R. C. Casella

Dr. Russell C. Casella has joined the U.S. National Bureau of Standards as a theoretical physicist in the Solid State Physics Group of the Institute for Materials Research. An expert in the theory of solids, he will be investigating the problem of penetration depths in the superconducting, heavily doped semiconductor, $SrTiO_3$. Dr. Casella has conducted investigations into the symmetry of electronic band structures, optical and superconducting properties of solids, and elementary-particle physics at the Watson Research Center of International Business Machines. From 1952 until 1958 he was a graduate student and research associate at the University of Illinois. He has also worked at the Air Force Research Center at Cambridge, Massachusetts. Dr. Casella was born in Framingham, Massachusetts, and received his B.S. from the Massachusetts Institute of Technology in 1951 and his Ph.D. from the University of Illinois in 1956. He is a member of the American Physical Society.

Sir Arthur Keith (1866–1955)

Among this year's centenaries of note is that of the birth, on February 5, of Arthur Keith, of Aberdeen.

Having gained his initial education in that city, Keith studied medicine in London and Leipzig, eventually becoming professor at the Royal College of Surgeons. As a noted research worker in anatomy and embryology, Keith was elected a Fellow of the Royal Society in 1913, and 4 years later he was appointed to the Fullerian chair at the Royal Institution. He was well known for his work on the development of early man and was an authority on fossil man. Keith wrote some attractive books, among them being *Human Embryology and Morphology* (1901), *Ancient Types of Man, Antiquity of Man* and *A New Theory of Evolution*, published in 1948. His presidential address to the British Association for the Advancement of Science in 1927 was an outstanding revision of the Darwinian theory. Despite his wide field of activities, Keith remained a staunch Aberdonian at heart, so that it is not surprising that he was Rector of Aberdeen from 1930 until 1933.

New Variable Energy Cyclotron at Harwell

THE first stages of commissioning a new variable energy cyclotron have been carried out successfully and rapidly at the Atomic Energy Research Establishment, Harwell. Within 24 h of switching on the machine, the beam was accelerated to the full radius. The difficult task of extracting the beam for experimental use has yet to come and the immediate programme is designed to explore the behaviour of the machine and to gain experience with it under various operating conditions. The cyclotron, with its buildings, research and development, will cost £1,650,000; it is to be initially used for studies of radiation damage, radiation chemistry and radiochemistry. The accelerator is a 70-in. spiral-ridge cyclotron designed to accelerate ions of up to about mass 40. The design provides for an internal beam of up to 1 m.amp. of 50-MeV protons (ultimately up to 100 m.amp. externally) and microampere beams of heavy ions, with energies in some cases up to 10 MeV per nucleon. A beam of 22·7-MeV ions was initially accelerated to the full extraction radius while the beam current was deliberately restricted to 2 μamp. Energies of as high as 50 MeV have since been achieved with currents of 20 m.amp. There are nine positions in which samples may be irradiated, one of which is on a probe in the internal beam. The external beam may strike a target in the cyclotron vault or be deflected by a bending magnet to any one of seven positions in three separate target rooms adjacent to the vault. The target rooms are shielded from one another and from the main vault so that experiments can be set up in one while irradiation proceeds in another. During operations the machine and targets can be observed from the control room through a water-filled window or by closed-circuit television.

Ex Opera et Industria

THE presidential address to the Institution of Mechanical Engineers at their meeting on October 27, 1965, was given by H. N. G. Allen, deputy chairman and joint managing director, W. H. Allen, Sons and Co., Ltd., Bedford, and was entitled *Ex Opera et Industria*, a tribute to the Allen family motto, signifying (with implied overtones) 'by service freely given and by diligent application'. This tribute, which constituted the first part of the address, was an appreciation of the life-work of two eminent engineers, the late W. H. Allen, who took over a small jobbing company, Newberry and Co., Cardiff, in 1865, thus founding the firm of W. H. Allen, and his eldest son, the late Sir Richard Allen. This first part is essentially historical, covering the years 1865–1955; but it sets the stage for what was, perhaps, the more important motive of this discourse, the second part, which considers "Unity of Purpose: Reflections and Queries". It is argued that in a rapidly changing world it may be questioned whether everyone concerned really understands what is meant by engineering, both in its own right and also as one vital organ of the complex living system which makes up a balanced industrial enterprise, and what kinds of engineer and engineering will be required in the future in industry. The answer to these and other pertinent questions raised by the president would appear to lie with the initiative for engineering co-ordination being within the province of the Council of Engineering Institutions, recently granted its Royal Charter. Mr. Allen's plea is for pooling of ideas to get these actions moving. Reappraisal of the term 'engineering' in depth, breadth and context is required, and implicit in this is the conception, creation or utilization of an engineering product. Next, investigation of the different kinds of engineering required and of the relationship of engineers and engineering technicians is needed along with re-examination of educational and training requirements; finally, to present a unified front to schools on the meaning of engineering, career patterns available, and to consider educational and training programmes to this end. "If Science is knowledge and Engineering is applied knowledge, surely Wisdom is knowing how and when to apply that knowledge." The title of this address is one which Mr. Allen thinks might well be adopted by all engineers. His remarks must have given his audience much to think about and will doubtless lead to healthy repercussions throughout the professions and industry. The address is in the course of publication by the Institution of Mechanical Engineers.

Scientific Research in Schools

THE *Report to Council, 1965*, of the Scientific Research in Schools Committee of the Royal Society has recently been published (Pp. 18. London: The Royal Society, 1965). The scheme, under which a committee of the Royal Society gives assistance to teachers of science to carry out research, has been in existence for 8 years. One hundred and seventeen teachers are now working under it, each being advised by a designated expert, and their investigations extend into ten major branches of science. Over the past year, £5,472 was expended in giving financial assistance to teachers, the strain on the Royal Society's own resources being in part relieved through contributions from eleven companies and the United Kingdom Atomic Energy Authority. In the opinion of the Royal Society, there is scope for further extension of the scheme, which is looked on favourably by head teachers and deserves to be more widely known.

South Australian Museum

The Records of the South Australian Museum includes an obituary and bibliography of the late Dr. H. M. Hale, director of the Museum from 1931 until 1960 (15, No. 1; 1965). Other articles include a revision of the Australian and New Guinea Dromini, Aboriginal rock poundings on Gallery Hill, North-western Australia, and new species of *Holachila* from Victoria and southern New South Wales. It is interesting to note that the rock poundings are the result of battering the surface of the rock with a rounded stone until the dark-coloured patina is destroyed, leaving a white mark as the shape of the design. The *Records* maintains its high standards of scholarship, illustrations and general format.

Auckland Institute and Museum

The Records of the Auckland Institute and Museum (6, No. 2, October 15, 1965) is, as usual, well illustrated and includes three papers by Mr. W. F. Ponder on the family Eatoniellidae, a revision of a recent species previously known as *Notosetia Iredale* and the recent and fossil species of *Estea Iredale*, respectively, all from New Zealand. The final paper, by A. W. B. Powell, deals with molluscan systematics.

University Research in Canada

THE *Annual Report on Support of University Research, 1964–65*, of the National Research Council and Medical Research Council of Canada gives detailed information on the former Council's programme of support for research in science and engineering and of the research awards of the Medical Research Council (Pp. vii+271. Ottawa: National Research Council and Medical Research Council, 1965. 50 cents). Direct university support totalled 17·8 million dollars, compared with 12·85 million dollars in 1963–64; of this, 1·86 million dollars was on postgraduate scholarships, 9·27 million dollars on operating research grants to university staff, and 2·1 million dollars on major equipment grants; 1·25 million dollars went to the Atomic Energy Control Board. Indirect support to universities totalled 1·29 million dollars. Lists of recipients of awards, scholarships and fellowships are included. Of the 7 million dollars expended on the extra-mural programme of the Medical Research Council (5·16 million dollars in 1963–64), 5·18 million dollars was on research grants, 610,109 dollars on fellowships, 348,823 dollars on scholarships, and 532,427 dollars on associateships. Lists of operating grants and of major equipment grants are included.

Eskimo Administration

THE Arctic Institute of North America has recently published the third in a series of studies designed to cover the administrations of all groups of the Eskimo race other than that in Siberia. The first two studies dealt with Alaska and Canada, and the third (Arctic Institute of North America. Technical Paper No. 16: *Eskimo Administration*: 3; *Labrador*. By Diamond Jenness. Pp. 94. Montreal, Washington, D.C., and New York: Arctic Institute of North America, 1965) covers Labrador. The history of the Eskimos of the Labrador Atlantic coast begins with a tale of conflict with white settlers from the sixteenth century, when European fishermen began to appear in numbers in the southern area; this unhappy situation lasted until the late eighteenth century when two Moravian missionaries negotiated a truce between the Eskimos and the settlers to the south. It is possible that the history of contact between Europeans and the Eskimos began earlier than this, if the recent recognition of Viking settlement in Newfoundland can be related to the Icelandic saga of a peaceful meeting with "small ugly men with broad faces", who left the Vikings in peace. In any event, after two centuries of struggle, the negotiated truce allowed gradual white penetration of southern Labrador, and the Eskimos there withdrew and concentrated in Hamilton Inlet area until they were absorbed into the white population. In northern Labrador the Moravian missionaries created an amicable atmosphere through their pressureless but successful evangelistic endeavours. Contact with the external world came with the Hudson's Bay Company and Newfoundland fishermen, and the history of the Eskimos since the mid-nineteenth century has been of increasing assimilation into and emulation of European and Canadian standards of dress, economy and customs. The outstanding lesson to be learned from this period in Labrador is simply that of the unqualified success of the Moravian missions, which, through devout yet eminently practical methods, painlessly achieved for the Eskimos a respected place in Newfoundland affairs; further advances will depend on the Eskimos of northern Labrador themselves.

Advances in Oceanography

A THIRD volume of *Progress in Oceanography*, edited by Mary Sears, of Woods Hole Oceanographic Institution, for the Pergamon Press, commemorates the seventy-fifth birthday of Prof. Hans Pettersson in 1963 (Pp. xxiii+407. London and New York: Pergamon Press, 1965. 105*s*. net). It contains thirty articles by his former students and colleagues. The majority deal with the sea floor and its sediments, to which a wide range of scientific techniques have successfully been applied. The remaining contributions deal with aspects of geophysics, marine chemistry and biology. The present volume departs somewhat from the systematic review of outstanding problems which was one of the main objects of the series, but it achieves the overall purpose of keeping oceanographers, and others interested in the sea, conversant with recent research and advances over a wide field. It is an appropriate and timely reminder of Prof. Pettersson's pioneering achievements. The third volume of *Oceanography and Marine Biology*, an annual review edited by Harold Barnes for George Allen and Unwin, Ltd., appears at the same time (Pp. 421. London: George Allen and Unwin, Ltd., 1965. 84*s*. net). Two-thirds is devoted to marine biology, particularly the systematics and distribution of chaetognaths, and the endocrine physiology of polychaetes and fishes. The remainder consists of review papers on waves, the carbon-dioxide system in the sea, exchange of chemical substances between sea and air, disposal of waste and pollution of the sea, conditions for life on the abyssal sea-floor, the biology of the north-east Atlantic herring population, and on the methods of determining phosphorus in sea water. Both books tend to serve as bound scientific journals containing some description of original work as well as review articles, and will probably be most useful in filling gaps between the journals and text-books and in emphasizing the unity of environmental science.

Effect of Duration of Curing on Slag–Portland Cement Concrete

THE use in concrete of ground blast-furnace slag mixed with Portland cement has in some countries been increasing during the past decade, and in view of the incomplete concrete curing procedures commonly adopted on construction sites in South Africa, it was decided that the effects of such limited curing of Slag–Portland cement concrete should be investigated. This has been done and the results are embodied in a paper by G. J. R. van der Meulen entitled *Effect of Duration of Curing on Slag–Portland Cement Concrete* (National Building Research Institute, South African Council for Scientific and Industrial Research, Pretoria. CSIR Ref. No. *R/BOU* 158. Reprinted from *Construction in Southern Africa*, **10**, 9; January 1965). The trend of this work was to compare the compressive strengths of various slag–Portland cement concretes with similar Portland cement concretes when they were continuously wet-cured until tested and when they were allowed to dry out after one day of wet curing. The object was to reproduce the effects of the best and the worst curing which concrete is likely to undergo in building practice. The results are not without a wider significance in other countries. From the experiments carried out, it was found that the effects of inadequate curing on slag–Portland cement concrete are severely adverse. The compressive strength of slag–Portland cement concrete is more sensitive to the effects of limited curing than that of similar Portland cement concrete. "The investigations showed that in nearly all cases the strengths of dry cured slag–Portland cement concretes at three months were not as great as those of similar Portland cement concretes developed after six days under the same curing conditions". It is concluded that it is therefore important to pay special attention to adequate wet-curing of slag–Portland cement concrete, particularly if it is used in thin sections. The paper includes details of mixes used, ages at test, and comparative graphs of compressive strengths of wet- and one-day-cured specimens of slag–Portland cement and normal Portland cement concretes, and also contains a short list of references to other workers who have been concerned with similar problems.

The Names of Early Man

Several publications during the past year have provided some guidance to the nomenclature of the fossil Hominidae. Dr. K. P. Oakley's *Frameworks for Dating Fossil Man* was the first to gather together some of the plethora of names that have accumulated around the finds of early man and his immediate family. Dr. Michael Day's *Guide to Fossil Man* indicated in greater detail some of the often confusing terminology used in published discussions on the physical relationships between these finds, some of which, by their fragmentary nature, were incapable of any degree of precise definition. Most recently has appeared *The Nomenclature of the Hominidae*, by Bernard G. Campbell (Royal Anthropological Institute of Great Britain and Ireland. Occasional Paper No. 22. Pp. v+34. London: The Royal Anthropological Institute of Great Britain and Ireland, 1965. 10*s*.). In this, he presents a full list of the nomina proposed for fossil hominid finds of Pleistocene age, and a review of these which establishes that a number of the existing names are invalid on the basis of the revised edition of the *International Code of Zoological Nomenclature*. Some idea of the complexity of the problem may be gained from the fact that sixty Pleistocene specimens have been made the types of new hominid taxa; these correctly include nineteen new genera and fifty-five new species and subspecies, with forty-four names which are synonyms, and twenty-one invalid names. The result is that almost every important fossil find is a distinct named species, and clearly this must disguise the varying degrees of relationships that must have existed within the general evolution of mankind. The recent finds of fossil man from Africa, Europe and Asia combine with the old to present a quite remarkable series of specimens falling within a broad distribution in time and space, and any future finds will improbably create new species, although they may extend the known habitat of man in early or mid-Pleistocene times; the recent finds at Vértesszollös in Hungary are important in this respect. The author may have been rash in prophesying the dismissal of the ten new hominid taxa created since 1955, but without doubt his paper represents an important advance in the control of hominid nomenclature.

Guide to Poisons and Therapeutic Substances

The new (eighth) edition of the *Poisons and T.S.A. Guide* incorporates all the relevant changes that have come into effect since the publication of the previous edition in August 1964 (*Poisons and T.S.A. Guide for Pharmacists in Retail and Hospital Practice*. Pp. 234. London: The Pharmaceutical Press, 1965. 10*s*.). The new requirements of the Dangerous Drugs Act, 1965, and the Dangerous Drugs (No. 2) Regulations, 1964, so far as they affect retail and hospital pharmacists, are explained, and the three separate classes of dangerous drugs are now clearly indicated in the Extended Poisons List. The *Guide* also includes the many changes in the classification of poisons brought about by the Poisons (No. 2) Rules, 1964, and the Poisons List Order, 1965, and Poisons Rules, 1965. Included for the first time is a short summary of the Drugs (Prevention of Misuse) Act, 1964, which makes unauthorized possession of certain substances, other than dangerous drugs, an offence. Such substances are now specially marked in the Extended Poisons List. Also new is the information on the supply of animal medicines to farmers, the information on animal feeding-stuffs containing poisons and therapeutic substances, and on antibiotics for horticultural purposes and for preserving raw fish. The explanatory text of the *Guide* has been completely rearranged, and the conditions for supply of drugs over the counter and on prescription are now clearly described in the same section for each of the following: Part I and Part II Poisons; First Schedule Poisons; Dangerous Drugs; and 'Therapeutic Substances'. The *Guide* includes, for reference, the full texts of the schedules to the Poisons Rules, and the schedule to the Dangerous Drugs Act. The "Extended Poisons and T.S.A. List", thoroughly revised and brought up to date, now includes nearly 5,000 entries, with a clear indication of the particular restrictions affecting each item. The list includes, so far as possible, all affected substances, proprietary and non-proprietary, which are likely to be called for in the ordinary course of retail and hospital practice.

Spices

The aromatic, often pungent, vegetable substances used to flavour food, grouped under the name 'spices', have, by one label or another, been household words for centuries, but few people are probably aware of what a wide range of products is involved or know much about their history. Certainly, most know little of the crude methods used by natives, for example, in rural Borneo, to produce them. A forthright article by Peter Humber entitled "The Biggest Thing in Spices since Vasco Da Gama" (*Albright Magazine*, 12; December 1965. 1 Knightsbridge Green, London, S.W.1) lifts the veil from some truly amazing circumstances in which some spices are prepared and brought to market in the Far East, from first-hand information gained during a recent visit to the spice division of the firm, Stafford Allen, a member company of the Albright and Wilson Group, who have been trading in spices for 132 years. He says: "In days gone by spices were as highly prized as precious stones. Men died violently or made their fortunes in their quest for exotic seasonings. Even now spices are used as currency in primitive parts of the world and the spice business is as exciting as it ever was".

This leads to a discussion of a product known as 'Saromex', originated and marketed by Stafford Allen 10 years ago, which has already revolutionized the world's spice industry. Mr. Humber regards the production of 'Saromex' as ". . . the biggest thing that's happened to spice since Vasco da Gama rounded Cape Horn and anchored off the coast of Calicut". 'Saromex' is a natural spice extract; it contains the whole of the available aromatic ingredients, incorporated on to a clean 'spreader base' such as salt, dextrose or flour; it contains all that is desirable in a particular spice without any of the drawbacks, some of which are mentioned in no uncertain terms in his article. It is pointed out that 'Saromex' is not sold direct to the consumer but is sold and used only in large-scale food production. Of 'Saromex' spices, he says: ". . . by virtue of their standardized flavour strength and quality, manufacturers can be sure that every batch of their product will taste exactly the same, whereas with natural spices the flavour can vary enormously depending on the conditions in which the spices were grown". Despite some rather terrifying remarks concerning 'infestation' of natural spices, the point is emphasized here that normal ground or whole spices (as supplied to-day) are perfectly safe for domestic usage. This is a very interesting and, in some ways, provocative article which, however, still leaves some questions regarding natural spices unanswered. It is followed by another entitled "Experiments with Spice", in which what are called 'spice-conscious' recipes are detailed. As a practical gesture, certainly an innovation in an industrial house-journal, there is included a generous sample of 'Saromex', so that the experiments may be carried out successfully.

Geological Society of London : Awards

The Council of the Geological Society of London has made the following awards for 1966: *Wollaston Medal*, to Prof. F. R. Shepard, formerly professor of submarine geology, Scripps Institute of Oceanography, University of California, for his researches in sedimentology and submarine geomorphology, and the light they throw on

the problems of ancient rocks; *Murchison Medal*, to Prof. K. C. Dunham, professor of geology, University of Durham, in recognition of his contributions to geology and especially the study of ore deposits and their genesis; *Lyell Medal*, to Prof. S. I. Tomkeieff, professor emeritus, University of Newcastle upon Tyne, for his contributions to mineralogy and petrology and his exposition of the works of Russian geologists; *Prestwich Medal*, to Mr. D. Curry, managing director (joint), Currys, Ltd., for his work on the English Lower Tertiary rocks and his contributions to the geology of the English Channel and Western Approaches; *Wollaston Fund*, to Miss E. M. Guppy, senior experimental officer, Geological Survey and Museum, for contributions to the work of the Geological Survey, especially in petrology; *Murchison Fund*, to Prof. J. G. Ramsay, Department of Geology, Imperial College of Science and Technology, for his contributions to structural geology, particularly in the Scottish Highlands, Transvaal and the Alps; *a moiety of the Lyell Fund*, to Dr. M. J. S. Rudwick, lecturer in geology, University of Cambridge, for his substantial work in developing the field of functional morphology in palaeontology and for his contributions on the history of geology; and to Mr. J. R. L. Allen, lecturer in geology, University of Reading, for his work in sedimentology.

Philosophical Society of Washington

Dr. M. M. Shapiro, head of the laboratory for cosmic-ray physics at the U.S. Naval Research Laboratory, has been elected president of the Philosophical Society of Washington. Dr. W. J. Youden, of the George Washington University, and Dr. E. Montroll, vice-president for research at the Institute for Defense Analyses, have been elected vice-presidents of the Society.

Technion Achievement Award

The Chicago Chapter of the American Society for Technion, Israel Institute of Technology, Inc., has announced the establishment of a Technion Achievement Award. The award, including an honorarium of 1,000 dollars, will be given for some successful application of technology to an important social problem. Nominations for the Award, which will be given this year for an achievement noted in 1965, should reach Dr. Herbert H. Hyman, secretary, Technion Achievement Award Committee, 59 E. Van Buren Street, Chicago, not later than July 1, 1966.

Annual Prize for Women Undergraduates

A prize of £15 is offered by the British Federation of University Women, Ltd., for an essay on one of the following subjects: (*a*) expectation and reality on entering a University; (*b*) the responsibilities of teachers and students. Competitors should be women students reading for a first degree or diploma at any University or College of Advanced Technology in England, Scotland or Wales. Further information can be obtained from the Secretary, British Federation of University Women, Crosby Hall, Cheyne Walk, London, S.W.3, to whom entries should also be sent. The closing date for the competition is May 1.

Esso Chemical Limited

Esso Petroleum Company, Ltd., has announced that the management and operation of its present chemical business will shortly be transferred to a new company, Esso Chemical, Ltd., which will also be responsible for the future development of the chemical business of Esso in the United Kingdom. The new company will be wholly owned by Esso Chemical Company, Inc., of New York, which is in turn a wholly owned subsidiary of Standard Oil Company (New Jersey).

"A Wave Mechanical Approach to the Plasma Interaction Problems"

Prof. V. C. Liu (Department of Aerospace Engineering, College of Engineering, University of Michigan, Ann Arbor) has notified the Editor of the following corrections to "A Wave Mechanical Approach to the Plasma Interaction Problems", which appeared on p. 883 of the November 27, 1965, issue of *Nature*: "Equations (7*a*), (7*b*), (8) and (9) should read:

$$\frac{d^2\psi_1}{dx^2} + \frac{8\pi^2 m}{h^2}[Ee - \varphi(x)]\psi_1 = C_1^2\psi_1 \qquad (7a)$$

$$\frac{d^2\psi_2}{dy^2} = -C_1^2\psi_2 \qquad (7b)$$

$$|\psi|^2 = (1 - e\varphi/E\cos^2\theta)^{-\frac{1}{2}} \qquad (8)$$

and

$$x - x_0 = \int_{\varphi_0}^{\varphi} (8\pi n_0)^{-\frac{1}{2}}[2E\cos^2\theta(\sqrt{1 - e\varphi/E\cos^2\theta} - 1) - \varkappa T_e(1 - e^{e\varphi/\varkappa T_e})]^{-\frac{1}{2}}\, d\varphi \qquad (9)$$

respectively".

University News: Edinburgh

The following lecturers have been appointed: Dr. A. J. Crosbie (geography): Dr. J. S. Kelly (pharmacology). The status of reader has been conferred on the following: Dr. J. H. Bowie (bacteriology); Dr. R. M. Gaze (physiology); Dr. F. L. Mitchell (clinical chemistry); Dr. P. H. Davis (botany); Dr. J. H. Knox (physical chemistry); Dr. J. B. Sissons (geography).

London

Prof. K. G. Denbigh, at present Courtaulds professor of chemical engineering at the Imperial College of Science and Technology, has accepted the office of principal of Queen Elizabeth College in succession to Miss M. J. Sargeaunt, who will be retiring on September 30.

Newcastle upon Tyne

The following lecturers have been appointed: Dr. D. F. Roberts (human genetics); Dr. H. C. Noltimier (geophysics); Mr. P. Baldwin and Dr. J. P. Baty (engineering mathematics); Mr. A. I. B. Moffat (civil engineering materials).

Announcements

Prof. A. R. J. P. Ubbelohde has been appointed to fill a vacancy in the membership of the Agricultural Research Council, which has arisen on the resignation of Prof. W. T. Williams.

Mr. A S. Foot, head of the Department of Dairy Husbandry of the National Institute for Research in Dairying, has been appointed deputy director of the Institute.

A three-day course on "Temperature Measurement" will be held at the Woolwich Polytechnic during March 16–18. Further information can be obtained from P. R. Pallister, the Woolwich Polytechnic, Wellington Street, London, S.E.18.

A symposium on "Early Crop Production in the British Isles" will be held in the University College of Wales, Aberystwyth, on March 9. Further information can be obtained from J. A. Taylor, Department of Geography, Llandinam Building, Penglais, Aberystwyth.

A meeting of the Welsh Soils Discussion Group on "Soil as a Biological Environment" will be held at the Welsh Plant Breeding Station, Gogerddan, on February 16. Further information can be obtained from C. Rudeforth, Soil Survey, National Agricultural Advisory Service, Trawscoed, Aberystwyth.

MONOLAYERS AT LIQUID INTERFACES

AN informal discussion of the Faraday Society on monolayers at liquid interfaces was held at Loughborough College of Technology during September 9–10, 1965. The meeting was attended by 68 people, among whom there was strong representation from European research laboratories. There were 27 from industrial organizations.

The chair for the session on September 9 was taken by Dr. J. L. Moilliet (Imperial Chemical Industries, Dyestuff Division). After a brief welcome from Prof. R. F. Phillips (Loughborough College of Technology) the opening paper was given by Prof. G. M. Bell (Chelsea College of Science and Technology) and Dr. S. Levine (University of Manchester). This was concerned with the surface-free energy and the surface pressure of monolayers. The relation between the part of the surface Helmholtz-free energy, F, due to dissolved components and surface-active species, and the corresponding part of the surface pressure, Π, was discussed. When all surface-active substances are soluble in the substrate, and F is defined as the change in surface-free energy density during a process in which they are brought into the surface region from the bulk of the substrate, then it was shown that $\Pi = -F$. This is not true if any of the surface-active substances are insoluble and an expression for $\Pi + F$ was obtained in this case. The form of Π obtained in this way was shown to be identical with that derived by integration of the Gibbs adsorption isotherm (as used by Onsager and Samaras and others for air-electrolyte interfaces). The authors concluded with a brief mention of the various contributions to the spreading pressure of an insoluble partially ionized monolayer on an aqueous electrolyte substrate.

The second paper was given by Drs. J. Mingins, J. W. Brooks and B. A. Pethica (Unilever Research Laboratory, Port Sunlight) and described investigations of ionized monolayers. Previous data on the surface pressures of insoluble and soluble ionized monolayers at both non-polar oil–water and air–water interfaces were critically reviewed and compared with recent data obtained at Port Sunlight. Discrepancies among the data were discussed in terms of solution of the monolayer, poor spreading, and impurities in the surface-active agents, electrolytes and water. Results on films spread from crystals indicated that solvent retention had occurred in some of the earlier published work on gaseous monolayers spread at the air–water interface. The cohesive pressure due to hydrocarbon chain interaction was shown to be negligibly small at non-polar oil–water interfaces over the range of areas per molecule studied (50 Å^2). However, at air–water interfaces the cohesive pressure was a complex function of chain-length, area per molecule and head group. The validity of previous empirical equations describing this term was questioned.

Dr. E. H. Lucassen-Reynders (Unilever, Vlaardingen) presented a thermodynamic approach to the surface equation of state. This included consideration of ionizing surfactants and non-surface active electrolytes. The resulting equations are similar to those for non-ionizing surfactants, except that the molar concentration in the solution is replaced by the geometric mean of the product of the concentrations of the long-chain ions and counter-ions. The general equations give the surface tension as a function of the mean ionic product in terms of three types of parameters. These are, first, the interfacial activity coefficients, γ_i^s, of the surface mixture of surfactant and water; secondly, the saturation adsorption, Γ^∞ (the total values for long-chain ions and counter-ions); and thirdly, a parameter, a, measuring the free adsorption enthalpy at infinite dilution. The equations were applied to published surface and interfacial tension measurements on adsorbed and spread monolayers of long-chain alkylsulphates, trimethyl ammonium bromides and carboxylates, in the presence of various amounts of non-surface-active electrolytes. At oil–water interfaces, the ionized surfactants were shown to form ideal surfaces ($\gamma_i^s = 1$), irrespective of the presence of inorganic electrolyte with a common ion. At the air–water interfaces especially the longer-chain surfactants were shown not to form ideal surfaces. In particular, carboxylate soap solutions show regular surface behaviour. The γ_i^s values obtained from the experimental data suggested that the main factor responsible for the non-ideality of a surface was the interaction between the hydrophobic chains of the surfactant.

A further paper on the thermodynamics of interfaces was presented by Dr. A. J. Spaull (Brunel College). Methods for obtaining thermodynamic parameters for adsorption at the air–water interface were considered. It was maintained that the initial linear region of the adsorption isotherm is important, but it was suggested that use of the slope, α, of the plot of surface pressure as a function of concentration, can lead to incorrect values of the thermodynamic parameters. Analysis has shown that, to first order, the plot of the ratio of surface pressure to concentration as a function of surface pressure can be represented by the equation:

$$\Pi/c_x = (\Pi/c_x)_0(1 - a/RT)$$

where a is a two-dimensional second virial coefficient, and $(\Pi/c_x)_0$ is the extrapolated value of the ratio of surface pressure to concentration at zero surface pressure. To calculate correct thermodynamic parameters it is $(\Pi/c_x)_1$ rather than α that is required, except when a is zero. Experimental methods for testing this argument were indicated. Certain thermodynamic parameters obtained for the adsorption of 1-butanol and 1-hexanol were used to test a recent theory of Aranow and Witten, who claim that adsorption of long-chain hydrocarbons is essentially an entropic process arising from restricted rotation about carbon bonds in aqueous solution. Some difficulties of the theory in explaining the evidence of the nuclear magnetic resonance spectra of alcohol solutions and the solvation of rare gases were discussed.

Dr. J. B. Matthews (Loughborough College of Technology) proposed a new approach to the dynamics of adsorption from solution. The kinetics of adsorption from solution is still a controversial subject, owing to the uncertainties associated with the experimental data and the model for the adsorption process. A number of theories for the kinetics of adsorption have culminated in the Ward–Tordai theory, which now receives general acceptance, either in its original form or as modified by others. The model proposed by Ward and Tordai assumes the existence of a sub-surface from which solute molecules are adsorbed instantaneously and irreversibly to the surface, if there are available sites for them. The kinetics of adsorption are assumed to be controlled by the diffusion of solute molecules to the sub-surface from the bulk of the solution. Dr. Matthews stated that this model ignores the dynamic character of adsorption and transitions between the possible states of adsorbed molecules. The mathematical treatment of this model leads to kinetic results which are more consistent with experimental data than the Ward and Tordai theory.

The chairman for the second day was Prof. D. D. Eley (University of Nottingham). In opening the session Prof. Eley paid tribute to Dr. J. J. Kipling, who was a member of the organizing committee of the discussion and whose untimely death occurred at a conference in Moscow earlier in the year.

The first paper was given by Prof. A. Scheludko (Institute of Physical Chemistry, Sofia, Bulgaria), who reported work carried out with Dr. D. Tissen on the damping of ripples on water by soluble surfactants and insoluble monolayers. A novel apparatus has been developed for this problem, consisting of a cylindrical vessel in which standing waves are generated by a vertical sinusoidal vibration. The amplitude of ripples smaller than 30μ could be determined optically with an accuracy of 1 per cent and the damping measured by changing the energy supplied to the vessel. Results were presented for the damping coefficient as a function of concentration (or area) for caproic, capric, myristic, palmitic and propyldodecyl acetic acids and for cetyl alcohol on water. With all except the first, a sharp maximum was found at intermediate surface densities and damping coefficients at the maximum exceeded (for a factor of 2–3) the value predicted by the hydrodynamic theory of Levich for damping by a condensed monolayer.

The next paper, by Drs. J. M. Corkill, J. F. Goodman, S. P. Harrold and J. R. Tate (Procter and Gamble, Newcastle upon Tyne), was concerned with mixed monolayers of both soluble and insoluble salts of the type alkyltrimethylammonium-alkyl-sulphate at the air-solution interface. Insoluble monolayers were examined using a Langmuir trough. It was demonstrated that although second-order effects are present due to the substrate or the particular molecular species involved, maximum interaction invariably occurs at a 1 : 1 ratio of long-chain cation to anion. The composition of the surface layers of the soluble members was measured by radio-tracer techniques. When the long-chain cation and anion had the same chain-length an equimolar surface composition was obtained. However, if the ions had different chain-lengths, the longer chain ion was preferentially adsorbed giving an asymmetric surface layer, the asymmetry being enhanced by the addition of inorganic electrolytes. It was concluded that adsorption is promoted principally by the elimination of alkyl chain–water interface, but this is modified by the electrical interaction between the positive and negative head groups, which is a maximum when the surface composition is equimolar.

A paper by Dr. J. J. Kipling and Dr. C. A. J. Langman (University of Hull) dealt with the surface tension of two component liquid mixtures and the adsorption from these mixtures at the liquid–vapour and liquid–solid interfaces. The surface tensions of a number of binary liquid mixtures of molecules of similar sizes were measured and the results were shown to fit quite well an equation based on a regular solution model, relating surface tension to composition. The adsorption from the mixtures at low-energy solid surfaces was studied and the surface excess of the more strongly adsorbed molecule compared with the corresponding surface excess at the liquid–vapour interface. In all cases presented, these two excesses were similar in magnitude.

Another paper on the surface tensions of binary liquid mixtures, by Dr. R. Aveyard (University of Cambridge), concluded the conference. The surface tensions of a range of two-component mixtures of *n*-alkanes have been measured. The applicability of various equations to the experimental data was tested and it was found that a theory, devised by Prigogine and Maréchal for athermal polymer solutions, was the most satisfactory of those considered. Using this theory, the surface tensions of all the mixtures investigated could be predicted to within 1·0 per cent with the use of physically realistic and self-consistent parameters. Further, the applicability of this theory to the surface tensions of methanol–*n*-decanol and methanol–glycerol mixtures was tested. The agreement between experiment and theory was poor for the former system, but reasonable for the latter. A possible explanation for these differences, and its bearing on the agreement obtained for the *n*-alkane systems, was discussed.

D. A. Haydon
M. J. Jaycock

PHYSICS IN MEDICINE

TECHNOLOGY, rather than science, was the main theme of the first International Conference on Medical Physics, held at Harrogate during September 8–10, but several topics of a more fundamental kind were also illuminated in a programme of more than 100 papers. A majority of the contributions dealt with the clinical and biological applications of ionizing radiations, reflecting a continuing preoccupation with the problems which brought physicists into the hospital service in significant numbers some 30 years ago.

Though the radiotherapist's armamentarium has not changed substantially in recent years, two areas of research at present give promise of greater accuracy in the measurement and control of radiation dosage for clinical purposes. The first problem here is to find a device of small size (preferably in the millimetre range) with a sensitive response independent of radiation energy. Lithium fluoride dosemeters, based on the thermoluminescent effect, satisfy these requirements reasonably well. Large-scale practical applications are, however, restrained by inadequate understanding of the basic processes involved. The preparation of lithium fluoride powder and its treatment before and after irradiation are still largely determined by empirical knowledge, which necessitates caution in the interpretation of experimental findings.

Thermoluminescent dosemeters have been used for the estimation of surface dosage in radiotherapy, but not so far for internal measurements. F. W. Spiers (General Infirmary, Leeds) described methods for the study of radiation dose in specimens of dry bone, using fine-grain lithium fluoride powder which is introduced into the narrow cavities of trabecular bone and removed for assay after irradiation. Work of this kind is useful in assessing clinical, occupational and environmental hazards from radioactive materials. B. E. Bjarngard (Controls for Radiation, Inc., Cambridge, Massachusetts) reported that some of the difficulties associated with the preparation and handling of thermoluminescent powders may be avoided by incorporating the lithium fluoride phosphor in polytetrafluorethylene, which can then be fashioned into rods, disks or foils for various applications. The combination has an effective atomic number close to that of tissue, and its response is independent of radiation energy over a fairly wide range.

Interesting applications are now being found for solid state devices as radiation detectors. P. R. Parker (Institute of Cancer Research, Sutton, Surrey) suggested that many of the desirable properties of the gas-filled ionization chamber may be reproduced, with the advantage of very small bulk, by *p–n* surface barrier detectors. Used as pulse counters, these devices will measure dose-rates of about 10 μrad/h, approximately equal to the natural background radiation in Britain. The radiation-induced potential across the *p–n* junction is found to be a logarithmic function of dose-rate and the short-circuit current is approximately a linear function of dose-rate. Silicon

surface barrier detectors offer high sensitivity in small size and may be useful in estimating radiation dosage (from internally administered isotopes) inside the body. The field effect transistor, having high input impedance and high transconductance, may replace an electrometer valve in the input stage of a current amplifier used, for example, in association with an ionization chamber. J. Keller (Central Laboratory for Radiological Protection, Warsaw) described an amplifier, using a single transistor, with a gain of 5×10^5 and excellent temperature stability.

A second problem of immediate interest in radiotherapy concerns the use of electronic computers to tackle the tedious calculations involved in treatment planning and dosage control. The great output delivered by machines such as the linear accelerator makes rapid arithmetic essential, and the complexities of radium dosage are not always adequately recognized in the rules or methods based on manual calculation. Papers presented at the Harrogate Conference showed a relatively unsophisticated approach to these problems and left the impression that a much larger effort is needed (from mathematicians as well as from physicists) if the computer is to make a realistic contribution to the technique of radiotherapy.

Diagnostic and physiological investigations using radioactive tracers are now commonplace, though still capable of extension in a number of clinical specialities. Interesting technical problems are met with in the design and operation of whole-body monitors. In measuring the natural radioactive content of the body (for example, potassium-40 as an index of lean body mass) elaborate electronic equipment and a well-shielded room will be necessary, at a cost which may approach £30,000. When it is desired to investigate, over a period of months, the retention of a particular isotope in the body, a simpler installation can be built for £2,000–£5,000.

Though knowledge of the whole-body content of an isotopic tracer is important, a detailed picture of the distribution in a particular organ or region may be significant (for example, in thyroid disease or in the localization of a tumour). The scintillation scanner and the scintillation camera (which uses the principle of the pinhole camera) are well known in this connexion. Another basically simple device, likely to have useful applications in clinical science, is the spark chamber. The design described by B. R. Pullan (St. George's Hospital, London) has two parallel plates, one of lead and one of aluminium mesh, in an enclosure filled with argon and alcohol vapour. When an appropriate potential difference is maintained between the plates, a spark will occur wherever ions are produced in the gas. A chamber of this kind (used in conjunction with a suitable collimator) should be useful in thyroid studies with radioactive iodine as a tracer.

Among the other topics discussed were (*a*) the use of analogue computers and mathematical models in the study of renal function, urinary excretion and control of ventilation, (*b*) objective measurement of X-ray image quality, (*c*) electron spin resonance in living tissue. A series of eight survey papers, illuminating areas of common interest among physicists, biologists and clinicians, included an interesting account by S. Rowlands (St. Mary's Hospital Medical School, London) of basic studies on blood flow. The apparent viscosity of blood depends on its composition and on the shear rate applied in the experimental equipment. These properties are shared by other biological materials and non-Newtonian fluids. Blood has a unique property, discovered by measurement of flow rates through tubes of less than 0·5 mm diameter. Here it is found that the narrower the tube the smaller the apparent viscosity. It seems likely that hydrodynamic forces drive the red cells from regions near the wall (where the shear rate is high) into the central stream, where the shear rate is lower. In very narrow tubes, of diameter comparable with the diameter of a red cell, further complications occur and the deformability of the cell has an important influence on flow phenomena.

In the realm of medicine there is abundant opportunity for work of social and economic value, amply testing the physicist's experimental skill and mathematical insight. In several countries (including Britain) this opportunity is not being sufficiently exploited at the present time. Many hospital physicists are dispersed in small departments, mainly occupied with work related to the practice of radiotherapy, and not adequately equipped (with staff, material resources and scientific contacts) for work of more basic importance.

During the next decade, it seems likely that the large and well-justified expenditure from public funds in support of scientific research in universities and government laboratories will come under closer scrutiny and its continued growth may even be restrained. If, in these circumstances, some of the less costly fields of endeavour receive increased attention, the collaboration of physics and medicine might be cultivated with advantage to the world of science and to the whole community.

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ENERGY SPECTRUM OF PRIMARY COSMIC-RAYS

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During the past decade or so, several measurements have been made of the flux of the cosmic-ray particles in the wide range of energy from 10^9 to 10^{20} eV. These were based at the lower energy end on the flux of nuclei observed at different geomagnetic latitudes and at energies greater than 10^{15} eV on the results of extensive air shower experiments. The data in the intermediate energy range were not so reliable. The pioneer experiments of Lal[1] and Kaplon and Ritson[2], which have played a useful part in giving an estimate of the primary flux in the energy region 10^{12}–10^{13} eV, made use of detectors of alternate sheets of 3 mm brass and 100 μm *G*5 emulsions mounted on 1·3 mm glass. In such a detector the distinction between electromagnetic cascades initiated by electrons or γ-rays entering from outside and those resulting from interactions of nuclear active particles in the detector itself could be quite ambiguous. Further, energy estimates were rather unreliable and the results were based on a small number of events. Values of the flux, at 4×10^{13} and 2×10^{15} eV, due to Barrett *et al.*[3], were obtained from underground measurements on μ-mesons, and therefore depend on the assumed model of high-energy interactions. This article presents our results on the primary flux, both

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of protons and heavier nuclei, in the energy range $2{\cdot}6 \times 10^{12}$–$2{\cdot}6 \times 10^{14}$ eV, and makes a comparison with other results over the entire range 10^9 and 10^{20} eV. At the lowest energies, the charge composition of the primaries is well known[4] and in our work some direct evidence on heavy nuclei also may be obtained. Extensive air shower experiments measure the flux of all nuclei above a given total energy and there is no direct evidence that the charge composition of the primaries is the same as at 10^{10} eV. We have, therefore, chosen to present a spectrum of all nuclei above a given total kinetic energy as distinct from kinetic energy per nucleon. The difference between the kinetic energy, E, and the total energy, T, is insignificant except at the very lowest energies considered where the former is a more informative quantity.

In our experiment, we used a tungsten-emulsion assembly of area $0{\cdot}7$ m^2, eight radiation lengths thick. This was flown for 28 h at an effective atmospheric depth of 22 g cm^{-2} (ref. 5). The assembly provided an efficient detector of electromagnetic cascades. As already described[5], the distinction between cascades initiated by electrons or γ-rays entering from outside and those from nuclear active particles interacting in the detector was good. Further, the cascade energy could be quickly and accurately obtained from photometric measurements of the density of electron tracks near the cascade axis. A total of 527 nuclear events, satisfying certain geometrical selection criteria and having a total cascade energy ($\Sigma E\gamma$) of more than 370 GeV, were recorded. Of these, 46 events originated in emulsion and the remainder originated in the tungsten sheets.

It is necessary to estimate the charge composition of the primary particles initiating interactions in tungsten. We assume that the interaction lengths and fragmentation parameters of multiply charged nuclei are the same as at low energies[4], and that the interaction length of nucleons in graphite is 73 g cm^{-2}. We take the attenuation length of nucleons in air to be 125 g cm^{-2} and their interaction length in the detector to be the geometric value, 14·5 cm. (*Note:* The value of 14·5 cm (or 176 g cm^{-2}) for the interaction length in the detector was obtained on the assumption that the interaction cross-sections of protons with different nuclei at these energies are the same as those observed at 24–30 GeV; an $A^{2/3}$ dependence was assumed wherever extrapolation was required. This value was checked experimentally using the depth of origin of events within the detector and was found to agree within 15 per cent.) The charge composition was then estimated in two ways: (*a*) From the observed charge composition of the forty-six emulsion events (thirty-four due to protons, three due to neutral particles, presumably neutrons, six due to α-particles and three due to heavy nuclei). (*b*) By assuming that at high energies the charge composition at the top of the atmosphere is the same as at low energies.

These two estimates are in good agreement and we find that the latter method also reliably predicts the charge composition of the observed events in the ICEF Collaboration[6]. There is thus no evidence that the charge composition is different from that observed at 10^{10} eV and we assume that the relative proportions of protons, α-particles and heavier nuclei are also fairly independent of energy in the range 10^{10}–10^{14} eV. We estimate that, of the observed tungsten events, 66 per cent are due to primary protons, together with nucleons from the interactions of these protons in the atmosphere, 13 per cent are nucleons from the fragmentation of nuclei with $Z \geqslant 2$, 15 per cent are due to α-particles and 6 per cent are due to heavier nuclei.

We find that the rate of production of cascades of total energy greater than 370 GeV by protons at the top of the atmosphere is $(1{\cdot}2 \pm 0{\cdot}3) \times 10^{-8}$ per sterad sec per gram of the detector (predominantly tungsten). We cannot exclude a systematic error in the energy determination of up to 15 per cent. This leads to the 25 per cent error quoted here, compared with which the statistical error is negligible. The integral energy spectrum of nuclear cascades[5] can be represented by a power law of the form $(\Sigma E\gamma)^{-1.44 \pm 0.05}$ in the range $370 \leqslant \Sigma E\gamma < 3 \times 10^4$ GeV.

To transform the cascade energy to primary energy we assumed that $E = 7\Sigma E\gamma$; this corresponds to $K\pi = 0{\cdot}43$ and $K\gamma = K\pi/3$. Dobrotin and Slavatinsky[7], working at 300-GeV primary energy, obtained a mean inelasticity $K\pi = 0{\cdot}35 \pm 0{\cdot}05$, and the foregoing figure was derived from their result allowing for the fact that the primary spectrum is a power law and that events are detected by their cascade energy. To obtain the flux of primary protons we have multiplied the production rate by the interaction length for protons in the detector (see '*Note*' above), 176 ± 26 g cm^{-2}. We thus obtain in the range $2{\cdot}6 \times 10^{12}$ to $2{\cdot}6 \times 10^{14}$ eV the expression

$$n(>E) = (1{\cdot}6 \pm 0{\cdot}5) \times 10^{12}\, E^{-1.44}, \text{ where } E \text{ is in eV}$$

The charge composition being taken to be that observed at lower energies, the flux of all nuclei at the top of the atmosphere above the same total energy is double this flux. About half these multiply charged primaries are α-particles and half are heavier nuclei. Thus, in Figs. 1 and 2, our results for the flux of all nuclei are represented by:

$$N(>E) = (3{\cdot}2 \pm 1{\cdot}0) \times 10^{12}\, E^{-1.44}$$

Fig. 1 is the spectrum of the number, N, of all nuclei above a given kinetic energy, E, in a conventional plot. The scale is so small that it is difficult to both present errors and to read values from it. We therefore give Fig. 2 in which the same data are plotted in the form log $(NE)^2$ against log E; in Fig. 2 a horizontal line corresponds to a spectrum of negative index $\gamma = 2{\cdot}0$.

In Figs. 1 and 2, the curves marked (1) represent the results of many workers as reviewed by Waddington[4]; we assume that the charge composition of the primaries does not change in this energy region and that the spectrum of total energy between 1·3 and 100 GeV is a power law of index $\gamma = 1{\cdot}50$ with a cut off below 1·3 GeV/nucleon. Lal[1] and Kaplon and Ritson[2] measured the proton flux, and their results have been scaled by the factor of 2·0 to allow for other nuclei. Baradzei *et al.*[8] used ionization chambers and observed the energy radiated as γ-rays in the interactions of nuclear active particles with carbon

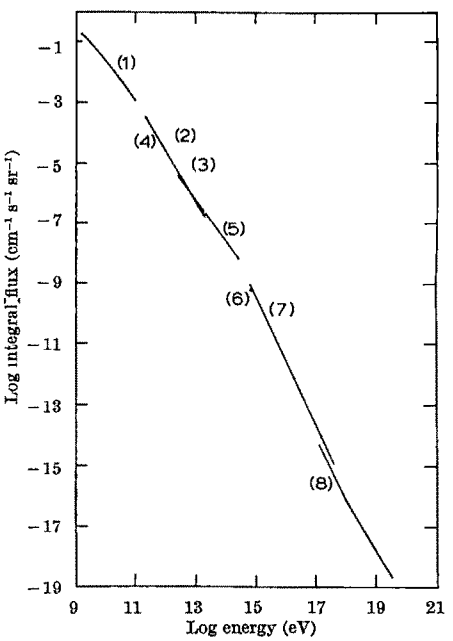


Fig. 1. Integral energy spectrum of all nuclei incident on the atmosphere. The data are collected from (1) geomagnetic measurements as reviewed by Waddington, 1960, (2) Lal, 1953, (3) Kaplon and Ritson, 1952, (4) Baradzei *et al.*, 1962, (5) present experiment, (6) Zatsepin *et al.*, 1963, (7) Clark *et al.*, 1963, (8) Linsley, 1963

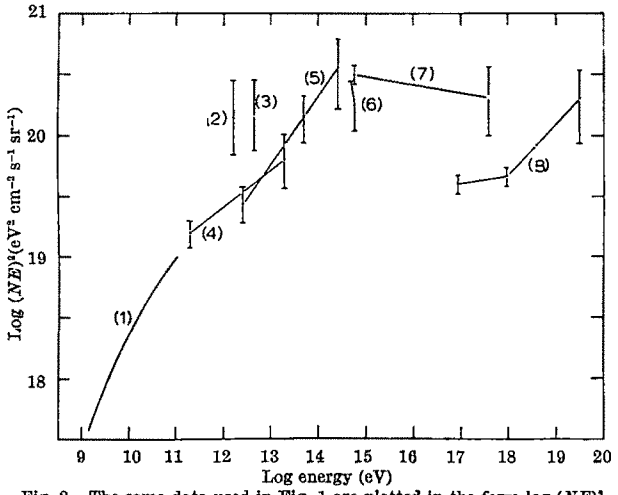


Fig 2 The same data used in Fig. 1 are plotted in the form log $(NE)^3$ against log E. A horizontal line in such a plot would correspond with a spectrum of negative index $\lambda = 2{\cdot}0$

at an atmospheric depth of 200 g cm^{-2}. The flux obtained by them refers to all nucleons; in order to obtain the flux of all nuclei we have increased it by a factor of 1·5 (this figure is the product of 0·75—ratio of protons to all nucleons—and 2·0—ratio of all nuclei to protons).

Finally, above 10^{15} eV, we give the results of Zatsepin *et al.*[9], Clark *et al.*[10] and Linsley[11]; these results are as given by their authors. The difference between the fluxes reported by Zatsepin *et al.* and Clark *et al.* at 6×10^{14} eV may be partially due to the different factors used by the two groups in converting number of particles into primary energy (1·7 and 2·0 GeV per particle respectively).

The present results, and those of Baradzei *et al.*, confirm that the flux values given by Lal and by Kaplon and Ritson are overestimates. It now seems clear that the energy spectrum cannot be represented by a single power law over the energy range 10^{10}–10^{20} eV. Between 10^{11} and 6×10^{14} eV it is consistent with a power law of negative index $\gamma = 1{\cdot}60 \pm 0{\cdot}02$ and the flux of all nuclei is:

$$N(>E) = (3{\cdot}9^{+3{\cdot}4}_{-1{\cdot}8}) \times 10^{14} \times E^{-1.60}$$

where E is in eV. (Preliminary results on the flux of all nuclei measured above the atmosphere in the satellite *Proton I* indicate a much lower flux (between 10^{10} and 10^{13} eV) than those quoted in this article. The results, communicated by Prof. Grigorov at the London Conference of the International Union of Pure and Applied Physics, September 1965, are a factor of about 7 lower than the results given here. It is as yet uncertain whether the difference arises from the observed flux or the measurement of primary energy.) Above 10^{15} eV the spectrum is significantly steeper and, at the very highest energies recorded, there is disagreement as to the absolute value of the flux.

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GENESIS OF RAPAKIVI

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A REVIEW of the work on the Pre-Cambrian basement of South Greenland has recently been published[1]. Investigations in the Tasiussak area (Fig. 1), carried out under the auspices of the Greenland Geological Survey, have revealed a rapakivi granite massif displaying clear and distinctive features. Excellent exposures on the glaciated southern Greenland coast allow deductions on rapakivi genesis to be made, and these are set out below.

There is a general consensus of opinion at the present time that rapakivi granites crystallized from magma at high crustal levels[2] and it is widely accepted that one of the peculiar textural characters of rapakivis (the occurrence of big mantled felspars) is a direct result of the crystallization of magma. It is this conclusion which is challenged here. The Tasiussak massif is a rapakivi in which basic rocks were emplaced during the genesis of the granite, and it is by means of these basic rocks that two stages in rapakivi development can be demonstrated, with the typical texture a product of post-magmatic metasomatism.

Rapakivi, a term derived from the Finnish '*rapautuwakivi*' ('disintegrating rock'), was adopted by Sederholm[3] for a particular set of Pre-Cambrian granites in Finland which, while displaying their vulnerability to weathering processes, were also linked by petrological characteristics. These characteristics include post-tectonic intrusion and a porphyritic texture formed of ovoid crystals of potash felspar each mantled by a shell of oligoclase. The latter feature has become known as 'rapakivi texture'. Many rapakivi

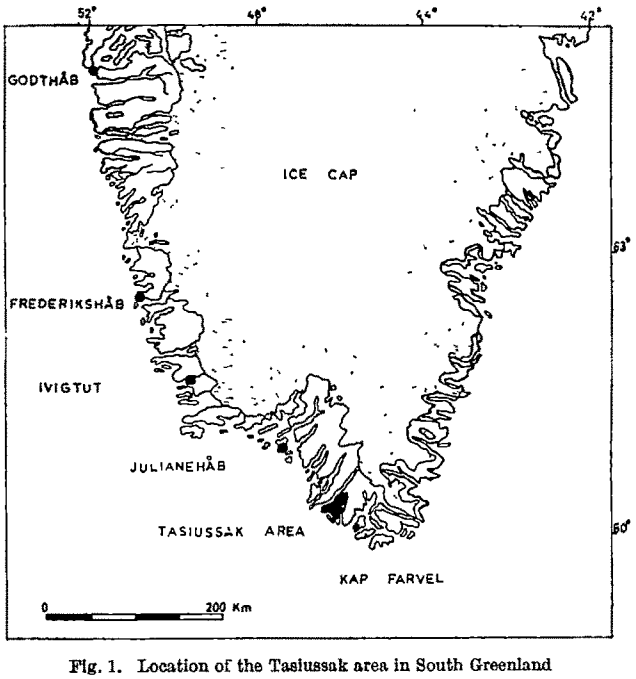


Fig. 1. Location of the Tasiussak area in South Greenland

granites, however, do not display prominent mantling of potash felspar crystals, and there is a transition from rapakivi granites in which the majority of the potash felspar megacrysts are mantled (for example, most of the Finnish rapakivis), to those in which only a few felspar individuals have an oligoclase shell (for example, the Swedish rapakivis). Terzaghi[4] restricts the use of the term rapakivi to granites characterized by mantled felspars, while von Eckermann[5] and Savolahti[6] do not regard this to be an essential feature and point to the importance of two generations of quartz, potash felspar and plagioclase.

The Tasiussak rapakivi covers an area of about 120 km. It post-dates the formation of the Ketilidian granulite facies gneiss and granites and the early Sanerutian granite with its associated aplites and pegmatites[7]. It has sharp, discordant contacts to the country rocks and is ethmolithic. The rock is a biotite–hornblende granite with felspars which vary from mesostasis size up to megacrysts 17 cm long. The megacrysts vary in shape from ovoid to euhedral, and they commonly display a mantling of oligoclase-albite. Concentric rings of oligoclase-albite are occasionally present in both ovoid and well-shaped megacrysts. In places the abundance of megacrysts is so high that the crystals have coalesced to form a continuous network with the groundmass of the granite occupying interstitial spaces. Such a fabric approaches the equilibrium texture of Voll[8]. The microcline of the megacrysts is perthitic, the string and patch perthite of Andersen[9] being the most common form. An earlier generation of potash felspar occurs in the groundmass, but this is in smaller grains and is non-perthitic. Early forming albite occurs in the groundmass and quartz forms at least two generations, first in the groundmass and later genetically connected with myrmekite in association with the microcline megacrysts.

The granite lacks an overall foliation or lineation, but it has widespread occurrences of oligomict and polymict inclusion-breccias composed of the principal rock types of the country rocks. These are quartz-felspathic gneiss, pelitic gneisses and schists, pyriclasites, pegmatites, quartzites (including psephites) and earlier granites. Local basin-shaped layering occurs. The breccias are indicative of strong movement since in the polymict types inclusions of different compositions have been aligned and drawn out. The inclusions, commonly elongated or spindle-shaped, are aligned with their long axes parallel in the form interpreted by Balk[10] as showing that they were "rotated by the magma current until they reached the position of kinematic equilibrium". Rotation of enclaves in the oligomict breccias is further indication of magmatic conditions. The local basin-shaped layering indicates settling under gravity of mafic minerals in peaceful conditions. Similar layering has been described by Harry and Emeleus[11] in other granite massifs in South Greenland.

From the structure it is concluded that the granite came into existence by an intrusion of magma into older Ketilidian gneisses and granites producing the typical sharp, discordant contacts to the country rocks. During this mobile stage the inclusion-breccias were formed when little crystallization had taken place and this was followed by quiet conditions permitting mafic layering and finally the main crystallization of the granite.

A dolerite-gabbro-norite suite of rocks was intruded as dykes and small bodies into the granite after the formation of the inclusion-breccias and the initial mobility. These were net-veined by acid material before their complete consolidation, and they also underwent later granitization. A critical fact is that these basic rocks pre-date the formation of the felspar megacrysts of the granite—a felspathization which resulted in the contacts between the basic rocks and the granite and the basic rocks and the net-veins being locally obscured. Felspar megacrysts are present in the basic rocks either singly or in clouds in a situation which excludes all possibility of magmatic formation either by direct crystallization from a magma or by diffusion of alkalis from the magma, since the crystallization of the granite was complete before the basic rocks were intruded.

'Rapakivi texture' has from the early days of granite research drawn the attention of many workers, and Sederholm[3,12], Holmquist[13], Vogt[14], Harker[15], Wahl[16], Popoff[17], Hackman[18], von Eckermann[5], Terzaghi[4], and recently Gates[19], Savolahti[3,6], Stewart[20], Tuttle and Bowen[21] and Marmo[22] have all concluded that the texture is directly bound up with the crystallization of magma. Experiments by Tuttle and Bowen[21] have confirmed the earlier work of Gates[19] on the crystallization of 'rapakivi magma', and they conclude that, since the rapakivi granites are richer in potash than the average granite, their composition lies in the alkali felspar field, and thus alkali felspar crystallizes before plagioclase. This sequence of felspar crystallization has been grasped as the significant feature in the genesis of rapakivi granites, and ideas from it have been adopted to explain the texture. Typical of these is the comment of Savolahti[6]: "As a consequence of the inverse crystallization of the rapakivi magma, the rapakivi structure and texture and the formation of the potash feldspar ovoids enveloped in a plagioclase shell are self-evident". While the possibility of mantling resulting from magmatic crystallization is not disputed, it is evident from the Tasiussak rapakivi, which is strikingly similar to the celebrated rapakivis of Fennoscandia, that this is a post-magmatic phenomenon. How do the theories of Stewart[20], Tuttle and Bowen[21], or Savolahti[6] explain the potash felspar–plagioclase relationship in a non-magmatic environment?

'Rapakivi texture' occurs in both the felspars of the granite and in the basic rocks and inclusions in the granite. In all these milieu the relationship of the potash felspar to the plagioclase is identical. The albite-oligoclase of the mantles and of the concentric rings in the megacrysts is in optical continuity with the plagioclase of the perthite, and there is evidence to indicate that the plagioclase of the mantles has locally replaced the microcline. It is considered that the formation of the plagioclase of the perthite and that of the 'rapakivi texture' has taken place by the same process; that is, exsolution during an overall post-magmatic potash metasomatism (see Eskola[23]).

It has been demonstrated by Tuttle and Bowen[21] that the alkali felspars form a complete solid solution series above 660° C and at lower temperatures homogeneous synthetic felspars unmix to give perthite. Spencer[24] has shown that up to 30 per cent of soda can be accommodated into solid solution within the potash felspar at a temperature of 700° C, and it is also known from the work of Tuttle and Bowen[21] that the unmixing and mixing of alkali felspar are readily reversible and dependent on water vapour and composition of the felspar. It is postulated that due to the fluctuating temperature in the late stages of the rapakivi genesis during potash metasomatism a number of exsolutions occurred which produced the perthite and the 'rapakivi texture'. The concentric rings of plagioclase represent retarded exsolutions, and the mantles the final exsolution to attain equilibrium.

Many theories have been advanced to account for the rounded shape of the felspar megacrysts of rapakivi, including impurity of the felspar[3], liquation[13], resorption[20], corrosion[14], and viscosity of the magma[12]. Savolahti[3] relies on four processes: slowness of diffusion, the slow and balanced crystallization, corrosion and automorphic processes. The megacrysts of the Tasiussak rapakivi have grown in the solid state, and the theories of magmatic origin are thus untenable. The observation of Sederholm[12] that only those crystals which are ovoid display 'rapakivi texture' is not universal, and in the Tasiussak rapakivi mantling occurs in both euhedral and non-euhedral megacrysts. The relationship between mantling and shape is thus not genetic. The presence of concentric rings of plagioclase in some megacrysts indicates that the two extremes of crystal shape persisted throughout growth.

It seems that the shape of the megacrysts was controlled by the immediate environment, and in places there has been coalescence to form a network. Buerger[25] discusses the problem of crystal form, and draws inferences from the crystallization of metals, suggesting that crystals without faces are quite normal under certain conditions. The presence of crystal faces in effect depends on the surface energy of the growing crystal and that of the milieu, and if a crystal develops in a medium the surface tension of which is equal to its own it is impossible for crystal faces to develop. Similar explanations have been used by Harker and Parker[26] for the polygonal shape of crystals. In the Tasiussak rapakivi, there is a range in megacryst shape from rounded to polygonal and euhedral, and it is suggested that conditions of equal surface tension between the environment and the growing crystal enable ovoidal crystals to form. This condition is realized in cases where felspar growth has been profuse and the fabric approaches the equilibrium texture of Voll[8].

The development of the Tasiussak granite can thus be divided into two stages: the magmatic stage giving rise to its form with discordant and sharp contacts, inclusion-breccias and mafic layering; and the non-magmatic stage producing the observed granite texture. The distinction between the magmatic and non-magmatic effects is clear and recognizable. The striking similarities between the Tasiussak rapakivi and the other rapakivis of the world, in particular the celebrated Fennoscandian and North American examples, suggest that this mode of origin operated generally but the stages have not been recognized because of the absence of comparable basic rocks. Basic rocks occur in association with other rapakivis of the world, but their relation to the granite is not the same as in the Tasiussak area. Sederholm[27] recognized basic intrusions both before the youngest and after the oldest rapakivi granite in some of the Finnish massifs, and Savolahti[6] has demonstrated from the Ahvenisto massif that a suite of olivine diabases was emplaced after the formation of the biotite rapakivi but before the intrusion of the hornblende rapakivi; but the formation of the first was complete before the basic rock intrusion. In contrast, the basic rocks of the Tasiussak granite were emplaced during rapakivi genesis.

The final comment might be reserved for Spencer[28], who states: "The occurrence of potash–soda porphyroblasts in xenoliths and similar metamorphosed rocks, under conditions which preclude an origin by direct crystallization from a magma, raises the question as to how far it is justifiable to regard holocrystalline rocks, even of typical granitic appearance, as products of direct magmatic crystallization". The Tasiussak rapakivi with the occurrence of basic rocks enables the magmatic effects to be distinguished from the later non-magmatic. The Fennoscandian and North American rapakivis without similar basic rocks, but with felspar megacrysts in xenoliths and the country rocks, have hitherto been regarded as products of direct crystallization from a magma.

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ODOUR AND MOLECULAR VIBRATION

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A MOLECULE is an assembly of mass points and elastic forces, and when energy is put into it, it vibrates in various more or less complicated ways. The complex oscillations can, however, be analysed into a finite number of vibratory motions which are mutually independent (in the absence of substantial anharmonicities) and which constitute the 'normal modes' of the molecule. Each has a specific period and frequency which can be deduced from its spectra—at least in simple cases.

Until now, all attempts to show that vibrations are osmically specific have used either the observed spectroscopic frequencies or the more refined normal modes deduced from them, and these attempts have been quite unsuccessful. Table 1, for example, shows the normal

Table 1. NORMAL MODES
100–500 cm^{-1}

C_6H_5-CN	C_6H_5-NO_2	C_6H_5-CHO
462	420	450
401	397	437
381	255	402
172		237
162	176	226
	139	130

modes for three substances with nearly the same almond-like odour, and quite evidently there is no substantial correlation.

This may be because the vibrational hypothesis of odour causation is wrong, or it may be because something important has been overlooked. That something appears to be the possibility that two normal modes can be excited in the same molecule at the same time. Thus, if a molecule has, for example, normal modes at 150, 200 and 400 cm^{-1}, each of these modes can be activated by collision at room temperature. Each of the modes can be populated. But—and this is the factor that has been overlooked until now—more molecules will be populated simultaneously in the 150 and 200 cm^{-1} modes than in the 400 cm^{-1} mode. Furthermore, when two normal modes are excited in the same molecule, the result must be a new frequency which is the difference between the frequencies of the two normal modes.

In terms of osmics, what is concerned here is the frequency of the vibrations that are actually occurring, that is, the populated energy-levels, and so the difference frequencies as well as the normal mode frequencies must

be taken into account—the basic requirement being that they must be populated at ordinary temperatures.

Benzonitrile is a substance with bond-defined molecules and known normal-mode frequencies—and it has a distinctive bitter-almond smell. Its possible 'populated frequencies' between 100 and 500 cm^{-1} are shown in Table 2. If the vibrational hypothesis is correct, some, but not necessarily all, of these numbers must hold the key. To identify the osmically active frequencies, we collate them with similar values for nitrobenzene and benzaldehyde, which have rather similar odours.

The frequencies that are common to all three fall near 175, 225 and 400 cm^{-1} and the agreement is within about 1 per cent, which is quite close. There are, however, many additional frequencies which conform to no pattern. Let us call them 'osmically inactive'. The first thing to note is that in benzonitrile, for example, if the 462 cm^{-1} frequency is called osmically inactive, all the difference terms that depend on it can be eliminated without destroying the pattern; and the same is true for the other 'inactive' modes.

Table 2. Normal Modes and Populated Combinations 100–500 cm^{-1}

C_6H_5-CN	C_6H_5-NO_2	C_6H_5-CHO
		450
462	420	437
401	397	402
381	281 (420–139)	320 (450–130)
300 (462–162)	258 (397–139)	307 (437–130)
290 (462–172)	255	272 (402–130)
239 (401–162)	244 (420–176)	237
229 (401–172)	221 (397–176)	226
219 (381–162)		
209 (381–172)		
172	176	176 (402–226)
162	139	165 (402–237)
	116 (255–139)	130
		107 (237–130)

Table 3

Mode	Assignment	Osmic quality
C_6H_5-CN		
462	Ring deformation, in-plane	Inactive
401	,, ,, out-of-plane	Active
381	,, ,, in-plane	Inactive
172	,, ,, out-of-plane	Active
162	Side-chain, flexing	Inactive
C_6H_5-NO_2		
420	Side-chain, out-of-plane bending	Inactive ?
397	Ring deformation, out-of-plane	Active
255	,, ,, in-plane	Inactive
176	,, ,, out-of-plane	Active
139	Side-chain, torsion	Inactive
C_6H_5-CHO		
450	Ring deformation, out-of-plane	Inactive ?
437	,, ,, in-plane	Inactive
402	,, ,, out-of-plane	Active
237	,, ,, in-plane	Inactive
226	,, ,, out-of-plane	Active
130	Side-chain, torsion	Inactive

Table 3 shows the assignments that various authorities have made to the 'active' and 'inactive' normal modes, and an interesting regularity is immediately apparent. All the osmically active frequencies are out-of-plane deformations of the benzene ring which is the main part of the molecule. Almost all the inactive modes are either in-plane ring deformations or localized oscillations of the side-chain. This does not apply to the 450 cm^{-1} mode of benzaldehyde, and it is not quite clear how far it is true for the 420 cm^{-1} mode of nitrobenzene, so that their inactivity is open to some question. This does not destroy the basic pattern and, in fact, the odours of nitrobenzene and of benzaldehyde are not exactly identical.

There is nothing intrinsically improbable in this apparent association of osmic activity with out-of-plane oscillations of these molecules. Benzene rings with these side-chains might be expected to lie flat when adsorbed on the receptor surface, and from this it is, perhaps, possible to deduce three criteria of osmically active vibrations: (1) they must have low frequency; (2) they must involve all or most of the molecule; (3) they must have substantial components perpendicular to the receptor surface.

Information theory suggests that the whole gamut of smells is derived from about twenty-five primary odours. If the almond odour is due basically to these three frequencies it is a three-primary odour. We can tentatively estimate the band-widths by dividing the 100–500 cm^{-1} region logarithmically into this number of non-overlapping intervals, in which case the frequency-specification of the almond odour is the simultaneous presence of osmically active vibrations falling roughly in the ranges 170–182, 218–232 and 380–405 cm^{-1}. Other odours would presumably correlate with other combinations of frequencies, but this is still to be demonstrated.

The odours of acetophenone and of phenyl isothiocyanate include an almond 'note', together with other 'notes' sufficient to modify, but not wholly disguise, the almond character. Table 4 shows their populated vibrational levels in the 100–500 cm^{-1} range. When the in-plane and the localized oscillations are taken out, using vibrational assignments from the literature, Table 5 shows the result.

Table 4. Normal Modes and Populated Combinations 100–500 cm^{-1}

C_6H_5-NCS	C_6H_5-$COCH_3$
491	464
437	406
396	366
355	301 (464–163)
257 (437–180)	243 (406–163)
245	225
216 (396–180)	203 (366–163)
180	181 (406–225)
175 (355–180)	163
151 (396–245)	141 (366–225)
110 (355–245)	

Table 5. Out-of-Plane Frequencies

C_6H_5-NCS	C_6H_5-$COCH_3$	
	LeBas[1]	Delorme[2]
491	464	464
437	406	406
396	301 (464–163)	366
311 (491–180)	243 (406–163)	301 (464–163)
257 (437–180)	225	243 (406–163)
216 (396–180)	181 (406–225)	203 (366–163)
180	163	163

The three almond-pattern frequencies are clearly present in phenyl isothiocyanate together with enough others to account for the other notes in its odour. With acetophenone the authorities differ with regard to certain of the assignments of in-plane and out-of-plane motions. The olfactory evidence, for what it is worth, seems to favour the LeBas assignments.

Table 6. Out-of-Plane Frequencies

C_6H_5F	C_6H_5Cl	C_6H_5Br	C_6H_5I	$C_6H_5CH_3$	$C_6H_5C_2H_5$	$C_6H_5OCH_3$
496	467	457	447	465	404	414
407	392	404	394	405	246*	390
240	197	277*	277*	217	158	209
167*	195	224*	224*	188*		205*
		180	170			181

* Difference frequency.

Table 6 shows the populated, whole-molecule, out-of-plane modes of seven substances the odours of which were presumed not to have a bitter-almond note. The three almond frequencies are, however, prominent in bromo- and iodo-benzene. A number of professional perfumers were asked, therefore, for expert opinions on toluene and on chloro-, bromo- and iodo-benzene with particular reference to any 'nitrobenzene-like' note. They were unanimous that toluene does not have it, but were divided as to the halo-benzenes. Some, but not all, recognized a coarse 'chemical-almond' or 'nitrobenzene-like' note in all three, and especially in iodobenzene. There are not enough frequencies outside the almond pattern to explain why this note should be so heavily overlaid, unless the halo-benzenes lie at an angle to the receptor surface, so that some of their in-plane oscillations (not shown in Table 6) might contribute to the pattern.

Orientation to the receptor probably explains the lack of pronounced odour (apart from trigeminal stimulation)

in phenol and aniline, for these compounds would probably be chemi-sorbed through their reactive substituent groups. Attachment at a point should allow for less vibrational interaction than attachment over an area.

This has been an attempt to find a precise answer to a very precise series of questions which may be summarized thus: (1) What property is common to C_6H_5-CN, C_6H_5-NO_2, and C_6H_5-CHO in addition to their rather similar odours? (2) Is this property found also in C_6H_5-$COCH_3$, C_6H_5-NCS and C_6H_5-I, the odours of which seem to have something in common with the first three? (3) Is this property absent from C_6H_5-CH_3, C_6H_5-OCH_3 and C_6H_5-NH_2, the odours of which are quite different from those of the first three?

In trying to answer these questions, the postulates of the vibrational theory of odour have been spelled out more precisely than ever before, and this can be expected to assist materially in stimulating further tests of the theory.

If difference frequencies do not appear in the spectra, it is not because they have no real existence, but because there is not sufficient anharmonicity to couple the two modes. Without such coupling, radiative transitions are not likely to involve both modes simultaneously. Deformations that accompany collisional excitation make multiple excitation both possible and probable, though subject to limitations from the energy available to excite them. The simultaneous excitation of two normal modes necessarily results in a complex oscillation with a new (difference) frequency. The fact that it may be osmically active without being spectroscopically active suggests that olfactory stimulation does not depend on dipole-dipole coupling. A near-synchronous 'throbbing' of the odorous molecule and the receptor site might, however, permit unusually close approach and allow greater-than-normal electronic (Van der Waals) interactions to establish themselves.

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MOLECULAR DISTRIBUTIONS OF NORMAL FATTY ACIDS AND PARAFFINS IN SOME LOWER CRETACEOUS SEDIMENTS

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SATURATED, straight-chain fatty acids (hereafter called normal fatty acids) and normal paraffin hydrocarbons are common organic constituents of Mowry and Thermopolis shales from the Powder River Basin of Wyoming. This article directs attention to a variety of molecular distributions of normal fatty acids and normal paraffin hydrocarbons observed in a few samples from the shales. The observations provide new evidence for possible relationships between acids and paraffins in sedimentary rocks.

Mowry Shale and Thermopolis Shale are two major shale units in the Lower Cretaceous section of the Powder River Basin. Mowry Shale is dark grey, generally siliceous, and is characterized by the presence of abundant fish scales and thin beds of bentonite. On weathering, Mowry Shale becomes medium-light grey and brittle. The formation ranges in thickness in the basin from 150 to almost 500 ft. Mowry Shale can be usually recognized in the field as a prominent, pine-covered hogback. Thermopolis Shale is soft and greyish black. At the surface, this unit forms grass-covered valleys between sandstone ridges. Exposures are generally poor. The thickness of the Thermopolis Shale varies from 125 to 250 ft. A sandstone is often present between Mowry Shale and underlying Thermopolis Shale.

Four samples of shale will be considered. Two samples of Mowry Shale were obtained from outcrops near Upton and Douglas. Two samples of Thermopolis Shale came from outcrops near Casper and Kaycee. Fig. 1 shows sample locations. Possible effects of surface weathering were kept to a minimum by collecting the samples from pits dug back from the exposed surface. Upton and Casper samples came from road cuts, and Douglas and Kaycee samples came from good, natural exposures.

The presence of acids and paraffins in sedimentary rocks has been noted by previous investigators. Normal fatty acids in rocks have been reported by Cooper[1], Abelson and Parker[2], and Cooper and Bray[3]. Normal paraffins in rocks have been described by Bray and Evans[4,5]. Lawlor and Robinson[6] described both normal fatty acids and normal paraffins in a sample of oil shale from the Green River Formation. These investigators have shown that in sedimentary rocks even-carbon-numbered normal fatty acids dominate odd-carbon-numbered normal fatty acids while normal paraffins usually show a preference for odd-carbon-numbered molecules or nearly equal distributions of odd- and even-carbon-numbered molecules. This report shows not only acid and paraffin distributions similar to distributions noted previously in sedimentary rocks, but also distributions of normal fatty acids with almost equal abundances of even- and odd-carbon-numbered molecules.

Normal fatty acids as well as normal paraffin hydrocarbons containing from about 12 to about 34 carbon atoms per molecule were found in Mowry and Thermopolis shales. Distributions of acids and paraffins from four samples are shown in Fig. 2 where the percentage of each acid (solid line) based on total acids recovered and the percentage of each paraffin (dotted line) based on total paraffins recovered is plotted against the appropriate carbon number. Carbon numbers for acids are shown at the top of the illustrations, and carbon numbers for

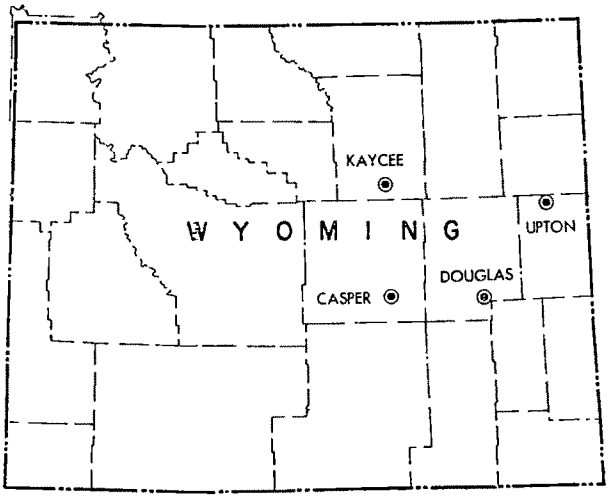


Fig. 1. Location map showing four localities

paraffins are shown at the bottom. Carbon numbers for an acid and a paraffin on the same vertical line are different by one: the acid carbon number is always one larger than the paraffin carbon number.

Relative molecular distributions of acids and paraffins can be characterized by distribution indices. A measure of relative distributions of normal paraffins was defined by Bray and Evans[4] as the mean of two ratios which are determined by dividing the sum of concentrations of odd-carbon-numbered normal paraffins by the sum of even-carbon-numbered normal paraffins over given carbon-number ranges. This measure of the relative abundance of odd- and even-carbon-numbered normal paraffins was called a carbon preference index (CPI)[3]. The relative distribution of normal fatty acids can also be described by a carbon preference index which is defined here as the mean of two ratios which are determined by dividing the sum of concentrations of even-carbon-numbered normal fatty acids by the sum of odd-carbon-numbered normal fatty acids over given carbon-number ranges. To distinguish between distributions of paraffins and acids, CPI_P will represent the carbon preference index for normal paraffins, and CPI_A will represent the carbon preference index for normal fatty acids. Fig. 3 shows formulae for calculating CPI_P and CPI_A. A CPI_P greater than one indicates that odd-carbon-numbered paraffins are more abundant than even-carbon-numbered paraffins. On the other hand, a CPI_A greater than one indicates that even-carbon-numbered acids are more abundant than odd-carbon-numbered acids. CPI_P and CPI_A values equal to one indicate that paraffins or acids have equal abundances of even- and odd-carbon-numbered molecules. Limits used in computing CPI_P and CPI_A are arbitrary. In this study fractionation of both paraffin and acid molecules containing less than about 16 carbon atoms is severe due to the analytical method used. Therefore, the lower limit for computation of CPI_P is C_{16} and of CPI_A is C_{17}. Upper limits for computation are C_{32} for paraffins and C_{33} for acids. Concentrations of molecules having more carbon atoms are low and approach the limits of detection of the analytical method.

$$CPI_P = 1/2\left(\frac{\Sigma \text{ odd-carbon paraffins from } C_{17} \text{ to } C_{31}}{\Sigma \text{ even-carbon paraffins from } C_{16} \text{ to } C_{30}} + \frac{\Sigma \text{ odd-carbon paraffins from } C_{17} \text{ to } C_{31}}{\Sigma \text{ even-carbon paraffins from } C_{18} \text{ to } C_{32}}\right)$$

$$CPI_A = 1/2\left(\frac{\Sigma \text{ even-carbon acids from } C_{18} \text{ to } C_{32}}{\Sigma \text{ odd-carbon acids from } C_{17} \text{ to } C_{31}} + \frac{\Sigma \text{ even-carbon acids from } C_{18} \text{ to } C_{32}}{\Sigma \text{ odd-carbon acids from } C_{19} \text{ to } C_{33}}\right)$$

Fig. 3. Formulae for calculating the carbon preference index for normal paraffin hydrocarbons and for normal fatty acids

Fig. 2 (upper left) shows the distributions of acids and paraffins recovered from a sample of Mowry Shale from near Upton. CPI_A is 2·49 and CPI_P is 1·63. Acids show an even-carbon preference, and paraffins show an odd-carbon preference. Another type of distribution of acids and paraffins from Mowry Shale is shown in Fig. 2 (lower left) for a sample from south of Douglas. Both CPI_A and CPI_P are near 1·00 (1·05 and 1·10). Little preference for either even-carbon-numbered acids or odd-carbon-numbered paraffins is observed. A sample of Thermopolis Shale from south-west of Casper, shown in Fig. 2 (upper

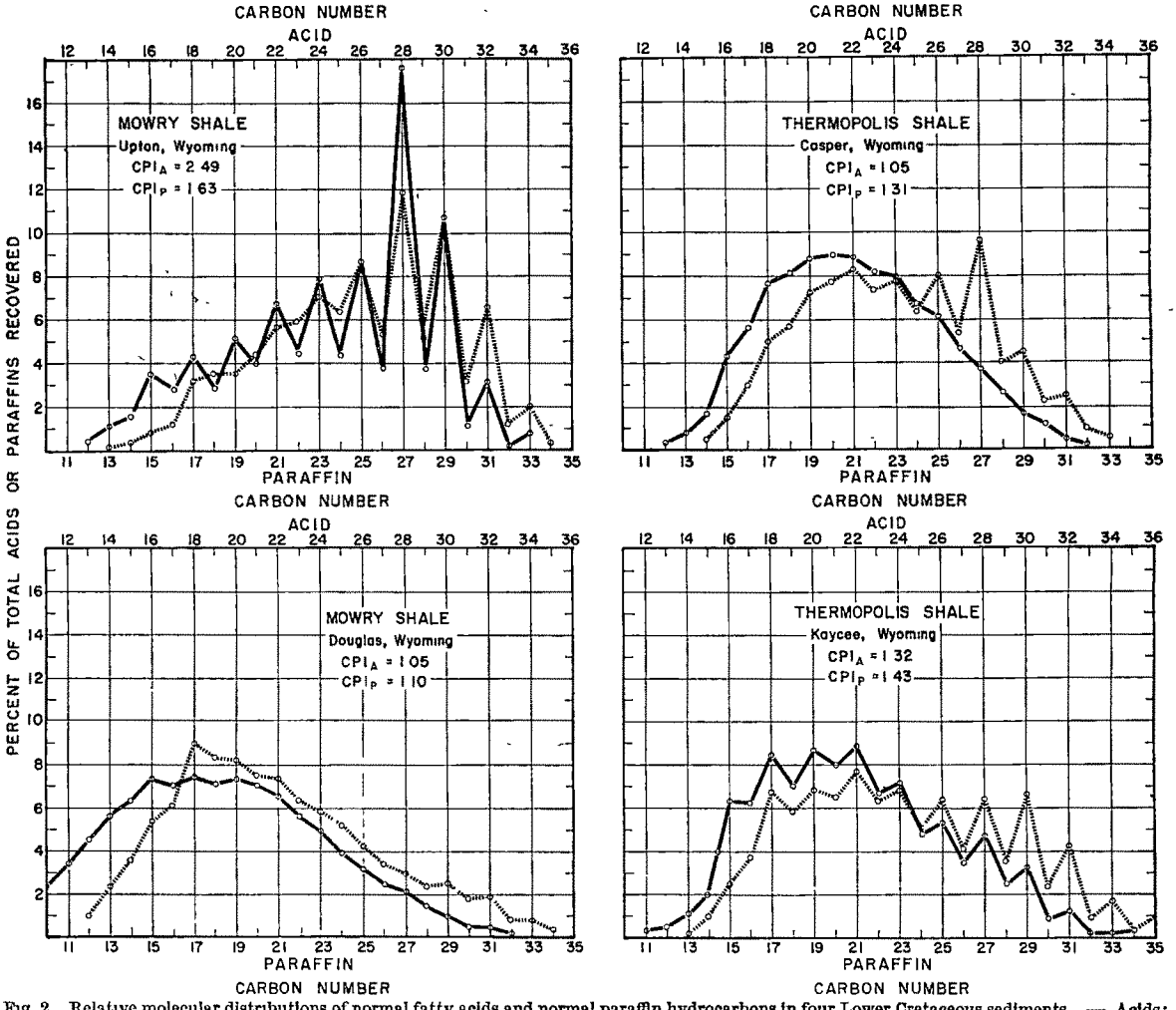


Fig 2. Relative molecular distributions of normal fatty acids and normal paraffin hydrocarbons in four Lower Cretaceous sediments. —, Acids; . . . , paraffins

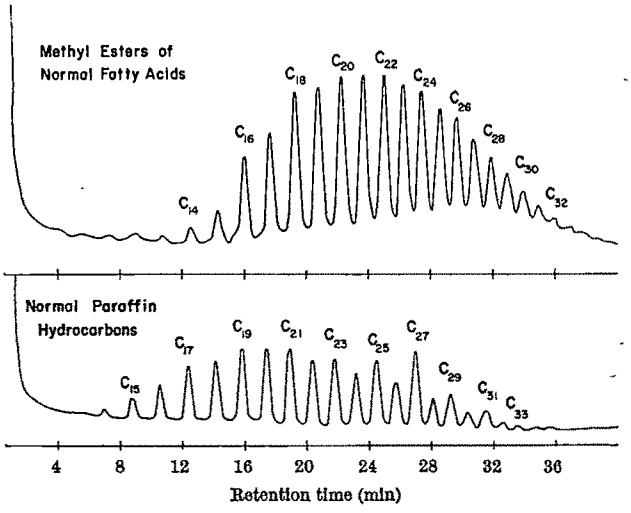


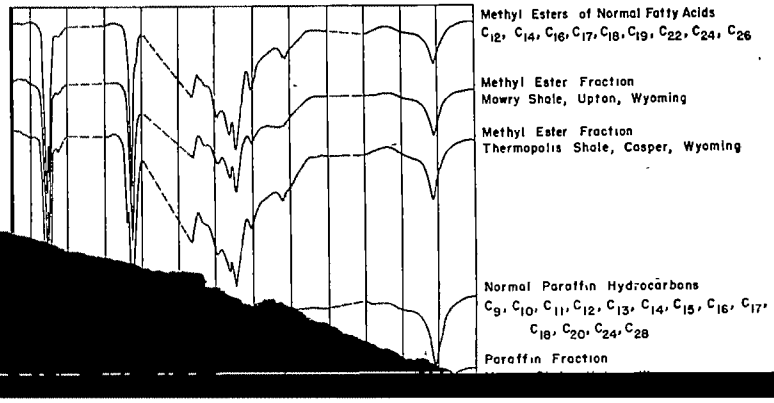
Fig. 4. Gas chromatograms of methyl esters of normal fatty acids and normal paraffin hydrocarbons in a sample of Thermopolis Shale from near Casper, Wyoming. F and M Model 500 gas chromatograph, 24 in. by 0·25 in. column packed with 0·5 per cent silicone grease (*DC*–11) on 60–80 mesh glass beads; temperature programmed from 75° C to 300° C at 5·6° C/min

right), contains acids equally distributed between molecules having even and odd numbers of carbon atoms (CPI_A is 1·05). Paraffins, however, have a definite preference for odd-carbon-numbered molecules (CPI_P is 1·31). Acids shown in Fig. 2 (lower right) from Thermopolis Shale near Kaycee show a distinct even-carbon preference (CPI_A is 1·32), and at the same time paraffins show a definite odd-carbon preference (CPI_P is 1·43). For any given sample the general trends of the molecular weight distributions are remarkably similar.

The analytical procedure used to isolate normal fatty acids and normal paraffin hydrocarbons is a modification of one used by Cooper[3] for isolation of fatty acids. Acids and paraffins were obtained from dried, crushed sediments by extraction with a solution of potassium hydroxide in methanol. Free acids, salts of acids, and acids combined as esters were probably recovered by this method. Some of the acids may have been derived from complex polymeric materials commonly called 'kerogen'. Acids and paraffins were extracted from the saponification mixture after acidification. Acids were converted to methyl esters. Normal fatty acid methyl esters and normal paraffins were separated from other lipid material by urea adduction. Acids were separated from paraffins by silica-gel chromatography. Identification of normal fatty acids as methyl esters and nor… raffin hydrocarbons was based o… procedure, infra-red and… and gas chromatogr… centrations of a… calculated fr… tograms… sh…

Infra-red spectrometry established the presence of methyl esters of fatty acids and normal paraffins in fractions obtained from silica-gel chromatography. Fig. 5 shows infra-red spectra of fractions from the Mowry Shale sample collected near Upton and the Thermopolis Shale sample collected near Casper. These spectra are compared with spectra obtained from standard solutions, one containing a mixture of methyl esters of normal fatty acids and the other containing a mixture of normal paraffin hydrocarbons. Spectra of ester fractions from the shales are very similar to the spectrum of the solution containing a known mixture of methyl esters of normal fatty acids as seen at the top of Fig. 5. At the lower part of Fig. 5 spectra of paraffin fractions from the shales compare favourably with the spectrum obtained from the known mixture of normal paraffins. Spectra of methyl ester and paraffin fractions show strong absorption bands in the 3·4–3·5μ region, indicating the presence of methyl and methylene groups, and a band at 13·9μ, indicating methylene chains with four or more carbon atoms. Spectra of methyl ester fractions have an ester carbonyl adsorption band at 5·75μ and a triplet pattern with adsorptions at about 8·0, 8·3 and 8·5μ which are characteristic of methyl esters of long-chain fatty acids[7].

Each mass spectral analysis of chromatographic fractions containing methyl esters of normal fatty acids as determined by gas chromatography and infra-red spectrometry showed a base peak at *m/e* 74. This peak is the base peak for methyl esters of higher normal fatty acids[8]. Parent peaks were present at *m/e* values equal to those of methyl esters of normal fatty acids (. . . 326, 340, 354, 368, 382 . . .). Mass spectral and gas chromatographic data for methyl esters of normal fatty acids in the Mowry Shale sample from near Upton and the Thermopolis Shale sample from near Casper are shown in Fig. 6. Parent-peak heights from mass spectra are compared with peak heights from gas chromatograms. Peaks at C_{18} and C_{26} are enhanced because of the presence of internal standards. Comparison of mass spectral and gas chromatographic data for methyl esters of normal fatty acids in these samples shows a parallelism between ester distributions given by parent–mass peaks and gas chromatographic peak heights. Mass spectral data confirm the presence and distribution of normal fatty acid esters as established by gas chromatography.



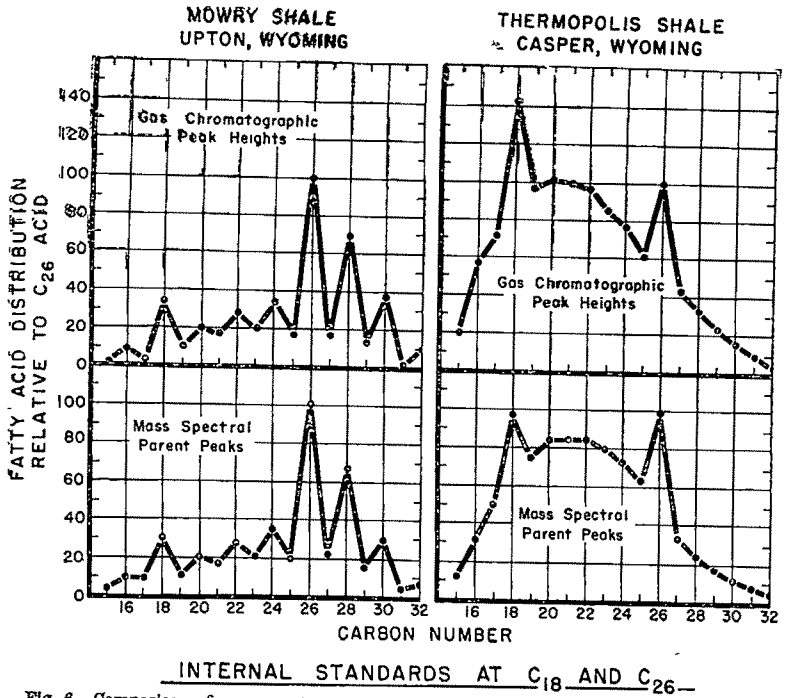


Fig. 6. Comparison of mass spectral and gas chromatographic analyses of methyl esters of normal fatty acids in Mowry and Thermopolis Shales

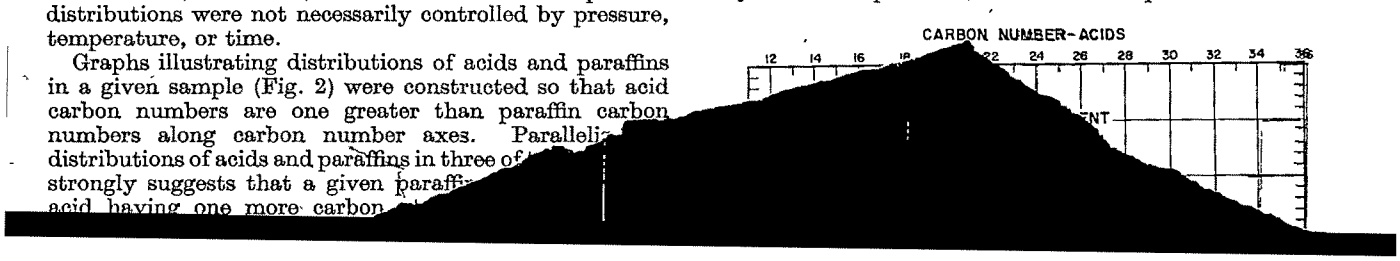
One result of this investigation is the observation that distributions of normal fatty acids as well as normal paraffin hydrocarbons can be different in similar lithologies of the same geological unit. For example, one Mowry sample has a CPI_A of 2·49 while the other has a CPI_A of 1·05. Respective CPI_Ps are 1·63 and 1·10. Thermopolis samples also have contrasting CPI_A values of 1·32 and 1·05. Distributions of paraffins in the Thermopolis samples differ little with CPI_Ps of 1·43 and 1·31. Different CPI_As and CPI_Ps in the same rock units suggest that factors controlling the distributions of acids and paraffins are not necessarily reflected in the gross lithology. Original distributions of acids and paraffins may have been different at various places in time intervals represented by a given lithology, or environments for chemical changes may have been more favourable or less favourable at various localities. All four samples experienced about the same pressures and temperatures for approximately the same time, because these samples are from outcrops of about the same geological age on the rim of the Powder River Basin; therefore, differences in acid and paraffin distributions were not necessarily controlled by pressure, temperature, or time.

Graphs illustrating distributions of acids and paraffins in a given sample (Fig. 2) were constructed so that acid carbon numbers are one greater than paraffin carbon numbers along carbon number axes. Parallel distributions of acids and paraffins in three of strongly suggests that a given paraffi acid having one more carbon

likely to be attacked. Furthermore, the rate of oxidation of any oxidation product would probably exceed the rate of oxidation of the paraffin.

Distributions of normal fatty acids and normal paraffins in these Lower Cretaceous sediments contrast greatly with distributions of acids and paraffins in a modern sediment. Fig. 7 shows normal fatty acids and normal paraffins in a modern sediment from San Nicolas Basin off the coast of southern California. CPI_A is 7·06 and CPI_P is 4·85. Large CPI_As and CPI_P values can be expected in most modern sediments because of the ubiquity of even-carbon-numbered normal fatty acids and odd-carbon-numbered normal paraffins in biological systems. Not only are carbon preference index values for acids and paraffins much larger in the modern sediment than in the ancient sediments, but also general trends of the molecular weight distributions of acids and paraffins in the modern sediment are not similar as they are in the ancient sediments. If, in the modern sediment, normal paraffins were derived directly from normal fatty acids, the relative percentage of C_{15}-paraffin especially and also C_{25}- and C_{23}-paraffins should have been larger. At the same time the relative percentages of C_{30}-, C_{32}- and C_{34}-acids should have been larger to provide for paraffins at C_{29}, C_{31} and C_{33}. These observations suggest that acids and paraffins in the modern sediment are not related, at least not in the same way as are acids and paraffins in the ancient sediments.

If in Thermopolis or Mowry time original distributions of normal fatty acids and normal paraffins were somewhat similar to distributions found at present in a modern sediment, observed acid–paraffin distributions, especially for molecules larger than C_{20}, in the Lower Cretaceous rocks can generally be explained by schemes involving originally deposited fatty acids and normal paraffins as starting materials. In a scheme proposed by Cooper[1] step-by-step decarboxylation of fatty acids occurs forming alkyl radical intermediates which react to give normal paraffins with one less carbon atom and fatty acids with one less carbon atom than the parent acids. Newly generated fatty acids react in the same manner as original acids. Operation of this scheme on originally deposited fatty acids, under conditions favouring formation of more fatty acids than paraffins, reduces carbon preference index



values for both fatty acids and paraffins. As a consequence of Cooper's scheme absolute concentrations of fatty acids should decrease while normal paraffins formed from acids should accumulate with initially deposited normal paraffins. Detailed mechanisms of chemical reactions which may take place in sediments to account for various acid–paraffin distributions are not known. Free radical as well as other kinds of reactions should be considered. Normal paraffins from other sources such as kerogen are possible, but parallelism of distributions of normal fatty acids and normal paraffins in the Lower Cretaceous sediments seems to indicate that a major source of normal paraffins could be normal fatty acids. The total concentrations of acids and paraffins in the examined samples (Table 1) suggest that in initially deposited sediments sufficient normal fatty acids can be present to provide normal paraffins which can modify the distribution of originally deposited normal paraffin hydrocarbons.

Table 1. Concentrations of Normal Fatty Acids and Normal Paraffin Hydrocarbons Recovered from Sediments

	Normal fatty acids (μmol/kg)	Normal paraffin hydrocarbons (μmol/kg)
Modern sediment, San Nicolas Basin	132·0	5·9
Mowry Shale, Upton, Wyoming	9·9	5·2
Mowry Shale, Douglas, Wyoming	97·7	113
Thermopolis Shale, Casper, Wyoming	22·0	6·9
Thermopolis Shale, Kaycee, Wyoming	27·4	3·7

The various molecular distributions of fatty acids and normal paraffins in the Lower Cretaceous sediments (Fig. 2) can be interpreted in light of the proposed scheme for obtaining paraffins from fatty acids. Fatty acids in the Thermopolis sample from near Casper and the Mowry sample from near Douglas have degraded to such an extent that they have lost most of the even-carbon preference they may have had initially. Paraffins in the Douglas sample have lost most of the odd-carbon preference they may have had originally, but paraffins at the Casper locality still have a distinct odd-carbon preference. At the Casper locality initial sediments may have had an unusually high concentration of odd-carbon-numbered normal paraffins relative to even-carbon-numbered normal fatty acids. Paraffins generated from acids have been unable to swamp-out the odd-carbon preference at the Casper locality. The slight preference for C_{27}, C_{29}, C_{31} and C_{33} normal paraffins in the Douglas sample is probably a reflexion of initially deposited normal paraffins. Fatty acids from the Upton and Kaycee samples appear to be less degraded than fatty acids from the Douglas and Casper samples. The reason for this may be due to higher initial CPI_A values for acids in the sediments from Upton and Kaycee localities or due to more favourable environments for degradation in the sediments from Casper and Douglas localities.

Various distributions of acids and paraffins in sediments may reflect different degrees of maturation in the paraffin-forming process of petroleum generation. Normal paraffins in petroleum usually have almost equal abundances of odd- and even-carbon-numbered molecules[5]. In any petroleum generation scheme final normal paraffins, therefore, must have a CPI_P near 1·0. In this investigation a CPI_P near 5·0 was determined for normal paraffins in a modern sediment. Normal paraffins of the ancient sediments had CPI_P values ranging from 1·63 to 1·10. The final CPI_P value approaches values found for paraffin distributions in crude oils of the Lower Cretaceous section of the Powder River Basin. At the same time, a reduction of CPI_A values is observed going from the modern sediment with a CPI_A near 7·0 to the ancient sediments where values ranged from 2·49 to 1·05. Although no report is known of normal fatty acids in crude oils of the Lower Cretaceous, small concentrations of normal fatty acids (from C_8 to C_{18}) have been found in a California petroleum[9]. These normal fatty acids have only a slight predominance of even-carbon-numbered molecules over odd-carbon-numbered molecules. The paraffin-forming process of petroleum generation, therefore, probably involves maturation in which distributions of normal paraffins approach equal abundances of odd- and even-carbon-numbered molecules as a result of degradations of normal fatty acids which produce distributions of fatty acids with nearly equal abundances of even- and odd-carbon-numbered molecules.

Molecular distributions of normal fatty acids and normal paraffin hydrocarbons in these Lower Cretaceous sediments suggest that many of the normal paraffins now found in these sediments could have come about through transformations of normal fatty acids involving loss of carbon and oxygen atoms. Specific reactions for fatty acid transformations in sediments are not known. It is likely that different degrees of degradation, suggested by the samples, of normal fatty acids to lower-carbon-number normal paraffins and normal fatty acids reflect different initial acid–paraffin distributions and different local chemical environments for degradation. Acid–paraffin distributions in sediments may reflect degrees of maturation in the paraffin-forming process of petroleum generation.

I thank J. E. Cooper, whose original investigations form the foundation of this work; J. T. Wall, who did the laboratory work; and D. F. Cardinal for advice and co-operation in selecting and collecting samples.

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EVIDENCE FO[redacted]FIBRE' ORGANIZATION IN

somes occurs by a tight but irregular folding of the interphase fibres into compact metaphase 'packages'. At least two corollaries are implicit in this hypothesis: (1) metaphase and anaphase chromatids are not expected to contain pairs of half-chromatids; (2) gross coiling, as observed in large chromosomes by light microscopy, is regarded as quaternary structure which is absent in small chromosomes and which, when it occurs, is superimposed on a fundamental, folded fibre structure. This article reports recent comparative observations made with larger chromosomes prepared from human cells by the same techniques previously used to investigate the honeybee chromosomes; these observations, for the most part, confirm the expectations of the folded-fibre model and support its applicability to chromosomes in general.

Two sources were used to obtain human chromosomes: (1) 3-day cultures of peripheral leucocytes in which the blood cells were donated by laboratory personnel and incubated according to the techniques of Moorhead *et al.*[3] (including an important modification by Dr. H. D. Moon[4], which requires only three drops of blood from a finger tip); (2) a commercial line of human liver cells (Microbiological Associates, Albany, California), which were subcultured according to the Chang technique[5] and incubated for 2 days before further treatment. Both blood and liver cells were prepared for the electron microscope in the same way: first, abundant metaphases were obtained by incubating the cells with colchicine for 12–18 h; the cells were then lysed in hypotonic medium (usually distilled water), floated on the clean surface of a trough filled with distilled water, and picked up directly on 'Formvar'-coated grids; after the grids had been dehydrated through an ordinary series of ethanol dilutions, they were passed from absolute alcohol into amyl acetate and finally dried by the critical point method of Anderson[6]. Fixation and staining, as well as sectioning, were omitted in most instances. Electron micrographs were made as a through-focus series with an RCA *EMU-3G* after careful normalization of the lenses.

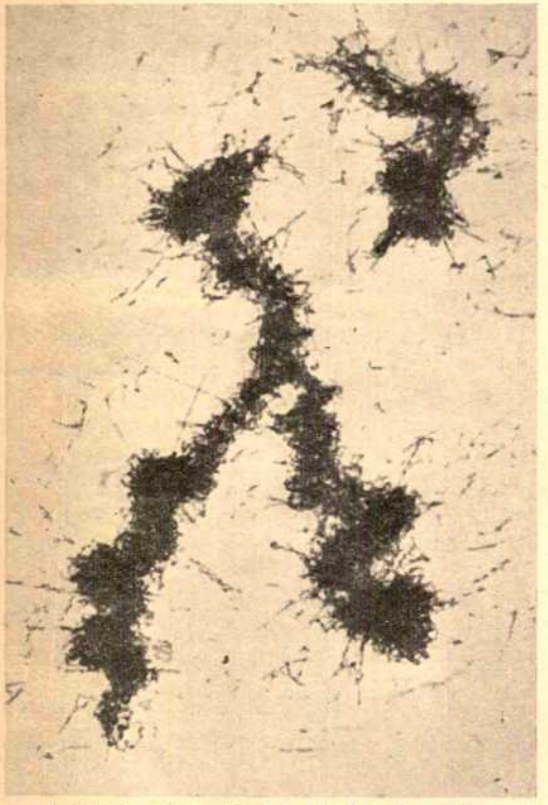


Fig. 1. A metaphase chromatid pair isolated from human liver culture cells. Note the apparent quaternary coiling which is superimposed on a basic, folded-fibre structure. The broken ends of three fibres protrude from the stump of the fractured arm (× *c.* 16,470)

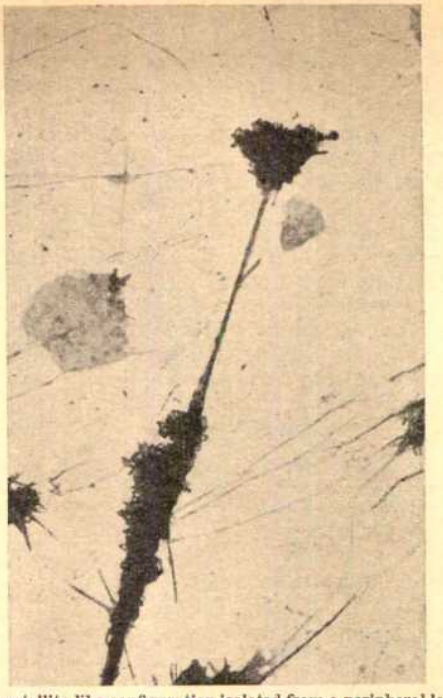


Fig. 3. A satellite-like configuration isolated from a peripheral leucocyte culture. In the connecting strand, three lengths of fibre lie side by side (one of which has broken) (× *c.* 6,990)

Grids prepared in this way usually contain one to three separate groups of metaphase chromosomes, in addition to varying numbers of interphase nuclei. No one group of chromosomes includes the complete karyotype, and often the same group contains some chromosomes which are well preserved, others which are less well preserved, and still others which lie across one another or tangled together in ways that make them unsuitable for investigation. Although the same effects are found in light microscope preparations, the much greater abundance of material there makes it possible to select complete, well-spread plates for karyotype examinations. Unfortunately, the relative scarcity of material with present electron micro- [illegible] has thus far made karyotype analysis [illegible]eless, the fine structure of individual [illegible]ely accessible by present methods. [illegible] that the familiar morphology of [illegible] is preserved by the tech- [illegible]hology (like that of

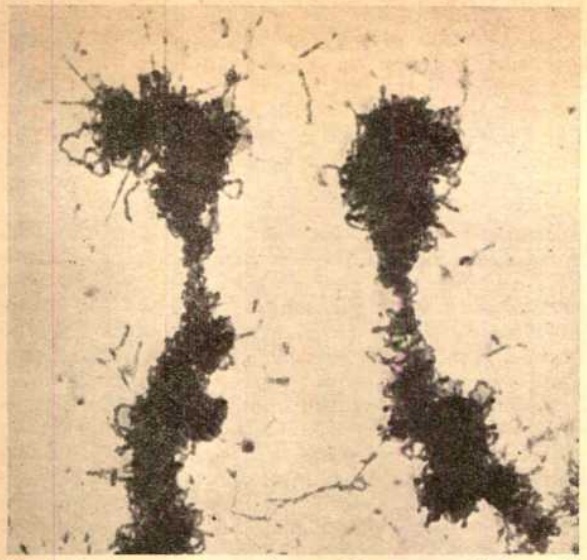


Fig. 4. Centromere regions and arms of two separated chromatids isolated from human liver culture cells. Note that the centromere fibres appear to be continuous with the chromatin fibres of the arms and resemble them in appearance. Some apparent quaternary coiling is also visible in the arms (× *c.* 17,690)

indicates that these vary around 230 Å at interphase and have a somewhat greater dimension (possibly exceeding 300 Å) at metaphase. The fibres have never been observed to form orderly rope-like complexes by winding together in pairs. Furthermore, human chromatids, like those of honeybees, lack any visible, paired half-chromatid structure.

On the other hand, the larger human chromosomes differ notably from honeybee chromosomes in showing clear evidence of quaternary coiling. This is especially conspicuous in Fig. 1, where the uncoiled portions of the chromatid arms (near the centromere) show a folded-fibre structure and diameters in the order of 0·3μ, whereas the coiled regions have apparent diameters exceeding 0·6μ and are very electron dense (an effect which is expected because of their greater thickness). Fig. 2 interprets this structure for a single chromatid, which is considered to have condensed first by an irregular folding of one or several 230 Å fibres, and thereafter by gross left- or right-handed coiling of the rod-form produced in the first phase.

In the intact chromosome the intricacy of three-dimensional folding usually makes it impossible to trace a given fibre for more than a short distance; nevertheless, significant clues regarding the arrangement of fibres within each chromatid have been found. For example, Fig. 3 illustrates a satellite-like configuration (either a natural satellite[7] or an accidentally-stretched chromatid arm), in which two separate, light-resolvable segments of the chromatid are connected by a narrow neck of unfolded fibres. The connecting strand, which would be invisible with the light microscope, appears to contain three parallel fibres (one of which has broken) and its continuous length is more than 4μ. In Fig. 1, three broken fibre ends can also be discerned protruding from the stem of the single broken chromatid arm. In Fig. 4 the diameter of each chromatid at its centromere actually falls below the limit of light resolution, and in this instance also the chromosome arms are seen to be connected by approximately three fibres. It is clear in Figs. 1 and 4, at least, that the centromere regions of human chromosomes are composed of fibres morphologically identical with the fibres of the arms, and that these centromere fibres are continuous with the arm fibres. Since some of the special properties of the centromere as observed in light microscopy (for example, reluctance to stain) may be accounted for merely by the difficulty in resolving the chromatid body at this point, there may be no real difference between centromere fibres and 'true' chromatin fibres.

On the other hand, it has not yet been determined how the centromere is organized to hold together the chromatids at metaphase and then to release them at anaphase. Originally it was suggested that, in a folded-fibre chromosome, the central, unreplicated portion of each chromosome might serve to hold together the previously replicated arms[2]; such a mechanism would not be inconsistent with the appearance of the centromere in chromosomes such as that illustrated in Fig. 1. However, most chromosomes resemble that shown in Fig. 5, where it is apparent that at least part of the centromere region has already been duplicated before the chromatids separate. In this bivalent the chromatids are held together at two points, and in the centromere proper, multiple lengths of fibre (about fifteen) pass from one chromatid to the other. Although it is not difficult to devise patterns of folding which can account for such chromatid pairs without violating the restrictions of a folded-fibre model, this question of centromere structure requires further investigation.

With respect to the number of separate fibres that are present in any given chromatid, there are also several possibilities open. As indicated in Figs. 1, 3 and 4, the narrowest chromatid regions observed so far still contain about three lengths of fibre lying side by side; nevertheless, all these lengths of fibre might actually be continuous with one another at the ends of the chromosome or elsewhere. Several possible patterns of folding for a very long, single fibre are illustrated in Fig. 6. In chromatid *A* the fibre is folded transversely along its entire length, in chromatid *B* it is folded longitudinally, and in chromatid *C* there is a complex combination of transverse and longitudinal folding. For most of the regions in chromatid *C*, stretching would seem to reveal three lengths of fibre lying side by side; a break in any one of these fibre lengths would very likely appear in the light microscope as a 'half-chromatid' break; yet this chromatid really contains only a single continuous fibre.

Some insight regarding the problem of chromatid 'strandedness' can be obtained by comparing the estimated total length of 230 Å fibre in a specific chromatid with the theoretical length of DNA present in the same chromatid. For example, in Fig. 1, one chromatid has a length of 4·9μ, a diameter of 0·6μ, and on a volume basis could contain approximately 3,330μ of fibre (assuming the volumes of cylinders). From its dimensions this chromosome is likely to represent one of the 6-*X*-12 group, in which case the measurements of Rudkin *et al.*[8] would

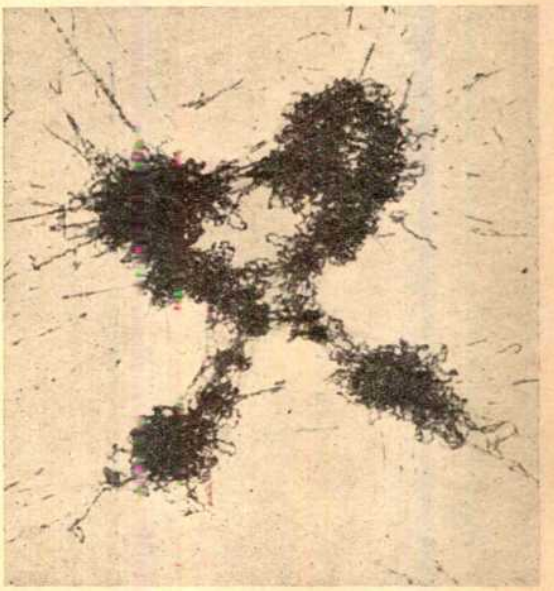


Fig. 5. A metaphase chromatid pair isolated from human liver culture cells. In the centromere region, multiple lengths of fibre pass from one chromatid to the other (× *c.* 14,270)

indicate that each chromatid contains 2·45 per cent of the diploid DNA content. Calculated from a total diploid DNA content equivalent to 180 cm of Watson–Crick molecule[2], this chromatid would then contain 4·4 cm of DNA, and the ratio of DNA length to fibre length would be about 13 : 1. Similar calculations for one of the tiny chromatids of the 21–22 group (having approximately the volume of a 1μ sphere) have yielded a ratio in the order of 9 : 1. The fact that these chromatids contain some ten times as great a length of Watson–Crick DNA molecule as of 230 Å fibre makes it less likely (though not impossible) that the apparent three-strandedness of the chromatids in Figs. 1, 3 and 4 represents a simple polytenic organization. Even if the side-by-side lengths of fibre were polytenic replicates, it would still be necessary to postulate either: (1) that each 230 Å fibre contains about ten DNA molecules lying side by side; or (2) that each fibre contains one DNA molecule which is coiled in a 10 : 1 secondary helix (as postulated in the folded-fibre model). In any event, the presence of only three strands fits better with the model illustrated in Fig. 6*C* than with a scheme for four-ply polyteny (though a fourth strand could easily be overlooked).

While it is true that the electron micrographs alone do not permit firm conclusions about chromatid strandedness, a somewhat clearer picture emerges if the fine structure is considered in relation to various recent experimental analyses. For example, autoradiography of human cancer tissue (HeLa) after exposure to tritiated thymidine has shown that replication of entire human chromosomes is semi-conservative[9], just as replication of individual DNA molecules is semi-conservative. A consequence of this fact is that each chromatid must possess two DNA-containing units which extend the length of the chromatid and which retain their structural integrity from one mitosis to the next. However, no such duplex units are visible in electron micrographs (for example, Figs. 1, 3, 4 and 5), either at the level of 230 Å fibres or at higher dimensions. This discovery supports Taylor's interpretation that the two units are the two polynucleotide chains of one or many (linked) DNA molecules, which in all probability form the cores of the 230 Å fibres[1]. If it is really true that duplex structural units larger than the 230 Å fibres do not exist within the chromatid, then it is difficult to understand how the side-by-side fibres could be polytenic replicates, or even separate fibres, while the chromatid as a whole continues to show semi-conservative replication. The only model that seems able to account for all these relationships simultaneously is that based on a single, long folded fibre.

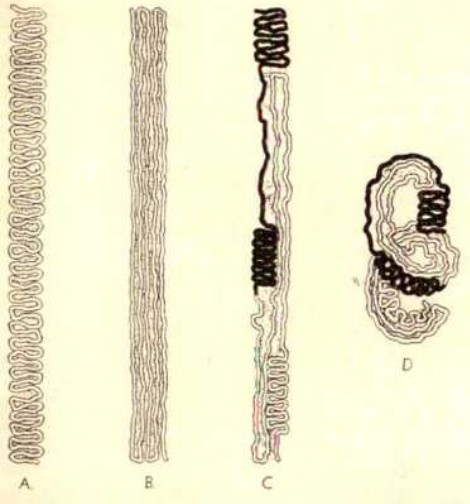


Fig. 6. Possible patterns of folding for a single, long chromatin fibre. (*A*) Transverse folding; (*B*) longitudinal folding; (*C*) combined transverse and longitudinal folding; (*D*) quaternary coiling superimposed on chromatid *C*. For further explanation refer to text

Recently, Solari[10] has confirmed the existence of single, long DNA molecules in higher organisms. In an electron microscope investigation of surface-spread sea-urchin sperm, he found that the DNA of these organisms occurs in the form of chromatin fibrils at least 50–93μ long. It would seem that the great length of these DNA strands must rule out general models of chromosome structure which postulate many short DNA segments in each chromatid (for example, Taylor's 'ladder' model[11]), and it makes unlikely those models which view the chromosome as a rope-like structure composed of parallel polytenic strands.

Evidence concerning the dimensions of interphase chromatids has been obtained by Neary *et al.*[12], using the chromosomes of *Tradescantia* (which are also known to replicate semi-conservatively[9]). Contrary to the results of earlier workers, these investigators found that chromatid breaks and exchanges are produced in efficient yield by low-energy, monochromatic X-rays, the photoelectrons of which have integrated path lengths of about 500 Å. Although their data can be interpreted on the basis of several models, one suggestion is that "the interphase chromatid diameter is less than half the range of (0·05μ) . . .". This would correspond rather closely to the dimension of a single 230 Å fibre, and would tend to support a folded-fibre model of chromosome structure.

Still another recent experimental investigation, which seems to provide striking support for a folded-fibre model, has been reported with synchronously dividing cells of the smut fungus, *Ustilago*[13]. The frequency of mitotic crossing-over in these cells can be increased by irradiation with ultra-violet light, and in synchronized cells this effect can be investigated at different times during the cycle of DNA synthesis. Holliday has found that, depending on the time of irradiation, crossing-over is induced between different genetic markers; of even greater significance, it appears that genes near the ends of the chromosome arms are affected earlier than genes positioned nearer the centromeres. These results strongly suggest that in *Ustilago* a telomere-to-centromere pattern of chromosome replication occurs, as postulated in the folded-fibre model[2].

An apparently sequential telomere-to-centromere pattern of DNA synthesis has also been detected in several types of plant and animal chromosome by autoradiography (see discussion in ref. 2). However, many other types of chromosome show patterns of incorporation which are complex and apparently irregular. That such irregular metaphase patterns do not necessarily imply an irregular interphase replication is illustrated in Fig. 6. The dark length of fibre in Fig. 6*C* shows a possible pattern of sequential incorporation as it would appear in a relatively extended folded-fibre chromatid. By contrast, Fig. 6*D* shows the same chromatid after it has gone through a process of quaternary coiling; though the sequence of incorporation is the same, the autoradiograph pattern would be entirely altered, possibly giving no hint of sequence at all. If a monomolecular connecting strand should occur (as in Fig. 6*C*), the presence of labelled thymidine in that part of the chromatid might easily go undetected by some autoradiograph techniques, and the appearance would then be of two separate loci synthesizing DNA simultaneously. Such patterns have been reported, but a much more sensitive technique of 'replicate autoradiography', recently introduced by Stubblefield[14], has revealed that chromatid loci of high specific activity are often connected by intermediate areas of lower specific activity.

The apparent folded-fibre organization of human chromosomes reported in this article, together with recent experimental investigations cited from other laboratories, would seem to extend the usefulness of the folded-fibre model well beyond the genetic material of honeybee embryos, where it was originally inferred. Although major problems, such as the organization of the centromere and the mechanism of replication in very long DNA

molecules, still remain to be clarified, essentially these same problems exist for any valid theory of chromosome structure. That DNA molecules many hundreds of microns long do replicate sequentially has been shown in micro-organisms by Cairns[15]; only further research can reveal the mechanism of this replication, which very probably occurs in the chromosomes of higher organisms as well. It is interesting to estimate that, if the folded-fibre model is correct, the average rate of DNA replication in cultured human leucocytes is about 27μ per min per replication site (based on diploid nuclei containing 180 cm of DNA[2], 46 chromosomes, two replication sites per chromosome, and a minimum DNA synthesis period of 12 h[16]). Although this result is only approximate, it is in good correspondence with Cairns's estimate of 20–30μ per min for the single replication fork of *E. coli*[15].

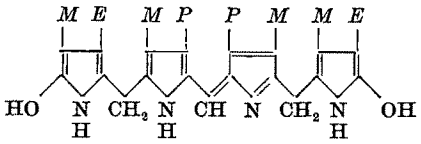
I thank Mr. Richard Jahn and Mr. Peter Rae for their assistance, and Mrs. Patricia Levy for drawing Figs. 2 and 6. This work has been supported by National Science Foundation grant *GB*–1924. A preliminary abstract has been published[17].

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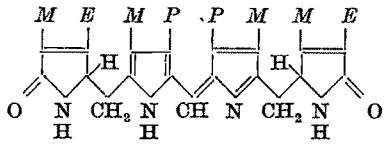
MOLECULAR SPECIES OF THE UROBILINS

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The Robert Robinson Laboratories, University of Liverpool
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THE urobilins are dipyrrylmethene compounds joined at the two α-pyrrolyl positions by way of methylene bridges to either pyrrolenone or pyrrolidone rings[1]. An optically inactive urobilin, usually designated *i*-urobilin or urobilin IXα, is formed by the dehydrogenation of mesobilirubinogen, the octahydrogenated product formed from bilirubin on reduction with sodium amalgam[2]. Urobilin IXα was synthesized by Siedel[3] and shown to have the structure (I) although subsequently the *bis*lactam structure (II)[1] has been accepted as more probable[4]. An inactive urobilin believed to be identical with urobilin IXα has been isolated from faeces[5].



(I)

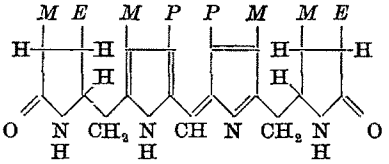


(II)

$M = CH_3$; $E = C_2H_5$; $P = CH_2CH_2COOH$

Stercobilin is readily isolated from normal faeces, in which it occurs as stercobilinogen[5]. The latter is a dihydrogenated colourless precursor formed by the bacterial reduction of bilirubin or bilirubin glucuronide in the intestines. Stercobilin is strongly laevorotatory[5], resists racemization even in concentrated hot alkali[6], and elementary analysis[7] suggests that its molecule contains four hydrogen atoms more than that of urobilin IXα. The products of degradation of stercobilin[1] suggest that it has the structure (III) which represents a compound also obtained by the extensive catalytic reduction of bilirubin[8].

A third pigment, *d*-urobilin, may be isolated from the faeces of subjects receiving broad-spectrum antibiotics[9]. This compound, which has an even greater optical activity than stercobilin, is dextrorotatory and contains two hydrogen atoms less per molecule than urobilin IXα. The site of the resulting extra unsaturation in *d*-urobilin has not been unequivocally located, but is probably present in a vinyl group attached to one end ring[1]. *d*-Urobilin is readily racemized in alkali[1] presumably with production of a laevorotatory enantiomorph, although the latter has never been isolated.



(III)

The chromogen obtained by the tetrahydrogenation of *d*-urobilin affords, on oxidation, a further urobilin, apparently isomeric with urobilin IXα: this product has been called *d*-urobilin IXα because, while it resembles urobilin IXα in containing only one readily reducible double bond, it has a *d*-rotation similar in magnitude to that of *d*-urobilin[1].

The possibility that mass spectrometry might throw light on the structure of these compounds led us to undertake their systematic investigation. The detailed structures of the various fragments are still being investigated and will be the subject of a separate report; the preliminary assessments of the molecular weights of the parent compounds may well demand revision of the present ideas of the structures of the urobilins.

Mass spectrometric studies were made in the A.E.I. '*MS* 9' mass spectrometer equipped with direct inlet and operating at temperatures up to 250°. Stercobilin hydrochloride was isolated from normal faeces by the method of Watson[5] and after successive recrystallization from chloroform had $[M]_D^{CHCl_3}$ $-24{\cdot}8° \times 10^3$ ε $93{\cdot}0 \times 10^3$ at 496 mμ. *d*-Urobilin hydrochloride, $[M]_D^{CHCl_3}$ $+27{\cdot}6° \times 10^3$ ε $93{\cdot}0 \times 10^3$ at 499 mμ, was obtained and purified similarly but with preliminary separation from a little violet pigment

by extraction of the latter from aqueous solution into ether. Faecal urobilin IXα ε $60{\cdot}8 \times 10^3$ at 496 mμ in $CHCl_3$ was obtained from the chloroform mother liquors of the *d*-urobilin preparations, by concentration and precipitation into petroleum spirit. The hydrochloride in chloroform was without optical activity and gave a characteristic violet colour on treatment with warm acidic dioxane[9]. Urobilin IXα was obtained also by the reduction and oxidation of bilirubin again being isolated as the hydrochloride with recrystallization from chloroform–acetone mixture. The product before the final crystallization had ε $61{\cdot}9 \times 10^3$ in chloroform.

The results of mass spectrometry show that bilirubin (Weddel Pharmaceuticals Ltd., ε $66{\cdot}0 \times 10^3$ at 450 mμ in chloroform) consists mainly of a single species of molecular weight 584 in accordance with the accepted structure; this was accompanied by trace amounts of some products of higher hydrogenation, possibly dihydrobilirubin or mesobilirubin. Results with the natural and synthetic urobilins are of particular interest. These, although hitherto regarded as pure compounds having analyses compatible with accepted structures, proved by mass spectrometry to be mixtures.

A specimen of *d*-urobilin isolated by Moscowitz, but of unspecified optical properties, was almost wholly of molecular weight 588 with a small amount of a component of weight 590 (ref. 15). The 588 component is in accordance with the presence of two reducible double bonds per molecule compared with one in urobilin IXα, as found earlier[1]. The *d*-urobilin isolated by us for mass spectrometry also had the compound of molecular weight 588 as the major component, but accompanied by a considerable quantity of pigment of molecular weight 590. The latter, considering the high optical rotation and molecular extinction of the *d*-urobilin, may be considered a *d*-rotatory urobilin, possibly identical with *d*-urobilin IXα[1].

Faecal urobilin IXα unexpectedly contained only a minor fraction of molecular weight 590 corresponding to its accepted structure (II); the major component was of mass 588, possibly identical with *d*-urobilin. On the other hand, the urobilin IXα prepared *in vitro* from bilirubin was mainly of molecular weight 590 in accordance with structure (II), accompanied by small amounts of a component of molecular weight 588. Both faecal urobilin IXα and that obtained from bilirubin contained a component of molecular weight 592. The *in vitro* product contained about 20 per cent of this, but the component may well have constituted about 50 per cent of the faecal urobilin IXα. The presence of this in the natural pigment is of particular interest; it represents a dihydro-urobilin IXα never before isolated or detected and intermediate between the structure formerly accepted for urobilin IXα (II) and stercobilin (III).

Stercobilin contained very little of a component of molecular weight 594 as required by the structure III. It consisted instead of two components, one of which was stable and of molecular weight 592. It is not at all certain that the 592 component is identical in stercobilin and urobilin IXα. An intermediate of this molecular weight between structures (II) and (III) would probably be readily oxidized to a violin. Urobilin IXα is oxidized in this way by ferric chloride, but stercobilin is quite unaffected. Either the 592 component in stercobilin differs from that in urobilin IXα or is stabilized by some peculiarity of molecular association. The second component of stercobilin was heat labile with a molecular weight 596, incompatible with any structure yet postulated for a urobilin. Stercobilin resembles *d*-urobilin and cryptopyrrolemethene in having a molecular absorption extinction of about $90{\cdot}0 \times 10^3$. The component of molecular weight 596 must, therefore, be a dipyrrylmethene and must be a derivative of III containing two extra hydrogen atoms in one end ring. Such a compound might have a β-methyl-β′-ethyl-hydroxypyrrolidine end ring or, less likely, a terminal open-chain aldehydoamino residue.

The ratio of the two components in stercobilin is not known with certainty but is compatible with their presence in a binary complex. The formation of this, if involving one end ring in a way precluding the extra hydrogenation referred to above, would account for the absence of a component of molecular weight 598. Any such complex must, however, be unstable under the conditions of mass spectrometry since the spectra show no component of molecular weight 1180. A binary composition for stercobilin might have been suspected from earlier unpublished studies in which X-ray analysis proved impossible because of crystal twinning. Similar indications result from studies[10] of the optical activity of the urobilins which arises from asymmetric carbon atoms in the end rings and from the non-coplanarity of the two middle rings[9,10]. Coordination of *d*-urobilin and *d*-urobilin IXα with zinc decreases optical activity, as would be expected from planarization of the molecule; the changes in optical rotation of stercobilin were complex[10], as were the accompanying spectral changes, and suggest that an intermediate complex was formed or that the free pigment was not a single entity.

Consistent with our results for urobilin IXα (ref. 11) is its recent optical 'resolution' by fractional crystallization into dextro- and laevo-rotatory forms[12]. Such a separation would be unlikely if the two components were simple enantiomorphs. The demonstration that the optically inactive urobilin IXα contains a component similar to or identical with *d*-urobilin would suggest that the component of molecular weight 592 might have a laevorotation equal and opposite to that of *d*-urobilin. Similarly the component of stercobilin with molecular weight 596 might be racemic, *d*-rotatory, or have a low *l*-rotation of sufficient magnitude to reduce the molecular rotation from $-31{\cdot}0^\circ \times 10^3$, numerically equal to that of *d*-urobilin, to the $-25{\cdot}0^\circ \times 10^3$ characteristic for stercobilin.

These results will undoubtedly lead to some revision of our ideas of the interrelationships of the three urobilins which may be isolated from faeces under various circumstances. It seems likely that the three components now identified are indeed formed in the gut, but that these might consist of *d*-urobilin molecular weight 588, an *l*-rotatory compound molecular weight 592 and a racemic, *d*-rotatory or weakly *l*-rotatory compound of molecular weight 596. Under some circumstances *d*-urobilin will predominate; it may otherwise become associated with an equimolecular amount of the 592 compound and the isolated material will consist of urobilin IXα. Under other conditions 592 and 596 compounds will be available and the material isolated will consist of the laevorotatory stercobilin. This would undoubtedly account for the unpredictable proportions of urobilin IXα, *d*-urobilin and stercobilin excreted independently of diet or disease[13]. If some of the molecular species are labile and only stable when associated with a second species it may also explain how the yields of these compounds are so poor during isolation from faeces.

We thank Prof. C. Djerassi and his colleagues (Stanford) for help with the initial determination of some of these spectra. This work follows on from a joint study of the mass spectrometric behaviour of mono-[14], di-, tri- and tetra-[15] pyrroles carried out at both Liverpool and Stanford Universities. We thank Prof. G. W. Kenner for his interest in this work.

Note added in proof. Since completion of this work a sample of racemic stercobilin obtained by Watson's method[8] has become available to us, and Prof. Plieninger has kindly provided a sample of a racemic, synthetic tetrahydro-*i*-urobilin. These on mass spectrometry also show the characteristic peaks at *m/e* 592 and 596 described for natural stercobilin and also a less intense peak at *m/e* 594. The possibility cannot, therefore, be entirely excluded that natural stercobilin is indeed wholly of molecular weight 594 as required by structure III and that the molecules of mass 592 and 596 appearing on mass spectro-

metry are artefacts arising as a consequence of a novel disproportionation which may be sterically controlled.

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HUMAN GASTRIN: ISOLATION, STRUCTURE AND SYNTHESIS

Isolation of Two Gastrins from Human Antral Mucosa

GREGORY and Tracy[1] have described the isolation from hog antral mucosa of two heptadecapeptide amides which are believed to represent the antral hormone gastrin; they were named gastrins I and II. There followed in this journal a report of the elucidation of their structures[2] and their total synthesis[3] together with an account of the physiological properties of a series of synthetic peptides structurally related to gastrin I[4]. It was shown that all the physiological actions displayed in appropriate experimental conditions by the gastrins (on gastric acid and pepsin secretion, pancreatic volume-flow and enzyme secretion, and gastro-intestinal tone and motility) were possessed by the C-terminal tetrapeptide amide, but that these activities were largely abolished by removal from this structure of the amide group masking the C-terminal residue. These observations were confirmed and extended by a subsequent study of the physiological actions of a series of synthetic analogues of the tetrapeptide[5].

In the ensuing accounts a description is given of the isolation from human antral mucosa of two heptadecapeptide amides (H. I and H. II). Structural and synthetic studies show that these differ from peptides obtained from hog mucosa only in that the methionine residue in position 5 is replaced by leucine; H. II is the tyrosine sulphate ester of H. I.

Antra were collected from Liverpool hospitals immediately after removal from patients undergoing partial gastrectomy. The mucosa was removed in strips, boiled for 5 min in water, and the cooked strips and liquor stored in a dry-ice container until material from approximately twenty antra had been accumulated. After thawing, strips and liquor were homogenized and boiled for 20 min in 5 l. of water. The extract was clarified by centrifugation and filtration and the gastrin recovered from it by adsorption on DEAE floc and elution with 0·1 N sodium hydroxide as previously described for hog gastrin[1]; after neutralization to *p*H 7 with dilute sulphuric acid the extract was freeze-dried. A similar procedure was followed in Los Angeles and the powder sent by air to Liverpool.

The subsequent procedure of isolation was as previously described for hog gastrin[1] except that the isopropanol extraction (from 100 ml.) was performed three times and the final aqueous residue was freeze-dried to a small volume (15 ml.) before precipitation at *p*H 4. The 'Sephadex' column was of smaller size (3 cm × 50 cm) and smaller samples were collected (2·5 ml. every 15 min). The aminoethylcellulose column was smaller (1 cm × 10 cm) and was packed with Whatman powder type *AE* 41; samples of 1·3 ml. were collected every 15 min and a gradient of 0·05–0·5 M ammonium bicarbonate solution was established by feeding the column through a magnetically stirred mixing vessel of volume 180 ml.

Fig. 1 shows a typical run; H. I emerges first, followed by H. II. The appropriate samples were pooled and freeze-dried until free from ammonium bicarbonate. Because

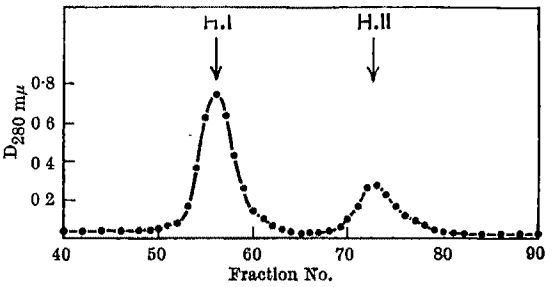


Fig. 1. Isolation of human gastrins (H. I and H. II) from an extract of antral mucosa by gradient elution on aminoethylcellulose in ammonium bicarbonate buffer

of the excellent separation and symmetrical form of the peaks, and the small amounts of material present, no attempts were made to re-run each fraction, or to weigh the products; the yields were calculated later from the data obtained during quantitative amino-acid analysis. Small aliquots were reserved for physiological studies. The highest yields obtained were from a batch of 23 antra all collected in Liverpool; this gave 1·35 mg of H. I and 0·79 mg of H. II. This predominance of H. I over H. II (the sulphated form) contrasts with a ratio of about 3 : 4 found in the hog gastrins[1].

Physiological studies. H. I. and H. II showed in apparently identical form and degree the range of physiological actions already described for the hog gastrins[1]; a further account of these will be presented elsewhere. In view of the previous investigations already mentioned, this was to be expected, since their structures are almost identical with those of the hog gastrins, differing in respect of only one residue which is situated in what has been shown to be the 'non-essential' portion of the molecule[4].

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Structures of Human Gastrins I and II

IN the preceding communication, Gregory, Tracy and Grossman[1] described the isolation of two gastrins (H. I and H. II) from human antral mucosa. Small samples of both hormones were generously made available to us by Prof. Gregory, and we now present evidence from degradative investigations supporting the assignment of

structures I and II to the human gastrins H. I and H. II respectively. These structures differ from those of the porcine hormones[2] G. I (III) and G. II (IV) only in the replacement of the methionine residues at position 5 by leucine residues. Synthetic evidence confirming the suggested amino-acid sequence of the human hormone H. I is described in the following section[3].

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad R \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ \text{pGlu.Gly.Pro.Try.}\textit{Leu}\text{.Glu.Glu.Glu.Glu.Ala.Tyr.Gly.Try.Met.Asp.Phe.NH}_2 \\ 1\quad 2\quad 3\quad 4\quad 5\quad 6\quad 7\quad 8\quad 9\quad 10\quad 11\quad 12\quad 13\quad 14\quad 15\quad 16\quad 17 \end{array}$$

I, R=H; II, R=SO_3H

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad R \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ \text{pGlu.Gly Pro.Try.}\textit{Met}\text{.Glu.Glu.Glu.Glu.Ala.Tyr.Gly.Try.Met.Asp.Phe.NH}_2 \end{array}$$

III, R=H; IV, R=SO_3H

At the outset of this work only very small quantities of the hormones had been isolated, and the approximate amino-acid compositions were necessarily determined on samples of only 0·02–0·03 μmole (0·04–0·06 mg) using a Beckman amino-acid analyser 120*B* fitted with a high-sensitivity optical system. Typical analyses of acidic hydrolysates of H. I and H. II are recorded in the first two columns of Table 1, and these may be compared with the known composition of porcine gastrin shown in the last column. These results immediately confirmed the close chemical relationship of the human and porcine hormones already suspected from their identical physiological and chromatographic properties[1]. However the human hormones each contained one residue less of methionine and one residue more of leucine than did the porcine hormones. The analytical results for glutamic acid were ambiguous, and initially no decision could be reached between the presence of five or six residues in each gastrin. It has since been realized that the discrepancies between the results in columns 1 and 2 of Table 1 and the strictly stoichiometric theoretical ratios were a consequence, not of the presence of impurities carried through the isolation procedure, but of the presence of traces of contaminants in the ammonium bicarbonate used in the preparation of buffer solutions. No method was found for the preparation of ammonium bicarbonate (even from gaseous ammonia and carbon dioxide) which did not eventually yield traces (*c.* 0·0001 per cent) of individual amino-acids after hydrolysis. Even these minute amounts were significant because the quantities of gastrin isolated were very small (less than 0·1 mg in the early experiments) and the volumes of buffer solution involved in the chromatographic procedures were relatively large. The amino-acid analysis of H. II, approximately corrected for this contamination, is shown in the third column of Table 1, and it strongly supports the presence of six glutamic acid residues, as in the porcine hormones. This result has since been confirmed both by enzymatic degradation (*infra*) and by concurrent investigations of syntheses.

Table 1. Amino-acid Analysis of Human Gastrin

	H. I	H. II	H. II corrected	G. I
Aspartic acid	1·05	1 01	1·01	1
Glutamic acid	5·46	4·92	5·83	6
Proline	1·08	0·79	0 97	1
Glycine	2 02	1·97	2 04	2
Alanine	1 00	1·00	1·11	1
Methionine	0·76	0 76	0 93	2
Leucine	0 95	0·88	1·02	0
Tyrosine	0·92	0·86	0·91	1
Phenylalanine	0 88	0 85	0 94	1
Tryptophan*	~2	~2		2

* By ultra-violet spectroscopy.

That the relationship between the human hormones H. I and H. II was the same as that between G. I and G. II (that is, H. II was the sulphated analogue of H. I) was suggested by their relative electrophoretic mobilities. Confirmation was provided by alkaline hydrolysis of H. II, which yielded tyrosine-*O*-sulphate in place of the tyrosine from H. I.

From the amino-acid compositions of the human gastrins, we adopted as a working hypothesis for investigations of syntheses two possible structures for each hormone. These were the structures which we had previously established for the porcine hormones (that is, III and IV)[2], in which one or other of the two methionine residues had been replaced by leucine. No experimental information was available favouring substitution at position 5 over position 15, although intuitively it was felt that the former was more likely since, in the porcine series, the structure of this part of the molecule has been shown not to be critical for biological activity[4]. While this synthetic work was in progress, however, a larger sample of human gastrin (*c.* 0·6 mg of H. I) became available, and was used in a single degradative experiment designed to locate the leucine residue.

In parallel experiments, approximately equal samples of G. I and H. I were digested by papain at *p*H 7·5 during 26 h at 37° (enzyme : substrate ratio 1 : 9). The resulting mixtures of peptides were separated by paper electrophoresis at *p*H 6·5. Development of end strips by the Ehrlich and ninhydrin reagents revealed fourteen distinct bands in the digest of H. I and thirteen in the digest of G. I. Apart from a single band at the origin in the electrophoresis pattern of H. I (which proved to be derived from a contaminant) the two patterns were quite similar, although differences in the relative intensities of corresponding bands were evident. The most prominent bands were eluted, corresponding eluates in the H. I and G. I series were compared and checked for purity by thin-layer chromatography, and analysed quantitatively for amino-acid content. The presence of tryptophan in peptides was inferred either from the ultra-violet spectrum or from a positive Ehrlich reaction. The following peptides were especially significant.

A strong, ninhydrin-positive band near the origin from the digests of both H. I and G. I corresponded approximately in composition to the expected mixture of neutral peptides derived principally from the C-terminus of the gastrin molecule. The H. I peptides, like those from G. I, contained methionine but no leucine. A strong, ninhydrin-negative, mono-acidic band from the H. I digest was pure by thin-layer chromatography and analysis, and had the composition (Glu,Gly,Pro,Try,Leu). It was clearly derived from the N-terminus (residues 1–5). The corresponding band from the G. I digest, although less pure, contained the expected fragment of composition (Glu,Gly,-Pro,Try,Met). A weak adjacent band in the H. I digest was not obtained pure, but had the approximate composition (Glu,Gly,Pro,Try) and contained a component which corresponded on thin-layer chromatography with a synthetic sample of the N-terminal tetrapeptide pGlu-Gly.Pro.Try. The foregoing results located the leucine residue at position 5, and this was further confirmed by the isolation of a pure, ninhydrin-negative di-acidic peptide of composition (Glu,Gly,Pro,Try,Leu,Glu) (that is, residues 1–6). Two strongly acidic ninhydrin-positive peptides from H. I had the compositions Glu_4,Ala,Tyr, Gly and Glu_5, Ala, Tyr, Gly, which established the presence of five residues of glutamic acid in the central part of the molecule and hence a total of six residues, as in the porcine hormones. No evidence was obtained for any difference between the human and porcine gastrins apart from the leucine-methionine interchange at position 5.

Although the foregoing results do not prove the structure of human gastrin I in detail, they provide very strong support for the suggested single-residue interchange relationship with porcine gastrin. Interchange of methionine and leucine residues has been encountered previously in cytochromes from different species[5]. It is interesting to note that sheep gastrin, which is also under investigation in these laboratories, appears to contain a valine residue at position 5 as well as replacement of other amino-acids. Methionine, leucine, and valine are all related to one

another by single-base exchanges in their respective triplet nucleotide codons[6], and the differences at position 5 in the gastrins of the three species may therefore be a consequence of single mutations during the differentiation of the species.

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Synthesis of Human Gastrin I

Analytical work[1] showed that human gastrin I contains one residue each of leucine and methionine, whereas porcine gastrin I contains two residues of methionine (at positions 5 and 15) but no leucine[2]. A plausible hypothesis was that leucine occupies either position 5 or 15, more likely 5 because 15 is part of the 'essential' C-terminal tetrapeptide sequence[3], and the rest of the molecule is identical with that of porcine gastrin I. However, because only minute quantities of the human hormone were available, there was initially ambiguity whether it contained five residues of glutamic acid or six, like porcine gastrin. Investigations of syntheses, concurrent with further degradative studies, were therefore directed towards three targets, namely: (i) the leucine-5; (ii) the leucine-5 des-glutamic-6; (iii) the leucine-15 derivatives of porcine gastrin I. This communication deals with the first two syntheses; the third will be described elsewhere.

For syntheses of the leucine-5 gastrins we required the N-terminal pentapeptide (Fig. 1, line 5). Benzyloxycarbonyl-tryptophan was condensed with leucine methyl ester by means of di*cyclo*hexyl carbodi-imide, yielding the protected (4–5) dipeptide (Fig. 1, line 1). After hydrogenolysis this was elongated by a procedure (Fig. 1, lines 2–5) exactly analogous to one used in synthesis of porcine gastrin[4]. This was merely the most convenient method with the materials available, and other routes are being explored.

Our first synthesis of porcine gastrin I[4] involved coupling the N-terminal pentapeptide with a central (6–13) octapeptide polyester by the azide method and then finally adding the C-terminal (14–17) tetrapeptide amide. Some difficulties were encountered in the first coupling, because reaction between the (1–5) azide and the penta-*t*-butyl monomethyl ester of the (6–13) octapeptide was sterically hindered and side reactions became important. In the meantime, the scheme has been improved by reversal of the order in which the three main components are joined, that is, a (6–17) dodecapeptide amide is constructed and then finally the (1–5) pentapeptide is added. The main advantage of this procedure is that all the protective *t*-butyl groups can be stripped from the dodecapeptide amide before the final azide coupling, which is no longer sterically hindered. Moreover, the (6–17) dodecapeptide amide (Fig. 1, line 6) could also be used directly in convenient syntheses of derivatives of porcine gastrin I differing near the N-terminus, for example the leucine-5 derivative, our target (1).

Preparation of the (6–17) dodecapeptide amide was accomplished by a few modifications of the previous scheme[4]. The (6–13) octapeptide polyester was provided with an N-terminal *t*-butyloxy-carbonyl group by condensation of α-2,4,5-trichlorophenyl γ-*t*-butyl *t*-butyloxycarbonylglutamate with the tetra-*t*-butyl monomethyl ester of the (7–13) heptapeptide (Fig. 1, line 2). Selective saponification of the methyl ester, in the manner already described[4], gave an acid (Fig. 1, line 4), which was coupled, by way of its mixed anhydride, with diphenyl phosphoric acid, with the (14–17) tetrapeptide amide. Treatment with 98 per cent trifluoroacetic acid for 1 h at 20° removed all the protecting groups and the resultant (6–17) dodecapeptide amide was purified by successive chromatography on 'Sephadex *G*25' (in 0·4 per cent ammonium bicarbonate) and aminoethylcellulose (gradient elution with 0·4–2·0 per cent ammonium bicarbonate). The homogeneity of this compound was checked by electrophoresis, thin-layer chromatography, and amino-acid analysis after hydrolysis with leucine-aminopeptidase. The lower homologue, the (7–17) undecapeptide amide, was prepared in the same way from the tri-*t*-butyl monomethyl ester of the (8–13) hexapeptide.

The triethylammonium salt of the (6–17) dodecapeptide amide was coupled in dimethylformamide at 0° C with two equivalents of the (1–5) pentapeptide azide (Fig. 1, line 6), prepared from the methyl ester by treatment with hydrazine and then aqueous diazotization[5]. After 18 h a second equal portion of the azide was added and the mixture

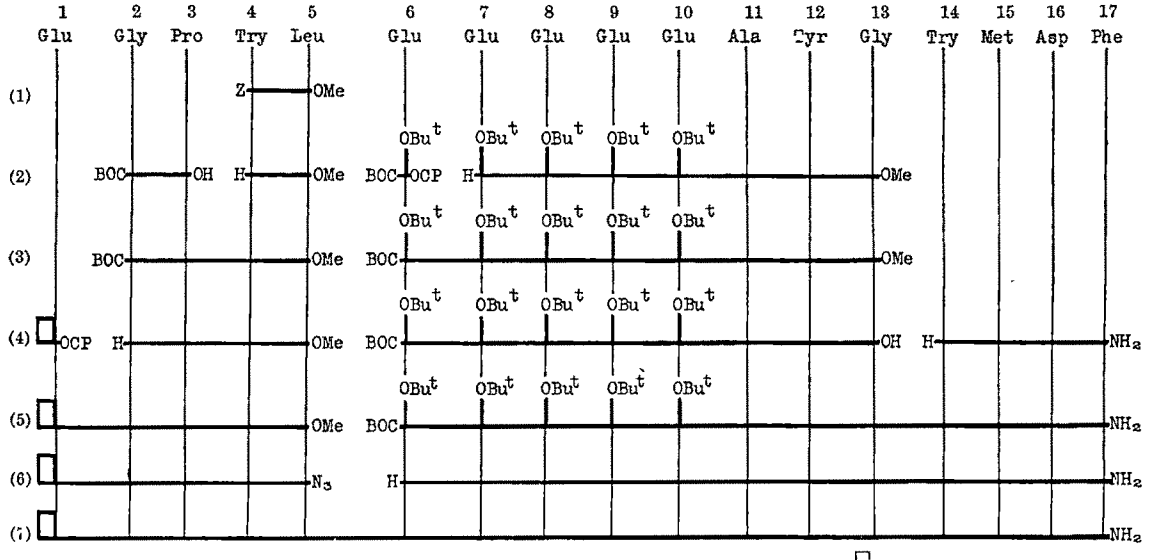


Fig. 1. *Z*, $C_6H_5.CH_2.O.CO$, *BOC*, $(CH_3)_3C\ O.CO$; *Bu*^{t}, $C(CH_3)_3$, *OCP*, $O.C_6H_2Cl_3(2,4,5)$; $^{\square}$Glu, pyroglutamyl

was left at 0° C for 3 days. The product was purified by chromatography on aminoethylcellulose. On rechromatography it was eluted as a single peak like natural human gastrin I, and was indistinguishable by electrophoresis. On the other hand, the lower homologue prepared in the same way from the (7–17) undecapeptide amide had clearly different electrophoretic mobility ($E^{6.5}_{glu}$ 0·65 instead of 0·70). The ambiguity concerning the content of glutamic acid was thus removed.

The heptadecapeptide amide (Fig. 1, line 7) was fully active in stimulating gastric secretion, and its identity with human gastrin I was confirmed by parallel degradations of the natural and synthetic hormones by papain, following the methods used in the structural analysis[1]. The peptides from the six most prominent electrophoretic bands were examined by thin-layer chromatography (three systems in each case); the chromatographic patterns were identical in the location of both major and minor components and their relative proportions. We regard this fingerprinting process as providing definite proof of the structure of human gastrin I. Experiments on the synthesis of human gastrin II are being undertaken.

Throughout this work the L-forms of amino-acids were used, and at each stage the peptides gave satisfactory amino-acid analyses (Beckman 120*B*). We thank Imperial Chemical Industries Pharmaceuticals Division for the gift of the analyser and for other generous support.

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ORIGIN OF URINARY METHYLATED PURINES

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ALTHOUGH the occurrence of 7-methylguanine in normal human urine was reported nearly 70 years ago[1,2] and subsequently, a variety of other methylated purines have been found as excretory products[3], their metabolic origin has remained obscure until recently. The meticulous investigations of Dunn *et al.*[4–6] established the presence of methylated purines and pyrimidines in transfer RNA as well as in DNA. The demonstration that all the methyl groups in methylated purines and pyrimidines in RNA (ref. 7) stem from the methyl group of methionine offered a possible new approach to the quantitative investigation of these excretion products. If after the administration of (methyl-^{14}C)-methionine the methylated purines of the urine become specifically labelled, this would offer a greater ease of identification and quantitation.

In this article we report that the methyl groups of the urinary purines stem from methionine. We also present the results of an investigation of the excretion of methylated purines in normal animals compared with animals bearing tumours originating from a thymic lymphoma or from a mouse mammary carcinoma. During the course of these investigations a previously unidentified excretion product—described as compound *S* by Weissmann *et al.*[3]—was tentatively characterized as 1-methyladenine.

Twenty-four male Wistar–Furth rats weighing 180–235 g were divided into two groups. One group served as the control and the other received subcutaneous implants of transplantable thymic lymphoma, strain *W*52. The animals were fed powdered Lablox rat food and water containing 10 per cent glucose and 1 per cent sodium chloride *ad lib.* Fifteen days later, 17 μc. of (methyl-^{14}C)-methionine (0·3 ml.; specific radioactivity 13·4 μc./μmole) were injected intraperitoneally into each of the twenty-four rats. The animals were housed in metabolism cages and the urine samples were collected over chloroform. The urinary output for the first 13·5 h of the normal and the leukaemic rats was pooled separately, and the output for the next 10·5 h was also collected and pooled as before. The preparation of the urinary purine concentrate and the identification of the purines were carried out essentially by the methods outlined by Weissmann *et al.*[3].

The urines were acidified to *p*H 2, diluted with an equal volume of water, refrigerated overnight and then filtered. The filtrate was then passed slowly through a column containing 100–200 ml. of 'Dowex 50 H^+' (20–50 mesh, 8 per cent cross-linked). The column was then washed with 2 l. of water and the purine bases were eluted with 1 l. of M ammonium hydroxide. The ammoniacal elute was concentrated to about 30 ml. and the *p*H adjusted to 2. Then 7 ml. of 1 M silver nitrate was added and the mixture was stored in the dark for a day at room temperature and for two more days at 5° to complete the precipitation of silver purine salts. The precipitate thus obtained was collected by centrifugation and was washed three times with 10 ml. portions of water. The silver purinate was decomposed by stirring in a boiling-water bath for 5 min with 100 ml. of 0·05 N hydrochloric acid. The precipitated silver chloride was removed by filtration and the filter was washed once with 50 ml. of hot 0·05 N hydrochloric acid. The filtrate and the washings were combined, concentrated to about 10 ml. and allowed to stand overnight at 5° to permit separation of additional insoluble material.

Aliquots of the purine concentrate were subjected to column chromatography on 25 ml. columns of 'Dowex 50 H^+' (8 per cent cross-linked; 200–400 mesh) according to the methods of Weissmann *et al.*[3]. The contents of the tubes contained within well-defined peaks were pooled, concentrated and subjected to two-dimensional chromatography in the solvent systems described by Weissmann *et al.*[3], the ultra-violet absorbing spots with corresponding blanks were eluted with 0·05 N hydrochloric acid and their spectra determined. An equal portion of each solution was then transferred to a stainless-steel planchet, dried and counted for determination of radioactivity.

For the investigation on normal mice and on mice bearing mammary carcinoma, 10 μc. of (methyl-^{14}C)-methionine (0·2 ml.; specific radioactivity 23 μc./μmole) were introduced intraperitoneally into each of ten normal *C*-57 mice and into ten mice which carried mammary carcinoma 755. The mice were then placed in metabolism cages and urine was collected for 24 h over toluene. The urines were pooled separately and processed

essentially by the methods described earlier (urine volumes, column volumes, elution volumes, etc., were smaller in this experiment). This time the purine concentrates were chromatographed on paper directly without prior separation on the second 'Dowex 50' column. In these experiments a windowless gas-flow counter with a high counting efficiency was used for determining total radioactivity in urine and purine concentrates and an end-window counter was used to measure total and specific radioactivities of purines.

The results obtained with normal rats and with rats with thymic lymphoma are summarized in Tables 1 and 2. The purine concentrate of the tumour-bearing rats has approximately twice as much radioactivity as that of normal rats. An analysis of the various purines present in this fraction shows that the content of radioactivity in major bases such as adenine and guanine is practically negligible and that most of the activity is located in the methylated purines. Moreover, the specific radioactivity of urinary 7-methylguanine is approximately 2·5 times higher than that of 1-methyladenine and 1-methylhypoxanthine in both the normal and tumour-bearing animals in the first 13·5 h after administration of the (methyl-^{14}C)-methionine. This is probably due to the greater turnover or depurination of 7-methylguanine as compared with other methylated purines. This view is supported by the finding that the specific activity of 7-methylguanine rapidly decreases after the first 13·5 h and falls to one-third of the initial value during the next 10·5 h (Table 2).

The higher levels of radioactivity in the urinary purine fraction of tumour-bearing animals may be a reflexion of a generalized increase in the excretion of the methylated purines studied or of any one of them.

Examination of the results (Tables 3 and 4) obtained with normal mice and mice bearing mammary carcinoma tumour reveals a pattern similar to that from leukaemia tumour-bearing rats. The purine concentrate from the urine of mice with mammary carcinoma had almost twice the radioactivity of normal controls. As before, the major bases contained negligible radioactivity. The specific radioactivity of 7-methylguanine in normal and tumour-bearing mice is at least 2–2·5 times that of 1-methylhypoxanthine and 1-methyladenine. Moreover, the total radioactivity present in the 7-methylguanine from the urine of tumour-bearing animals is approximately 1·8 times that obtained in the case of the normal mice. The total radioactivity in the other two methylated bases, that is, 1-methyladenine and 1-methylhypoxanthine, is similar in both normal and tumour-bearing mice and is considerably lower than that of 7-methylguanine. Thus, the increased radioactivity seen in the purine concentrate of mice with tumours can be attributed to a two-fold increase in the excretion of 7-methylguanine.

Several other ultra-violet absorbing excretion products (designated as compounds *S*, *V*, *W*, and *Z*) were found in human urine specimens by Weissmann *et al.*[3]. We have been able to identify tentatively one of these products—compound *S*—as 1-methyladenine[8] on the basis of the following criteria: (1) The specific radioactivity of compound *S* isolated from rat and mouse urine is comparable with that of 1-methylhypoxanthine (Tables 2 and 4). (2) The absorption spectra of compound *S* and 1-methyladenine are identical (Table 5). (3) The R_F values in three chromatographic solvent systems are similar (Table 5). (4) Preliminary isotope dilution experiments using chemically synthesized 1-methyladenine[9] also suggest that compound *S* is probably identical with 1-methyladenine.

DNA of the mammalian species contains only 5-methylcytosine as the minor methylated base[10]. On the other hand, a variety of methylated purines and pyrimidines are found in transfer RNA. Degradation of transfer RNA would result in the release of all of these minor methylated bases. It is surprising to find 1-methyladenine, 1-methylhypoxanthine, and 7-methylguanine as the predominant methylated bases in the urine of normal and tumour-bearing animals, because these three methylated purines are present in very small amounts in transfer RNA. This apparent paradox may be a reflexion of the absence of enzymes for the degradation of these bases.

Some of the other methylated purines such as 6-hydroxy-2-methylamino purine and 2-dimethylamino-6-hydroxypurine might be metabolized to the corresponding major bases by enzymatic hydrolysis with the concomitant

Table 1. Radioactivity in Urines and Purine Concentrates of Normal and Tumour-bearing Rats

Sample	Urine volume	Total c.p.m. in urine	Total c.p.m. in purine concentrate
Rats with tumours			
1st 13·5 h	310 ml.	$2·0 \times 10^7$	$9·7 \times 10^5$
Next 10·5 h	230 ml.	$3·5 \times 10^6$	$1·5 \times 10^5$
Controls			
1st 13·5 h	530 ml.	$1·5 \times 10^7$	$5·6 \times 10^5$
Next 10·5 h	225 ml.	$1·6 \times 10^6$	$8·1 \times 10^4$

Table 2. Specific Radioactivity of Urinary Purines of Normal and Tumour-bearing Rats

Compound	Controls		Tumour-bearing rats	
	1st 13·5 h (c.p.m./μmole)	Next 10·5 h (c.p.m./μmole)	1st 13·5 h (c.p.m./μmole)	Next 10·5 h (c.p.m./μmole)
Adenine	0	0	0	0
Guanine	1,000	0	0	0
Xanthine	420	250	100	0
Hypoxanthine	180	220	250	180
Compound *V*	500	0	450	0
Compound *S* (1-methyladenine)	6,000	8,000	6,500	4,400
1-methylhypoxanthine	6,200	6,000	5,800	5,300
7-methylguanine *	16,000	5,000	14,000	4,100
8-OH-7-methylguanine †	–	–	–	–
N^2-dimethylguanine ‡	+	+	+	+
1-methylguanine §	+	+	+	+

* 7-methylguanine occasionally gives rise to two spots on the 2-dimensional paper chromatogram. Both spots have similar ultra-violet spectra and specific radioactivity.

† 8-hydroxy-7-methylguanine is not present in rat urine.

‡ N^2-dimethylguanine is present in rat urine. It can be isolated as a highly radioactive purine. However, it is present in trace amounts and it is therefore difficult to obtain an accurate specific radioactivity.

§ 1-methylguanine is present in rat urine. However, it is present in trace amounts and it migrates very closely or together with 7-methylguanine. Consequently it is difficult to isolate it and determine the specific radioactivity.

Table 3. Radioactivity in Urine and in Purine Concentrates of Normal Mice and of Mice with Mammary Carcinoma

Sample	Urine volume	Total c.p.m. in urine	Total c.p.m. in purine concentrate
Control mice	250 ml.	$9·5 \times 10^6$	$1·07 \times 10^5$
Mice with mammary carcinoma	230 ml.	$1·1 \times 10^7$	$1·89 \times 10^5$

Table 4. Specific and Total Radioactivities of Urinary Purines of Normal Mice and of Mice carrying Mammary Carcinoma

Compound examined	Control		Mammary carcinoma	
	Total activity (c.p.m.)	Specific activity (c.p.m./μmole)	Total activity (c.p.m.)	Specific activity (c.p.m./μmole)
Adenine	—	—	80	140
Guanine	50	180	160	380
Xanthine	130	170	100	220
Hypoxanthine	200	190	160	140
7-methylguanine	4,080	7,570	7,240	8,070
1-methyl-hypoxanthine	320	3,890	320	2,900
Compound *S* (1-methyladenine)	320	4,370	390	3,040

Table 5. Comparison of Urinary Compound *S* with Synthetic 1-Methyladenine

Properties	1-methyladenine (synthetic)	Compound *S*
λ max, *p*H 1·0	259	259–261
λ max, *p*H 13	272	270
O.D. ratios at *p*H 1·0		
250/260	0·80	0·80
280/260	0·24	0·25
245/259	0·66	0·63
290/260	0·02	0
R_F values (descending)		
n-Butanol : H_2O : HCOOH (77 : 11 : 12)	0·20	0·15
n-Butanol : H_2O : NE_3 (86 : 14 : 5% vapour phase)	0·25	0·22
Isopropanol : H_2O : NH_3 (70 : 30 : 5% vapour phase)	0·53	0·60

production of mono- or di-methylamines. This may be responsible for their extremely low levels in normal urine. It is noteworthy, in this connexion, that the *t*RNA of liver contains only 1-methyladenine in contrast to *Escherichia coli* *t*RNA in which only 6-methyladenine is present[8].

The specific activity of 1-methyladenine is comparable with that of 1-methylhypoxanthine. This could be due to deamination of 1-methyladenine either at the nucleotide or at the polynucleotide level. Since both hypoxanthine and 1-methylhypoxanthine are present in *t*RNA (ref. 11) the interesting possibility of the formation of hypoxanthine and 1-methylhypoxanthine at the polynucleotide level suggests itself (a model for the enzymatic alteration at the polynucleotide-level exists in the methylation of *t*RNA). If such an enzyme for specific deamination of adenine to hypoxanthine should exist, the introduction of degeneracy in the coding properties of *t*RNA might be achieved by it.

The elevated excretion of the methylated purines from the tumour-bearing animals is highly significant in view of the relatively small size of those tumours in comparison to the whole animal. The metabolism which yields the methylated excretory products must be very much higher in the tumour tissue to produce the elevated levels in the total metabolic output.

Elevated excretion of 7-methylguanine and of 8-hydroxy-7-methylguanine has been found by conventional methods of analysis in the urine of human subjects with leukaemia by Weissmann *et al.*[3] and Park *et al.*[12]. It would be interesting to investigate whether the pattern of excretion of methylated purines is similar in other malignancies.

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CHORIOALLANTOIC MEMBRANE LESIONS PRODUCED BY INOCULATION OF ADULT FOWL SMALL LYMPHOCYTES

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THE lesions which develop on the chorioallantoic membrane (CAM) following the inoculation of adult fowl leucocytes represent the reaction of immunologically competent cells to histocompatibility antigens present in the membrane[1]. Using whole leucocyte preparations, Szenberg and Warner found that only with the large lymphocyte was there a significant correlation with the number of lesions formed[2]. They concluded that it was this morphological type which was responsible for the production of lesions on the CAM.

Although it was widely held for many years that the small lymphocyte was an end cell, incapable of further reactivity[3], in recent years evidence has arisen for the potential reactivity of some of these cells, and particularly for their important role in immune reactions[4]. Therefore it was decided to re-investigate the CAM phenomenon with the view of establishing whether or not the small lymphocyte could produce the lesions.

In order to obtain pure samples of small lymphocytes, the following procedure was adopted. Adult fowl blood was obtained by cardiac puncture, heparinized and centrifuged at 500 r.p.m. for 4 min to sediment the red cells. 1·0 ml. of the uppermost layer of the supernatant was passed through a column of glass wool and glass beads, following the method developed by Rabinowitz[5]. The first ten drops of the effluent were collected and a white-cell count was performed using a haemocytometer chamber. To ensure that the only cells present in the effluent were small lymphocytes, drops of the cell suspension were placed on glass slides, drawn along the slide by a fine capillary rod, air dried and stained with Leishman stain for 10 min. Cells with dark-staining nuclei and minimal cytoplasm were identified as small lymphocytes. Only aliquots containing 100 per cent pure cell suspensions were used. Such aliquots were inoculated on to the dropped membranes of 12-day-old embryos. The inoculated eggs were incubated at 38° C and the membranes collected at 4–5 days.

Initial experiments demonstrated that these small lymphocyte inocula could produce the characteristic nodular lesions, as shown in Fig. 1.

In subsequent experiments dilutions of cell suspensions were prepared to contain as few as 500 cells/0·1 ml. A summary of the results from seven experiments is given in Table 1.

A typical distribution, as observed in experiment (7), is shown in Fig. 2.

Inocula containing larger numbers of cells often resulted in the appearance of clumped, multi-nodular lesions and this precluded accurate counting. The smaller inocula also produced clumped reactions despite efforts to prevent

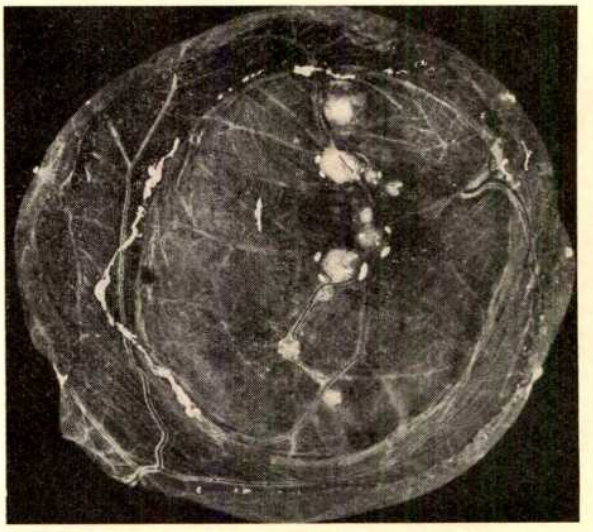


Fig. 1

Table 1

Experiment	Fowl No.	No. of eggs	Mean cell-focus ratio	Lowest cell-focus ratio
(1)	462	35	4,400/1	800/1
(2)	462	32	3,260/1	1,000/1
(3)	462	34	4,600/1	600/1
(4)	468	33	1,140/1	90/1
(5)	468	30	950/1	30/1
(6)	465	31	4,200/1	800/1
(7)	465	55	2,860/1	250/1

their appearance by rolling the eggs at the time of inoculation and frequent turning of the eggs during incubation.

Even with these smaller numbers of cells considerable variation occurred within each experiment in the number of foci produced by the same dilutions, and between each experiment in the mean and lowest cell-focus ratios. There are at least four possible sources of these variations.

The first concerns the genetic relation between the donor fowl and the recipient embryo. A correlation between CAM tests and the homograft response in inbred fowls has been established[6], and evidence presented for the proposal that three major antigens are responsible for histo-incompatibility[7]. From this it follows that the number of fowl cells potentially capable of initiating CAM lesions will vary directly with the degree of genetic disparity between donor and recipient. Also, since only a small number of antigens are responsible for the CAM reaction the variation in cell-focus ratios resulting from the use of genetically different fowls will be correspondingly great. Our results are consistent with these predictions in that, using three non-inbred fowls as donors, it was found that the ratios obtained using the second fowl differed greatly from those from the other two (see Table 1).

The second possible source of variation lies in the sampling of the potentially reactive fowl small lymphocytes. Unless there is a non-random distribution of these cells throughout the peripheral blood of the fowl, then sampling is probably not a major source of variation.

The third possibility arises from the technique of leucocyte separation. If this method involves more than mere physical filtration and some characteristic of the potentially reactive small lymphocytes results in their preferential passage through the column, then some variation might be expected in the number of foci which appear in response to a given number of cells. Possible cell characteristics which have been investigated in this system by Rabinowitz are motility, phagocytic activity and adherence[5]. Furthermore, if these cells have been exposed to some selective pressure resulting in the development of a sub-population of morphologically indistinguishable small lymphocytes which are highly competent for CAM reactivity, then the effect of their preferential passage through the column may result in an even more pronounced cell-focus variation. Alternatively, the facilitated filtration of some small lymphocyte sub-group, independent of its immunological competence, may result in more suitable conditions for CAM reactivity.

The final possibility comprises a group of factors which are peculiar to this biological system. Those related to the embryonic membrane are the varying presence and prominence of the histocompatibility antigens, and the local conditions necessary for potentially competent cells to initiate a reaction. In addition, it is known that all cells do not stay on the membrane, a variable number migrating to form typical nodules in the spleen and less frequently in other sites[8]. The cells that remain may have to be in a particular phase of their generative cycle before their capacity to initiate nodule formation is realized.

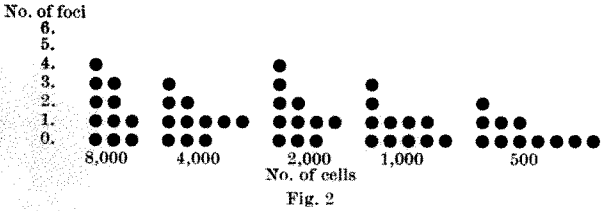


Fig. 2

The effect of any of the factors in this group would result in an under-estimation of the number of competent cells in any given inocula—that is, in a spuriously high cell-focus ratio.

In view of the numerous sources of variation and their likely effect, it is reasonable to attach greater importance to the lower cell-focus ratio for any immunological interpretation. This ratio was as low as 30 : 1 in one experiment (Table 1), a value which approximates to the lower limit of 40 : 1 quoted by Szenberg and Warner[2], for the large lymphocyte. In this connexion it is conceivable that cell suspensions comprised only of large lymphocytes, which have a higher metabolic rate than small lymphocytes and are probably their progenitors, would also be able to produce CAM lesions.

The CAM phenomenon has been proposed as an experimental model which is suitable for testing the clonal selection theory of immunity[9,10]. If each focus does represent the response of a single lymphocyte, and this claim has not yet been established with certainty, then the present results appear to be at variance with the ratios predicted on the basis of this theory. Clonal selection theory postulates a random distribution of patterns of specific reactivity among lymphoid cells permitting each to react only to the corresponding antigenic determinants. The immunological patterns are thought to arise by somatic mutation, and the magnitude of the ratio of cells carrying a genetically predetermined pattern to the total number of cells in a given population has been suggested to be, in the absence of selective pressures, of the order of one in 10,000 (ref. 10). For the observed low order of reactivity to be due to selection of donor fowl cell clones for reaction against allogeneic antigens present in the embryonic membrane, it would be necessary to postulate that such clones had been exposed to identical antigenic determinants during the course of the normal immunological development of the fowl. Although there is no direct experimental evidence on this point, the known specificity of antigens renders this requirement highly improbable.

However, even if some mechanism does exist which favours the development within the apparently uniform population of a sub-group of small lymphocytes, and if all the sources of variation operate so as to favour a low cell-focus ratio, then it is still only just conceivable that one reactive cell might occur every 30 or so cells. So numerous are the sources of variation and so great would be their resultant effect that it does not seem possible to establish whether the CAM phenomenon can be accounted for by an unmodified clonal selection theory.

A modification, which to us seems more consistent with the results obtained in the present experiments, is that a small lymphocyte or other lymphoid cell can be induced to proliferate in response to one or any of several closely related antigenic configurations—the extent of its responsiveness being genotypically determined, and the realization of its responsiveness being influenced by factors associated with the lymphocyte and the membrane cell and their interaction. It is doubtful whether investigations of the CAM phenomenon alone will enable this proposal to be unequivocally established.

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MECHANISM OF A HISTOCHEMICAL REACTION DIFFERENTIATING BETWEEN ADRENALINE- AND NORADRENALINE-STORING CELLS IN THE ELECTRON MICROSCOPE

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SOME months ago we published details of a method of fixation and staining recently developed in this laboratory[1], which makes possible the differentiation between adrenaline- and noradrenaline-storing cells in the light and electron microscope. Using this method, the separate identities of the adrenaline- and noradrenaline-storing chromaffin cells, which had been suggested on the grounds of the iodate-staining reaction[2], were established in the adrenals of the adult frog, mouse, rat, cat and domestic fowl. In the rabbit, the majority of adrenal chromaffin cells are adrenaline-storing, while in the extra-adrenal para-aortic chromaffin bodies they are noradrenaline-storing.

More recently work has now established the mechanism of the reaction. Aqueous solutions of noradrenaline react with glutaraldehyde with the production of a dense precipitate. The kinetics and product of the reaction have been examined by ultra-violet and visible light and by infra-red spectroscopy and paper chromatography using various solvent systems; melting-point determinations have been carried out and recrystallization attempted. All findings are in favour of the product being a polymer of glutaraldehyde and noradrenaline combined in a molecular ratio of 1 : 1.

If glutaraldehyde-fixed frozen sections of rat, cat, mouse and fowl adrenal glands are immersed in aqueous osmium tetroxide (1–2 per cent), 3 per cent potassium dichromate, or saturated potassium iodate, the same islands of cells give a colour reaction which is respectively black, yellow or brown. Furthermore, when the chromatograms of the reaction products of the noradrenaline–glutaraldehyde are sprayed, or when the precipitate is dried on filter paper with these oxidizing agents, similar colours are observed. We have been unable to demonstrate a similar reaction between adrenaline and glutaraldehyde by the foregoing methods.

No reactive islands are observed when frozen sections of glutaraldehyde-fixed adrenals of reserpinized Sprague–Dawley rats (5 mg/kg intravenously 24 h previously) are immersed in aqueous osmium tetroxide, dichromate or iodate solution. A reaction confined to specific islands is, however, observed in glutaraldehyde-fixed adrenals obtained from animals starved overnight and killed 2–4 h after an injection of insulin (10 I.U./100 g, subcutaneously). Under these conditions, the reserpine depletes both adrenaline- and noradrenaline-storing cells, while a depletion of the adrenaline-strong cells alone can be observed under the light microscope after treatment with insulin. These findings, together with the chemical evidence, establish the identity of the reactive material as a glutaraldehyde–noradrenaline complex.

In order to assess the importance of glutaraldehyde in this reaction as it relates to electron microscopy, adrenal glands have been fixed in (*a*) Caulfield's buffered (*p*H 7·4) osmium tetroxide-sucrose mixture, (*b*) 4 per cent glutaraldehyde, buffered to *p*H 7·4 with 0·1 M cacodylate, (*c*) 4 per cent buffered glutaraldehyde followed by post-fixation in either 1 per cent buffered (*p*H 7·4) osmium tetroxide or 3 per cent potassium dichromate. Tissue blocks were afterwards dehydrated in ethanol, cleared in 1 : 2 epoxypropane and embedded in 'Araldite'. In view of the recent communication from Benedeczky, Puppi, Tigyi and Lissák[3], only the findings relating to the domestic fowl adrenal will be considered, though similar results have been obtained with rat adrenal glands.

Buffered osmium-tetroxide fixation. In well-fixed and adequately processed normal fowl adrenal glands all chromaffin cells contain large numbers of electron-dense chromaffin granules which exhibit a peripheral limiting membrane (Fig. 1). Many have a target form with an intensely electron-dense centre and a periphery of a more diffuse granular nature.

Glutaraldehyde fixation only. Two distinct types of chromaffin cells may be identified (Fig. 2) in unstained sections and after staining with potassium permanganate or after brief staining with Reynold's lead citrate (20 min). Electron-dense granules, usually of homogeneous nature, represent the noradrenaline-storing elements, while the adrenaline-storing cells contain granules which have a washed-out appearance and show internal granularity. Peripheral limiting membranes of the granules are not visible.

Glutaraldehyde fixation followed by post-fixation in osmium tetroxide or potassium dichromate. Two distinct types of granule are again visible in unstained sections and in sections stained with permanganate or briefly with lead citrate (Fig. 3). One type is intensely electron-dense and homogeneous, the other has a washed-out appearance and shows internal granularity. The former is found in the cells giving a positive colour reaction for the noradrenaline–glutaraldehyde complex, the latter in others presumed to store adrenaline. In tissues post-fixed in osmium tetroxide, both types of granules show a peripheral limiting membrane, while in those post-fixed in potassium dichromate typical peripheral membranes are not seen.

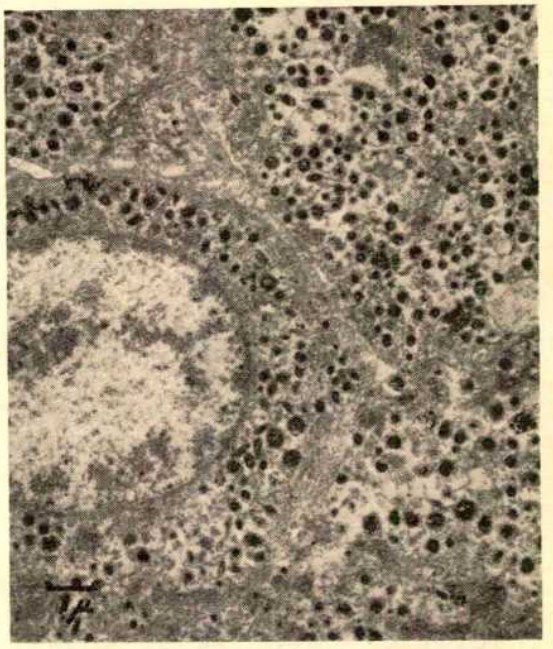


Fig. 1. Chromaffin cells of domestic fowl adrenal fixed only in buffered osmium tetroxide. All cells contain strongly electron-dense chromaffin granules

Since the previously mentioned findings indicate that glutaraldehyde is the essential reactive chemical in this method of differentiating adrenaline- and noradrenaline-storing cells, it was of interest to determine the fate of adrenaline, which is apparently unaffected by glutaraldehyde.

Various tests on mixtures of adrenaline and glutaraldehyde revealed that, for about 1 h after mixing, the secondary amine was still oxidizable with iodine and indeed that the method of Euler and Hamberg[4] could be applied to determine the quantity present. Glutaraldehyde had no effect on this colorimetric method. This method was, therefore, used to determine whether the adrenaline remained *in situ* or diffused out of the tissues during the course of fixation. Pieces of adrenal gland about 1–2 mm³ in volume were fixed 4 h in ice-cold cacodylate-buffered 4 per cent glutaraldehyde. During the fixation period the stoppered specimen jar was occasionally inverted to ensure adequate contact of fixative and tissue. Tissues were then dehydrated in the normal way in ethanol. Fixative and alcohols were assayed for adrenaline and noradrenaline and the results compared with those obtained by assaying homogenates (in 0·01 N hydrochloric or 0·4 per cent perchloric acid) of pieces of the same gland. Catecholamines present in homogenates were adsorbed on an alumina column[5], and eluted with 0·2 M acetic acid prior to colorimetric assay. The results, corrected for column yield (80 per cent), are shown in Table 1, and indicate that the majority of the adrenaline is lost during glutaraldehyde fixation and dehydration. This fact, together with the production of the glutaraldehyde–noradrenaline polymer, accounts for the differential staining reaction.

Table 1. CATECHOLAMINE CONTENT OF 4–6-MONTH-OLD COCK ADRENALS

Homogenate CA μg/g adrenal		Fixative + alcohols CA μg/g adrenal		Loss A
A	N	A	N	
4,310	3,180	4,100	0	95
4,800	2,300	4,700	0	98

CA = catecholamines, A = adrenaline, N = noradrenaline.

It is worth noting that the staining reaction of the glutaraldehyde-fixed noradrenaline-storing cells can be obtained many months after tissue fixation, provided the cells are stored in water, saline or glutaraldehyde. Hence,

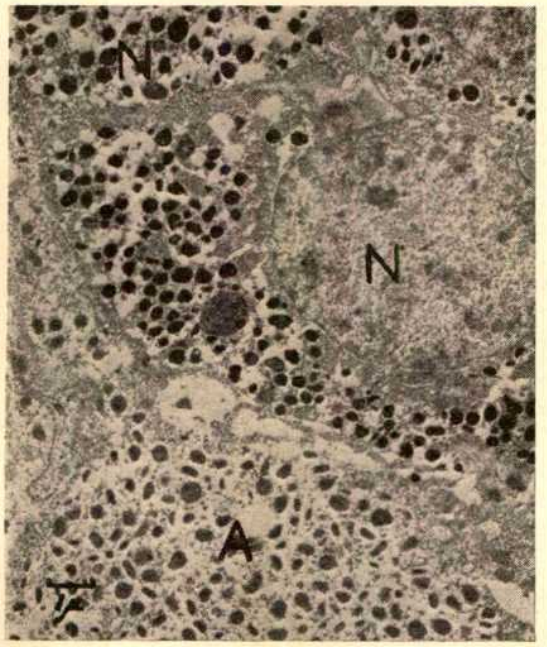


Fig. 2. Chromaffin cells of domestic fowl adrenal fixed only in buffered glutaraldehyde. Chromaffin granules of noradrenaline-storing (*N*) cells are strongly electron dense, while those of adrenaline-storing (*A*) cells show only moderate electron density

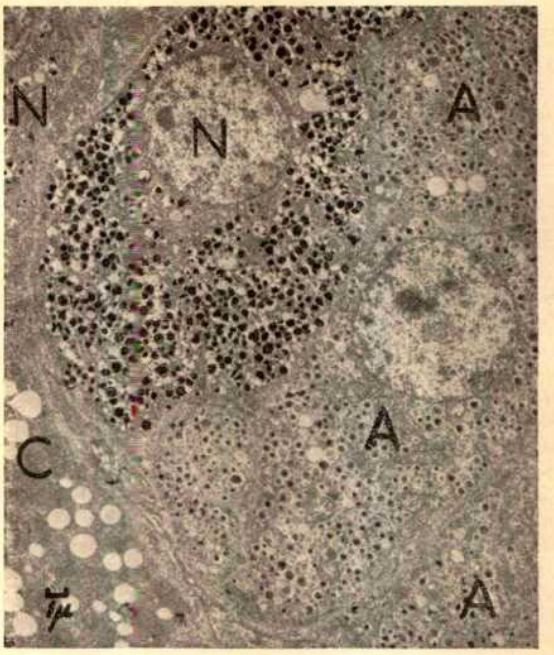


Fig. 3. Domestic fowl adrenal gland fixed initially in buffered glutaraldehyde and afterwards in osmium tetroxide showing noradrenaline-storing (*N*) cells with very strongly electron-dense granules, adrenaline-storing (*A*) cells with moderate electron-dense granules and cortical (*C*) cells

a delayed chromium, osmium or iodate reaction may be performed on frozen sections of this material. If the tissues are embedded in paraffin prior to treating with oxidizing agent, the reaction is reduced considerably and is of little histological value so far as the light microscope is concerned; block impregnation of glutaraldehyde-fixed adrenal with osmium tetroxide or potassium dichromate prior to embedding in paraffin does, however, result in a good demonstration of the noradrenaline islands, and this technique, or the use of frozen sections, is the method of choice for light microscopy.

The glutaraldehyde method for distinguishing between noradrenaline- and adrenaline-storing cells has recently been criticized by Benedeczky *et al.*[3], who claim, quite rightly, that adrenaline is more readily oxidized than noradrenaline; this fact has been generally accepted since 1949, when Euler and Hamberg introduced their colorimetric method for assay of the two amines. Unfortunately Benedeczky *et al.*[3] took no account of the fact that all tissues used by us had been fixed initially in glutaraldehyde, which for reasons given here is the most essential part of the technique. Cells showing morphological characteristics of "the light cell" illustrated by Benedeczky *et al.* may be observed frequently in adrenals fixed only in buffered osmium tetroxide, including glands obtained from animals such as the guinea-pig or rabbit which contain little or no noradrenaline. They are, however, artefacts due to bad fixation and have a specific zonal distribution in the tissue block. Although with care and experience the fixation artefacts associated with buffered osmium-tetroxide fixation of chromaffin tissue can be considerably reduced, more uniformly well-preserved chromaffin cells are obtained by initial fixation in glutaraldehyde followed by osmium tetroxide, which also has the advantage of allowing one to differentiate between the adrenaline- and noradrenaline-storing elements. Adrenaline- and noradrenaline-storing elements cannot be differentiated with certainty in tissues of the rat, mouse, cat, cock or frog fixed only in osmium tetroxide.

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VARIOUS CELL TYPES IN THE ADRENAL MEDULLA

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MOST electron-microscope and histochemical investigations of the nature of the adrenal medulla have been concerned with the reliable identification of adrenaline-producing and noradrenaline-producing cells. In 1950, and later in 1954, Euler[1] stated that "these two hormones (adrenaline and noradrenaline) are produced in different conditions and it could hardly be imagined that both were synthesized in one and the same cell". This theory was experimentally proved for the first time by Bänder[2] and Eränkö[3], who succeeded in demonstrating the existence of the assumed types of cells by histochemical methods. But with light-microscopy and histochemical methods it was not possible to obtain a satisfactory answer to the question of a possibly different sub-microscopic representation of adrenaline- and noradrenaline-containing granules in these two types of cells. The problem was attacked in a number of different ways by several workers. Hagen and Barrnett[4] isolated, by means of differential centrifugation, a fraction containing 'large granule'-noradrenaline, and one containing 'small granule'-adrenaline from a homogenized granule suspension of the adrenal medulla. Yates *et al.*[5] soon afterwards showed, by electron-microscopic and phase-contrast-microscopic methods, that granules of two orders of magnitude exist in different cells in the adrenal medulla of the golden hamster. Similar observations were made in the grass snake[6]. These workers also demonstrated, by means of differential centrifugation and electron-microscopic examination, the existence of large (2000–3500 Å) noradrenaline-containing secretory granules, and smaller (500–1500 Å) adrenaline-containing secretory granules in the homogenized adrenal medulla of the dog. However, the two types of cells described in the snake could not be identified in ultra-thin sections taken from dog and rat. An entirely different approach to the problem was used by Wassermann and Tramezzani[7]. Using various methods, they found only noradrenaline in the anatomically separable ribbon of the adrenal gland of the snake. With the electron microscope, they found and described noradrenaline granules of the polymorphic type in the medullary cells of the snake. In the central part of the adrenal medulla they found typical circular, membrane-limited adrenaline granules. They also mention that a reliable electron-microscopic demonstration of secretory granules is possible only after prefixation with glutaraldehyde, and that the size of noradrenaline granules is larger than that of the adrenaline granules.

The application of glutaraldehyde prefixation resulted in new possibilities, not only in electron microscope preparation techniques, but also in the electron-histochemical exploration of the adrenal medulla. The possibility of identifying the adrenaline and noradrenaline granules using the electron microscope was described by Coupland[8] and Wood[9] about the same time. According to Coupland[8], the noradrenaline-containing granules have a high internal density in the osmiophylic noradrenaline-storing cells, while the less electron-dense adrenaline granules are typical in the less osmiophylic, adrenaline-storing cells. Wood *et al.*[9], on the other hand, emphasize that, when prefixed with glutaraldehyde, the noradrenaline granules in the adrenal medulla of the golden hamster are highly electron-absorbent after oxidation with dichromate at *p*H 4·1, while if dichromate oxidation occurs at *p*H 6·5, both types of granule become highly electron-absorbent.

Coupland's findings were commented on by Benedeczky *et al.*[10], and they state that the "osmiophylic, noradrenaline-storing cells" ("dark cells" in Benedeczky's nomenclature) can also be identified using formalin prefixation in several animals (frog, grass snake, dog, rat). Benedeczky *et al.* see a relationship between the high internal density of the cells and the secretory granules in the cells, and the 'physiological and ontogenetic' state of the cell. In their recent investigations, Coupland *et al.*[11] have made a detailed analysis of the mechanism and circumstance of the noradrenaline–glutaraldehyde reaction. They suggest a number of proofs showing that this chemical reaction is specific for noradrenaline, and is thus suitable for a sub-microscopic identification of noradrenaline-containing granules, because the substances of adrenaline-containing granules react much less intensely to glutaraldehyde. In Coupland's opinion, the less electron-dense nature of adrenaline granules is due to the fact that the adrenaline content of the granule is dissolved in the course of fixation with glutaraldehyde and subsequent alcoholic dehydration. This circumstance makes it possible to preserve the glutaraldehyde–noradrenaline complex on the electron-microscopic level. Recent photographs[11] have also been taken of the adrenal medulla of the domestic fowl, but it is reported that in the adrenal medulla of the rat, treated with reserpine, it was not possible to identify reactive isolated spots. Thus, the question arises as to whether or not the electron-histochemical method used by Coupland *et al.*[11] is suitable for reliable identification of adrenaline and noradrenaline-containing granules not only in a given species but in the chromaffin cells of any animal, and whether the attempts of other research workers to explain the sub-microscopic appearance of the two types of hormone-producing cells with other methods and interpretations are tenable.

Naturally, it would only be possible to answer this question satisfactorily with a large amount of experimental data. Coupland's method and results seem very convincing, but some circumstances deserve consideration. It is primarily the evaluation of the results obtained with the ultracentrifuge that presents a problem in connexion with Coupland's recent findings. A large number of experiments seem to support the conclusion that the 'large granules', that is, those having a larger diameter and a higher sedimentation rate, contain noradrenaline. After differential-centrifugation of the homogenized adrenal medulla of the domestic fowl we found that 76 per cent of the fraction of large granules (showing a sedimentation rate of 3,000*g*/30 min after the nucleus fraction had been removed) contained noradrenaline. On the other hand, 48 per cent of pellets which settled at a rate of 24,000*g*/min proved to be adrenaline. Entirely similar results were obtained when the homogenized adrenal medulla of the dog was tested[12]. No conclusions on granule dimensions can be drawn from Coupland's illustrations because, owing to the dissolution of adrenaline, the contours of the granules are dimmed. Thus it cannot be decided whether the 'dense noradrenaline' granules show any difference in dimension compared with the adrenaline granules. The problem of how to reconcile the foregoing facts with Coupland's recent results remains, therefore,

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unsettled, since he has made available no data on the size of adrenaline and noradrenaline granules.

Coupland's results would, in our opinion, be more convincing if he gave data to show whether he has found any parallel between the number of noradrenaline-containing cells and the noradrenaline content measured in the adrenal medulla of the domestic fowl. It is well known that the adrenal medulla of the hen has a relatively high noradrenaline content. According to references in the literature, and our own observations[13], the noradrenaline/adrenaline ratio is about 2 : 1 (5,170 μg/g noradrenaline and 2,609 μg/g adrenaline). Accordingly, we found that the number of cells with small granules (those which, in our opinion, contain adrenaline) was rather low in the adrenal medulla of the domestic fowl after prefixation with formalin. But when prefixation with glutaraldehyde was applied we were unable to find any predominance of 'dense granule' cells (which, in Coupland's opinion, contain noradrenaline). Although Coupland *et al.*[11] give no numerical data on the ratio of adrenaline and noradrenaline cells, in Fig. 3 of their paper it is clear that there are six adrenaline-containing cells for every three noradrenaline-containing cells. But on the basis of our biochemical investigations a ratio of 1 : 1 might have been expected. Despite contradictory data and conclusions, it would seem that there is a possibility of reconciling the views of the two 'schools', if the following observations are taken as a basis. In one of our recent publications[6] we described the finding of cells containing 'small' and 'large' granules in the adrenal medulla of the grass snake after prefixation with formalin. The light transmission of the granules of adjacent cells (on the same photographic plate) was then measured, using a microphotometric apparatus. The results are given in Fig. 1. It can be seen from Fig. 1 that the light transmission of the small granules (500–1500 Å diameter), which, in our opinion, contain adrenaline, is much lower than that of the noradrenaline-containing large granules (2000–3500 Å diameter). Accordingly, the small adrenaline granules in Fig. 2 appear in a much less dense form than the noradrenaline granules of larger diameter, which are highly electron-absorbent. This fact is also clearly shown in the photographs by Wassermann and Tramezzani[7], who applied glutaraldehyde-prefixation. It would seem, therefore, that the noradrenaline granules, whether they are prefixed with formalin or glutaraldehyde,

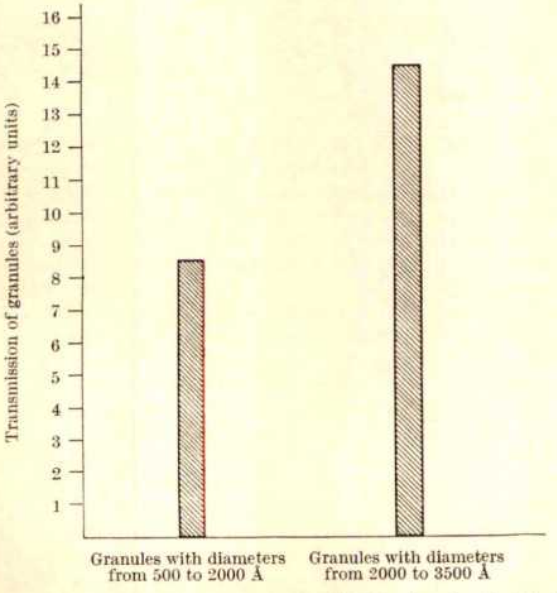


Fig. 1. Light transmission of the 'small' (left column) and of the 'large' (right column) catecholamine granules in the adrenal medulla of the grass snake. It can be seen that the light transmission of the small granules (those which, in our opinion, contain adrenaline) is much lower than that of the noradrenaline-containing large granules

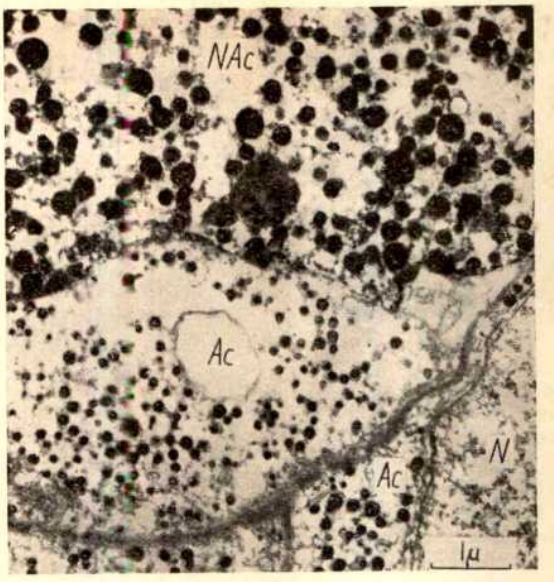


Fig. 2. Chromaffin cells of the grass snake adrenal, fixed in buffered formalin and afterwards in osmium tetroxide. The secretory granules of the noradrenaline cells (*NAc*) are larger and more electron-dense than in the adrenaline cells (*Ac*) (× 10,000)

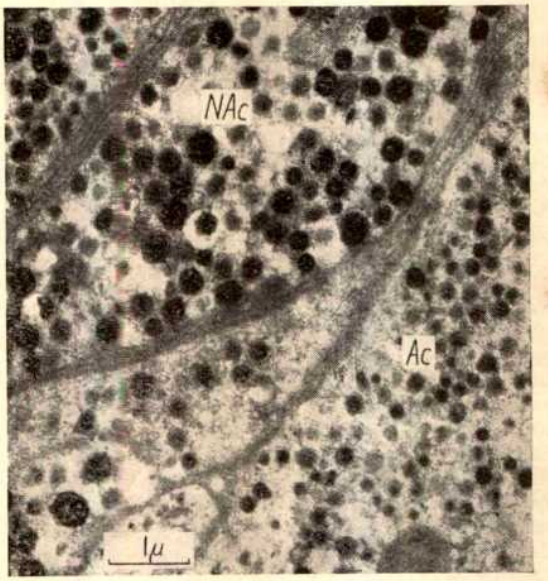


Fig. 3. Grass snake adrenal gland fixed initially in buffered glutaraldehyde and afterwards in osmium tetroxide showing noradrenaline cells (*NAc*) with 'large' (2000–3500 Å diameter) and very strongly electron-dense granules, and adrenaline (*Ac*) cells with moderately electron-dense and 'small' granules (500–1500 Å diameter) (× 10,000)

can be distinguished from adrenaline granules by their larger dimensions and higher internal density. But this electron-histochemical characteristic is not conclusively present in every species. It was not possible, for example, to identify the previously mentioned types of granules in the adrenal medulla of the rat with either formalin or glutaraldehyde prefixation. But both methods of prefixation of the adrenal medulla of the grass snake resulted in the appearance of typical 'small-granule' and less electron-dense cells, and, on the other hand, 'large-granule', highly electron-dense, noradrenaline-containing cells (Fig. 3).

Thus at present there exist some contradictions concerning the types of cells in the adrenal medulla, as different workers have used different techniques and investigated different animals, which makes the evaluation of results difficult. It is to be hoped that in the near

future workers will rely on identical experimental animals, and that it will be possible to resolve the persisting differences of opinion and obtain a more thorough knowledge of the types of cells in the adrenal medulla.

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A POSSIBLE FUNCTION FOR THE AXIAL ORGAN OF ECHINOIDS

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As any student of echinoderms is aware, the axial organ is a most puzzling structure. No function has yet been clearly shown for it. Though ideas have not been lacking, there has been much pre-occupation with oversimplified analogies with organs of very different character[1]. This situation arises partly because the living organ has been insufficiently observed and little experimental analysis has been attempted. Inferences as to its function have often been made from studies of its structure. This is especially dangerous where structure is imperfectly understood and it must be admitted that the histology of the organ is exceptionally difficult to interpret.

Recent investigations on echinoids have emphasized three cardinal features of the organ. First, it is permeated by an elaborate contractile structure[2]. Secondly, it is a central point of confluence of perivisceral coelom, 'haemal' system and water vascular system[1]. Thirdly, some echinoids can survive its removal, at least for several months[1].

The present investigation of the axial organs of *Arbacia punctulata* and *Strongylocentrotus dröbachiensis*, in addition to indicating a likely function, has clarified some important anatomical features. Thus, the numerous cavities at the periphery, which give the organ its spongy character, are not entirely 'haemal' as previously supposed, but are of two kinds. One type communicates directly with the perivisceral coelom by the canals already described[1,3]. These spaces constitute a system that is distinct from the 'haemal' spaces which surround them. The 'haemal' spaces can be distinguished by their dense content of cells, together with pigment and debris, some of which appears to be derived from disintegrating amoebocytes. The other spaces are lined by a characteristic shallow epithelium and, with the notable exceptions to be described, often appear relatively empty. The distinction is not absolute, for the walls which separate the two kinds of cavity disappear in places so that the contents of the 'haemal' spaces may enter the complementary spaces (Fig. 2). In the case of *Arbacia*, the peripheral 'haemal' spaces were traced into continuity with the central contractile vessel, through irregular, ill-defined channels permeating the strands of cells that traverse the central lumen of the organ to reach the vessel (Fig. 6). It is therefore evident that fluid from the contractile vessel might come into intimate relation with fluid from the perivisceral coelom at the periphery of the axial organ, since the two kinds of cavity are separated, at most, by the attenuated epithelium just mentioned.

Although the importance of the existence of canals by which the axial organ communicates with the perivisceral coelom has been emphasized[1], nothing tangible is known of their function. Amoebocytes and what appears to be secretion are found in them, but it is not clear in which direction they are passing. Initially, therefore, their openings were examined to see whether inward or outward currents could be detected.

Ciliary currents. Finely powdered carmine, sprayed from a fine pipette on to an excised axial organ of *Arbacia* examined in sea water, was quickly swept off by the powerful surface ciliation. The external openings of the peripheral canals were readily identified, but no inward or outward currents were detected, even when particles were sprayed directly into the openings. A powerful swirl of particles was sometimes seen on the outside surface.

Most significantly, however, an internal circulation was seen to occur in the clear peripheral cavities of the organ. This becomes evident when the axial organ is viewed by transmitted light because some of the cavities contain red amoebocytes which are swirled around in a minute whirlpool. The amoebocytes may aggregate so that a more or less compact ball or cyst is produced. When excised and examined alive, a cyst appears to consist of an envelope of red and colourless amoebocytes enclosing similar cells which are disintegrating (Fig. 1).

The rotating cysts, or circulating streams of amoebocytes, do not occur in the 'haemal' spaces but in localized swellings of the complementary system of peripheral spaces which communicate directly with the perivisceral coelom. Such dilated spaces may be termed follicles. Usually no more than two could be detected in a normal axial organ. The internal currents which produce the stream or rotation appear to be localized, for when cysts are displaced by gentle pressure into adjoining regions of the canalicular system, their rotation ceases and is resumed when they are restored to their original locus. Again, follic'es may be temporarily isolated by gently pinching them off with fine forceps, but the internal currents continue. When follicles are excised, however, their currents cease immediately.

It is remarkable that such appearances have not been described before, and so it was suspected that they might be artefacts due to immersion of the excised axial organ in sea water. This is not so, for they appear in excised organs examined in coelomic fluid taken from the same animal.

How the currents within the follicles are produced is unknown. Although they show every sign of being produced by internal ciliation, no such ciliation has yet been found in fixed material. In one case a small clot of coelomocytes that had formed in the coelomic fluid outside one of the follicles began to rotate in the same direction and with the same speed as the stream of amoebocytes within. It is difficult to envisage how this could occur except by the transmission of motion through one of the superficial openings, and this may be correlated with the rotary currents detected on the outside of the organ. Although not impossible, it appears unlikely that the

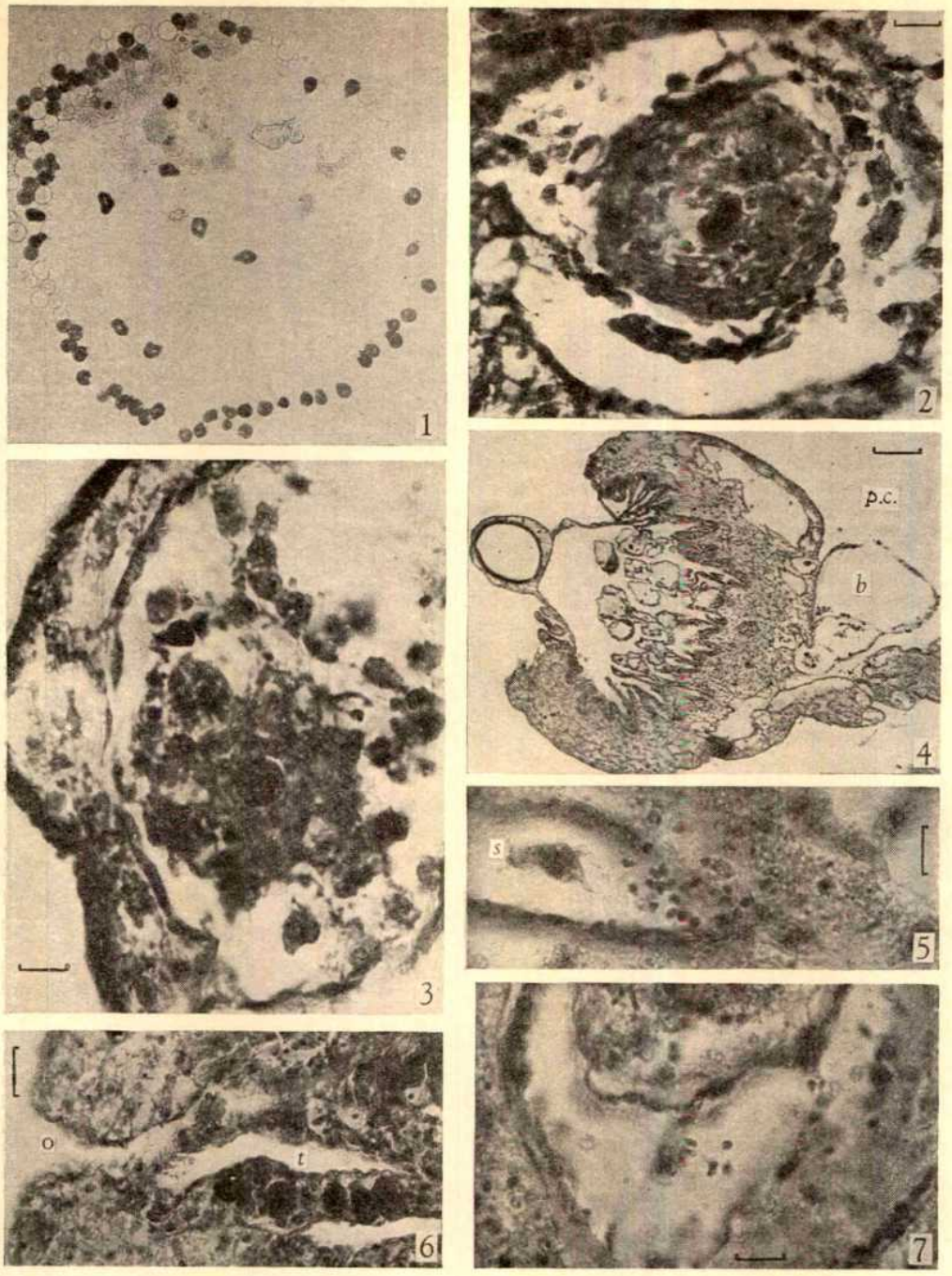


Fig. 1. A living, unstained cyst, excised, while still rotating, from a follicle of the axia organ of *Strongylocentrotus*. The dark bodies are red amoebocytes

Fig. 2. A follicle and its contained cyst in the axial organ of *Strongylocentrotus*, 73 h after part of the perivisceral coelomic fluid had been replaced by coelomic fluid from *Asterias forbesi*. Note the concentric layers of coagulum enclosing coelomocytes and the low flat cells forming the wall of the follicle which has broken down in places. Cells from the wall, together with those from the surrounding haemal space, are invading the follicle. Fixed with Susa in sea water and stained: Mallory. Scale: 13μ

Fig. 3. A follicle and cyst from *Arbacia* 3 days after introducing sperm of the same species into the perivisceral coelom. Note the centre of the cyst, formed of what appears to be compacted sperm heads, surrounded by phagocytes. Fixed and stained as in Fig. 2. Scale: 8μ

Fig. 4. Transverse section of the axial organ of *Arbacia*, 24 h after injecting a culture of *Uronema* with bacteria into the perivisceral coelom. Note the follicle that has distended into thin-walled bladders (*b*), containing amoebocytes and extending freely into the perivisceral coelom (*p.c.*). The bladders appear to have formed successively. No distension of the central lumen is evident in this region. Fixed and stained as in Fig. 2. Scale: 140μ

Fig. 5. A follicle from the same axial organ as that shown in Fig. 3, showing the presence of sperm, some of which (on the right) are free, so that their tails are visible. One sperm cell (*s*) has been ingested by a phagocyte. Fixed and stained as in Fig. 2. Scale: 9μ

Fig. 6. Portion of the axial organ of *Arbacia*, showing a channel within the shaft of cells (*t*), connecting the contractile vessel (on the right) with the peripheral 'haemal' spaces (on the left). The channel is filled with deeply staining amoebocytes. *O*, marks an opening into the perivisceral coelom. Fixed and stained as in Fig. 2. Scale: 20μ

Fig. 7. One of the canals leading from the perivisceral coelom into a follicle of the axial organ of the same urchin as that used in Fig. 3. The dark conical heads of sperm cells, which mostly appear free, can be seen within the canal. Fixed and stained as in Fig. 2. Scale: 8μ

motion is due to the effects of external ciliation spreading into the follicle, because internal currents were often seen below regions where no corresponding external currents could be detected.

Follicular activity appears to be phasic, for follicles were observed in both normal and experimental animals that showed no signs of such activity and contained static cysts. Moreover, the rotation of cysts has been seen to start and stop, lasting for some 30 min.

Experiments with markers. Knowing the phagocytic properties of echinoid amoebocytes, it was suspected that such an elaborate mechanism might be more than merely a means of disposing of effete amoebocytes. Accordingly, cysts were examined following the introduction of identifiable foreign matter into the perivisceral coelom. Initial crude experiments, in which suspensions of indian ink in sea water were injected, revealed nothing in so far as none of the substance appeared in the axial organ. Some was ingested by amoebocytes, but most remained in clots within the perivisceral coelom.

When foreign cells were injected these suspicions were confirmed. Thus when a portion of the coelomic fluid of *Strongylocentrotus* was replaced by an equivalent quantity of coelomic fluid freshly withdrawn from *Asterias forbesi*, examination of stained sections of an organ fixed 73 h later showed the presence in the axial organ of numerous cysts. They consisted of coelomocytes wrapped in coagulum, which appeared to be in concentric or spirally wound layers (Fig. 2), presumably as a result of the continued rotation. Such cysts were found in the central lumen of the organ, but more especially in the peripheral follicles. Comparison with a smear of starfish coelomic fluid, stained in the same way, indicated that many of the cells in the cysts were starfish coelomocytes.

Bearing in mind the difficulty of distinguishing such cells with certainty from some types of the urchin's own coelomocytes, especially after enclosure within a cyst and subsequent fixation, the experiments were repeated using readily identifiable cells. For this purpose, sperm freshly discharged from *Arbacia* was suspended thickly in sea water and a little was mixed with about 2 ml. of coelomic fluid freshly withdrawn from the same individual or from another of the same species. The mixture was quickly injected into the perivisceral coelom of the individual from which the coelomic fluid had been taken. Sections of the axial organ fixed 3 days later showed clearly the presence of sperm in the follicles (Fig. 5) and in the canals opening to the surface (Fig. 7). Some of the sperm appeared to be free; more was enclosed in aggregated amoebocytes which formed cyst-like masses within the follicles (Fig. 3), but most of it appeared in large clots adhering to the outside of the organ.

Such elaborate phagocytosis is frequently accompanied by grosser changes in the axial organ, such as distension of the central and peripheral cavities and stone canal. In *Arbacia*, the effect of sperm was so marked that the dilated peripheral spaces protruded from the surface as large thin-walled bladders. Even more striking were the changes induced by injecting into the perivisceral coelom 0·05–2·0 ml. of a culture of the ciliate *Uronema* with bacteria. Six to 48 h after injection, the axial organ, normally somewhat elongated, had swollen to become almost spherical, its central cavity being enormously dilated, while the peripheral cavities and stone canal had expanded into a crop of large thin-walled bladders (Fig. 4). Both bladders and central cavity showed vigorous circulatory streaming of amoebocytes. These cells sometimes aggregated in the perivisceral coelom in the vicinity of the organ, producing a conspicuous red cloud and in some instances a very obvious quantity of red pigment was deposited in the body cavity, presumably following their dissolution. Similar, but less extensive, changes followed the injection of bacterial culture alone.

The effect of clotting the perivisceral coelomic fluid. The indications of clotting and cell dissolution are suggestive, knowing the marked tendency of echinoid coelomic fluid to respond in this way, following injury, when exposed, or in the presence of foreign bodies[4]. The effect of clotting the coelomic fluid on the axial organ of *Arbacia* was therefore examined. About 2 ml. of perivisceral coelomic fluid were withdrawn into a sterilized hypodermic syringe, in which it clotted rapidly. To assist breakdown of coelomocytes in the clot, the syringe was shaken and in some cases air was briefly bubbled through it. The fluid, together with the clot, was quickly re-introduced into the perivisceral coelom of the same urchin. The effect became clear in 4–10 h. In 'mild' cases the organ was greatly swollen or bore a crop of large or small blisters. In others the whole syndrome appeared, the central cavity of the organ being greatly swollen, the follicles dilated and the blisters expanded into thin-walled bladders. An impressive streaming of amoebocytes was evident in all these cavities.

The specific importance of clotting was shown by repeating the foregoing procedure using a sterilized syringe which had been rinsed with a 10 per cent solution of disodium ethylenediaminetetraacetate in sea water. This inhibited clotting, and the effects on the axial organ of re-introducing coelomic fluid so treated were drastically reduced.

The significance of the reaction. It is clear that the axial organ can take up directly, or ingested in phagocytes, organisms or cells that contaminate the perivisceral fluid and deal with them in an elaborate, well-defined way. The ultimate fate of the cysts and 'foreign' cells has yet to be determined. Similarly, the way in which such extraneous matter enters the organ is unknown, but the presence of the injected cells, both free and ingested in phagocytes, in the canals leading into the organ from the perivisceral coelom, is suggestive. Nevertheless it will be remembered that inward ciliary currents have not yet been detected in these canals. Perhaps the rhythmic pulsations of the contractile vessel are significant in this connexion. Thus the pulsing of 'haemal' fluid through the vessel could exert changing pressures in the periphery of the axial organ and so be indirectly responsible for moving perivisceral fluid in and out of the openings on the surface and the associated system of canals.

Discharge of this decontaminating function is accompanied by equally well-defined overt changes in the axial organ and similar changes are induced by the presence of a clot of the animal's own coelomocytes in its perivisceral fluid. The circumstances are not disparate, in the sense that both are associated with injury. Thus wounds are rapidly invaded by amoebocytes and sealed by a clot formed by the change or breakdown of these cells. At such times invading organisms may enter the tissues or coelomic fluid and their presence in the latter may induce further clotting. The reactions of the axial organ could therefore be part of a defensive injury response in which the organ is 'alerted' or its activity is triggered by 'injury substances' released into the coelomic fluid by the breakdown of amoebocytes; but this has yet to be shown.

In conclusion, two points should be emphasized. The possible reactions of other organs to the experimental conditions described have not been studied, though cursory examination failed to reveal anything so obvious as those of the axial organ. No claim is made that this may be the sole function of the organ.

I thank Dr. G. Holz for cultures of *Uronema* and bacteria, Dr. R. Boolootian and Mr. Z. Podhorodecki for help with photography, and Drs. Boolootian and Farmanfarmaian for advice. I also thank Dr. W. D. Russell-Hunter for providing facilities for the work, which was supported by a grant from the National Science Foundation, *G.B.*-3447, made to the Department of Invertebrate Zoology of the Marine Biological Laboratory.

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FOUR SIBLING SPECIES INCLUDED IN *Simulium damnosum* THEOBALD (DIPTERA : SIMULIIDAE) FROM UGANDA

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THE black fly *Simulium damnosum* was described in 1903 by F. V. Theobald from material he collected in the Nile provinces of Uganda[1]. The name he chose, *damnosum*, reflects an opinion of the fly's voracious biting habits. In 1923, in Sierra Leone, on the other side of Africa, D. B. Blacklock identified *S. damnosum* as a vector of the filarial worm *Onchocerca volvulus* which produces in man the disease onchocerciasis, or 'river blindness'[2]. As the seriousness and widespread occurrence of this disease became apparent it was realized that this black fly was the most important vector of onchocerciasis. The habits of this fly have since been extensively studied in the hope of eliminating it and controlling the disease. From these investigations one point stands out, the extreme variability of its habits[3]. For example, in some areas the fly bites man at low altitudes but not at higher altitudes; in different regions the reverse situation seems to be true. On the Nile River larvae may be found at the brink of a waterfall or in quiet backwaters where the only movement is wave motion[3]. Because of this variability several field workers have begun to suspect that more than one species is involved[4]. Yet morphological variations between specimens from widely distributed localities are so slight that most taxonomists have considered *S. damnosum* to be a single species. One possible way of resolving whether or not *S. damnosum* is one or more species is to use the technique which has been successful in solving similar Holarctic black fly problems, the examination of the larval salivary gland giant polytene chromosomes[5–7]. In these insects they are large and often very clear (Fig. 2). This article describes how such a cytotaxonomic survey of larvae from Uganda, identified as *S. damnosum*, disclosed the presence of four distinct cytological categories.

In an ideal species, one which has only one giant chromosome banding pattern, all the individuals will share this pattern. The most frequent alterations to this standard pattern are two-break rearrangements in which the interstitial segment becomes inverted. Such inverted segments are called 'inversions'. Within a species these inverted and original sequences can be expected in homologous chromosome pairs in any of three ways: both chromosomes having either (1) the standard sequence or (2) the inverted sequence (the two alternate 'homozygous' arrangements), or (3) one chromosome being standard and the other inverted (the 'heterozygous' arrangement). The finding of this last category, the heterozygote, establishes that an inversion is part of the chromosomal polymorphism of a species, that is, it is an intraspecific ('floating') inversion[5]. Species have usually a few to many floating inversions.

When the banding patterns of closely related species are examined there are almost always, in black flies, one or two inverted sections which appear to be like the two alternate homozygous arrangements already mentioned. However, the intermediate heterozygous arrangement is never found. The best interpretation for this is that there are two non-interbreeding populations, that is, two species. These interspecific ('fixed') inversions are the main evidence for the establishment of the cytological categories which are characteristic of different species[5]. Secondary evidence for these categories comes from the floating inversions in that two species rarely share a floating inversion, although this is theoretically possible. In practical terms a floating inversion can be assigned to one specific category. Cytotaxonomic identification of a species is not dependent on variable (or in black flies, almost invariable!) morphological characters, but is dependent on the observation of the chromosomal banding pattern which undergoes virtually no phylogenetic change except by clearly defined, discrete steps, such as inversions. Precise cytological categories, therefore, are provided by the presence or absence of inversions.

There are also micromorphological characters in the chromosomes which are very useful in the identification of a species, a chromosome or even a chromosome arm. Most of these are prominent features within the banding pattern and have been given descriptive names (Figs. 1 and 2). Although their expression may vary, the real value of these characters is in their relative positions within the banding pattern where they serve as ready markers. Other micromorphological characters[8] include the chromosome number, the degree of pairing of homologous chromosomes, the expanded regions associated with centromeres and the degree of fusion, if any, of the centromeres.

The material used in this investigation came from three localities: (1) Sebwe River, Toro (Ruwenzori); (2) Nile River, Kagela Falls, 14 miles downstream from Jinja; (3) Kagagala River, near Kikagati, Kyansore Island, Ankole (Table 1). The larvae were

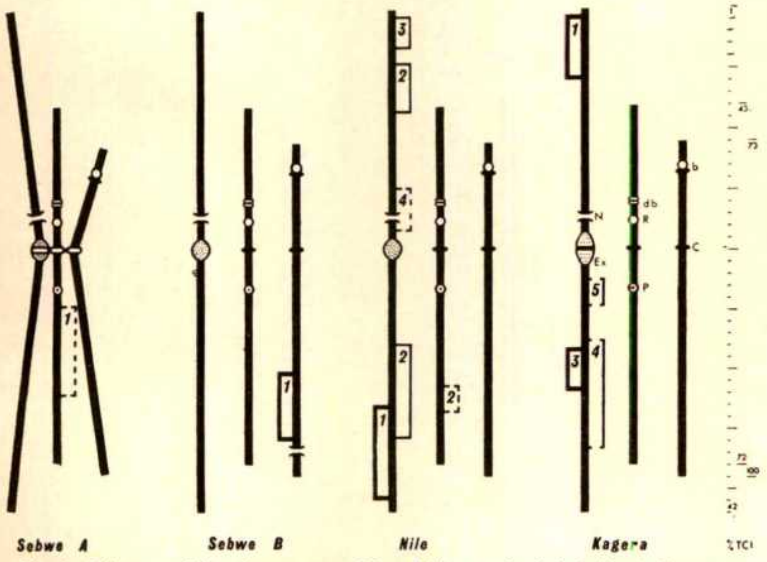


Fig. 1. Idiograms of the chromosomes of four sibling species included in *S. damnosum*. The inversions and chromosomal features are plotted according to their percentage position in the total complement length (percentage TCL). Brackets indicate inversions. Heavy brackets to the left represent interspecific inversions. Brackets to the right represent floating inversions; solid brackets, common floating inversions (more than 40 per cent heterozygotes); broken ones, uncommon floating inversions (less than 4 per cent heterozygotes); detached brackets, inversions restricted to *Y* chromosomes. *C*, Centromere (ref. 8); *N*, nucleolus (ref. 8). Descriptive names: *b*, 'blister' (ref. 13); *db*, 'double bubble' (refs. 7 and 14, 'bulge'); *R*, 'ring of Balbiani' (refs. 8 and 14); *P*, 'para-Balbiani' (ref. 6); *Ex*, 'expanded region' associated with centromere (ref. 8)

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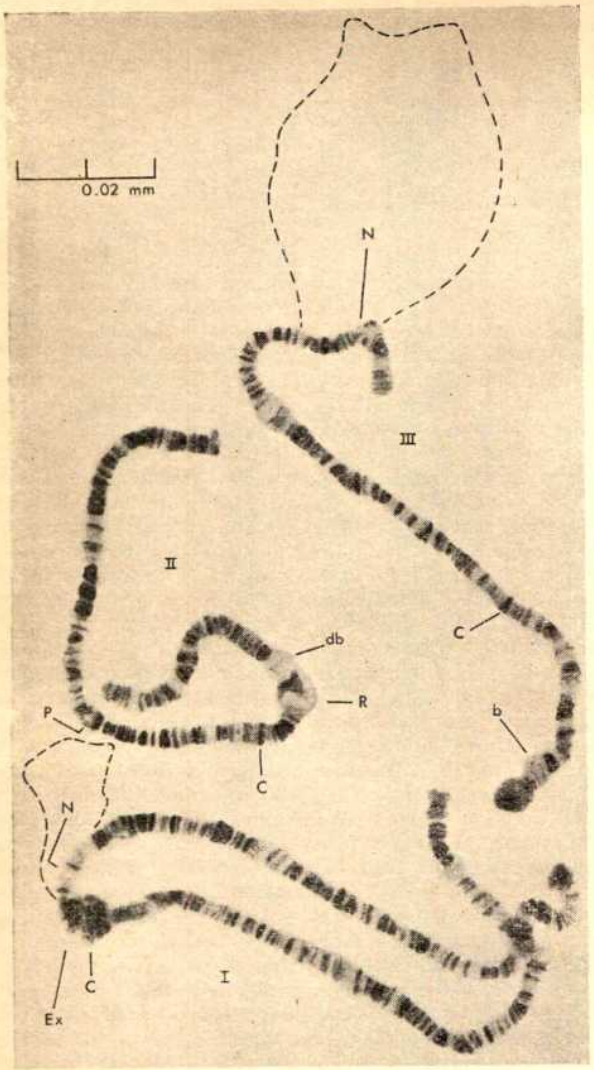


Fig. 2. Giant chromosomes of 'Sebwe *B*' (No. 1693.63). Broken lines show the outlines of the nucleolar masses

fixed in Carnoy's fixative, stained in leucobasic fuchsin, and the slides made permanent by the dry ice method[5,8,9]. A modified Leitz 'Ortholux-Orthomat' microscope–camera combination was used for analysis and photo-micrography. A photographic map was made of the chromosomes of a specimen (No. 1693.63) from the Sebwe River (henceforth referred to as 'Sebwe *B*'). All the slides were compared with this map. The four siblings were provisionally named after the rivers from which they came. They are identified as 'Sebwe *A*', 'Sebwe *B*', 'Nile' and 'Kagera'. The details of this analysis are summarized in the idiograms (Fig. 1) which depict graphically the features of the cytological categories to be described. The inversions and the micromorphological characters have been located by plotting their percentage positions within the total complement length.

The close relationship of these four siblings is shown by the basic similarities of their chromosomes. All four have three pairs of tightly paired chromosomes, and arm-length ratios about the centromeres of 1 : 1·1, 1 : 1·5 and 1 : 1·7. They share the same basic banding pattern except for the few interspecific inversions. They have the same relative positions for the main nucleolus and the micromorphological characters of the second and third chromosomes. In these siblings only the first chromosome has an expanded region associated with the centromere. The centromeres of the other two chromosomes are indistinguishable from a large band.

Table 1. Collections of *S. damnosum* Complex and the Number of Slides of Each Sibling Analysed

Location:	Sebwe River		Nile River		Kagera River		Total
Date:	31/1/63	10/3/63	28/3/64	8/4/65	10/3/63	11/4/64	
Sibling							
Sebwe *A*	11	25					36
Sebwe *B*	8	23					31
Nile			27	20			47
Kagera					4	44	48

Sebwe *A* was chosen, because of its phylogenetically central position, as the basis for comparisons (Fig. 3). Its banding pattern, therefore, is the standard against which the others are compared. This is almost a chromosomally monomorphic species since only a single heterozygous inversion configuration was found. It is in the second chromosome (No. II), short arm (*S*) and was the first inversion in any sibling found in that arm; it is inversion II*S*-1. Another feature of this sibling is that the centromeres are tightly fused, but no chromocentre is formed. The centromeres also appear 'glassy' rather than dark as in the other siblings.

Sebwe *B* differs from Sebwe *A* by a single interspecific inversion in the third chromosome, III*L*-1. This appears to be a monomorphic species since no floating inversions were found. Sebwe *B* has a second nucleolar organizer in the third chromosome distal to III*L*-1, the only sibling with this feature.

Nile differs from Sebwe *A* by a single interspecific inversion in the first chromosome, I*L*-1. This is a relatively polymorphic species with four floating inversions in the first chromosome, three of which are common, and one in the second chromosome, I*S*-2, I*S*-3, I*S*-4, I*L*-2 and II*L*-2.

Kagera differs from Sebwe *A* by two interspecific inversions in the first chromosome, I*S*-1 and I*L*-3. The only two floating inversions, I*L*-4 and I*L*-5, were always found heterozygously, together and only in males. Therefore this sibling has a distinct *X* chromosome with I*S*-1 and I*L*-3, and a distinct *Y* chromosome with I*S*-1, I*L*-3·4 and I*L*-5. It is the only sibling in which the *X* and *Y* chromosomes can be distinguished. The expanded region is slightly larger than in the other three siblings and has a distinct band marking the centromere.

Interspecific inversions not only define cytological categories, but they also show phylogenetic relationships[5]. If the interspecific differences between Sebwe *A* and the other three are plotted as in Fig. 3, then to find the difference between any peripheral pair it is only necessary to 'add' the inversions along the lines. Thus, for example, the difference between Sebwe *B* and Kagera is three interspecific inversions, I*S*-1, I*L*-3 and III*L*-1. This is precisely what is found. Unfortunately without further information it is impossible to determine phylogenetic direction[10].

Sebwe *A* and Sebwe *B*, because they are sympatric, are clearly a pair of species, and Sebwe *B*, because of its position in the phylogenetic scheme, is a species distinct from the others. However, there is a lack of evidence to eliminate entirely all possible arguments that certain of the remaining three siblings represent isolated populations of the same species[5]. The distance between the Sebwe and Kagera Rivers is less than 100 miles, and there is a little more than 100 miles between the Sebwe and Nile Rivers. With probable breeding sites for any of these siblings in the intervening areas, and considering the flight range of these insects, it is practically impossible that such large cytological differences could represent geographic variants of the same, but widespread, species

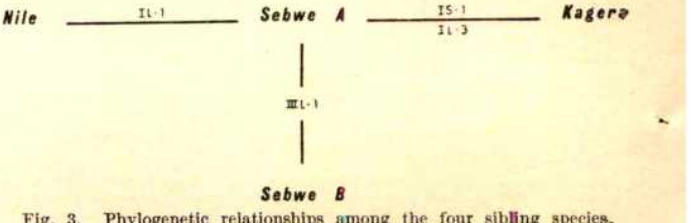


Fig. 3. Phylogenetic relationships among the four sibling species. Interspecific inversions are shown between the siblings

around Lake Victoria. These doubts could be dispelled by the examination of intermediate populations and the establishment of ranges for each sibling.

It is a reasonable conclusion, therefore, that these four siblings, Sebwe *A*, Sebwe *B*, Nile and Kagera, represent four distinct but closely related species. The sibling identified here as Nile may be *S. damnosum* since the type locality is the 'Jinja fly belt' at the mouth of the Nile. It also raises the possibility that some of Enderlein's names for species in this group may be valid[11,12].

Complete chromosome maps for these four species will be published at a later date. It is also hoped that for this future report it will be possible to examine materials of the *S. damnosum* complex from other parts of Africa.

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PLANARIANS AND MEMORY

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I. Transfer of Learning by Injection of Ribonucleic Acid

SEVERAL recent investigations of planarians have contributed suggestive evidence regarding the biochemical basis of memory. McConnell[1] and John[2] found that cannibal worms which had ingested classically conditioned worms later performed better than did cannibals which had ingested untrained worms. Corning and John[3] obtained evidence that ribonuclease may 'wipe out' conditioning in regenerating planarians. Two preliminary reports[4] have indicated that injection of ribonucleic acid (RNA) extracted from trained planarians may enhance responding in untrained planarians.

Two related factors have delayed full acceptance of these findings: the controversy concerning the validity of classical conditioning in planarians, and the absence of pseudo-conditioning controls in several of the aforementioned investigations (for discussion of these problems, see ref. 5). The experiment described here was carried out in an attempt to resolve these questions, at least in part, by showing that (*a*) classical conditioning effects can be easily distinguished from pseudo-conditioning effects in planarians, and (*b*) enhanced responding is produced by injection of RNA from classically conditioned but not from pseudo-conditioned or non-conditioned planarians.

675 planarians (*Dugesia dorotocephala*) of about 1 cm length were used, which had been obtained from West Coast Biological Supply Co., Los Angeles. They were maintained and trained in spring water and were not fed during the course of the experiment (the composition of the water, as provided by the laboratory of Sparkletts Drinking Water Corp., was 30 mg/l. calcium, 15 mg/l. sodium, 72 mg/l. chlorine, 12–14 mg/l. bicarbonate, 25 mg/l. silica, and 0·1 mg/l. fluorine). The planarians were handled with a sable brush. The room light was diffused and was on only during the day.

The planarians were trained in a rectangular plastic trough measuring 14·5 cm × 5·5 cm × 5·5 cm and filled with spring water to a depth of 1·5 cm. Aluminium plates which covered the two long walls of the trough served as electrodes. A potential of 10 V peak 60-c/s a.c. was connected to the electrodes by a clock device. Current flow through the volume of water was 2·5 m.amp peak. The conditioned stimulus (CS) was provided by a 75-W bulb in a 25-cm reflector suspended 22 cm above the trough. A piece of paper covered the reflector and diffused the light. The illumination at the trough measured 13 ft.-candles with the CS-light off and 400 ft.-candles with the CS-light on. All stimulus and interval durations were controlled by Hunter clocks.

Three groups of 200 planarians each were used in the first stage of the experiment. One group (non-conditioned, hereafter referred to as group NC) was simply permitted to rest in their home containers. A second group (classically conditioned, or CC) was given training consisting of paired presentations of light and shock. Before these training trials were undertaken, group CC was given fifty 3-sec presentations of light alone in two sets of twenty-five trials separated by a 10-min interval. The interval between light presentations within a set was 20 sec. Then, following another 10-min interval, training was instituted. A training trial consisted of a 3-sec presentation of light, during the last second of which the shock was also applied. The inter-trial interval was 20 sec. On the first day of training, group CC received two sets of twenty-five conditioning trials, with 10-min intervals between adjacent sets, in addition to the fifty light-alone trials. On the next day, group CC received three further sets of training trials.

The third group (pseudo-conditioned, or PC) was trained in the same fashion as was group CC, except that for group PC light and shock were presented in a fixed interspersed sequence, rather than being paired. Whereas group CC received one light-shock pairing every 20 sec, group PC received a stimulus, either light or shock, every 10 sec. The total number of lights and shocks received by group PC in a given trial set was equated to that for group CC, and shock was never given to group PC more than twice in succession. At the beginning of each day's sessions, all worms were adapted to the trough for 10 min before training was commenced.

On each presentation of light, each worm in groups PC and CC was scored according to whether or not it responded during the first 2 sec. A response was defined as a discernible longitudinal contraction of the body, this being the unconditioned response to shock. Other changes in locomotion, including stopping, or lifting, wagging, or turning the head, were not counted as responses. On eight separate occasions, two observers made independent judgments on a set of twenty-five trials. Agreement ranged from 23 out of 25 to 25 out of 25; overall, the two judges agreed on 192 out of 200 trials.

Twenty conditioned and twenty pseudo-conditioned worms (randomly selected) were scored individually for

the fifty light trials and 125 subsequent training trials. The performance levels of these animals are presented in Fig. 1. The differentiation between the two groups in their responsiveness to light is apparent and is further indicated by the ranges of scores for the last two training sets: on set 4, the range for CC was 10–17, that for PC 0–5; on set 5, the range for CC was 13–17, that for PC 0–6.

An additional five sets of trials were given to groups CC and PC, three sets on the day preceding extraction (described below) and a final two sets on the day of extraction. During the last trial set, four worms from each of the groups were scored individually. The four CC worms scored 14, 15, 16, 17; the four PC worms scored 1, 2, 3, 3.

After the completion of training, RNA was extracted from each of the three groups of planarians by a phenol-extraction technique (for details of the procedure, see ref. 6). The 200 worms of a given group were pooled together for an extraction. The RNA from each group was dissolved in 700 μl. of isotonic saline. The amount of RNA was determined from the optical density at 260 mμ (ε^p = 7,450 in 0·2 M sodium chloride). One extract was used for the determination. The yield was approximately 3·4 mg/1·0 g of tissue. During the extractions, the experimenters did not know the identity of any group.

Four hours after extraction, the RNA from each of the groups (NC, PC and CC) was injected into twenty-five untrained planarians. Fourteen hours before injection began, each of these 75 recipient worms had been given 100 light-adaptation trials, in two sets of fifty trials separated by a 10-min interval. The inter-stimulus interval within a set was 20 sec. The injections were performed under a binocular dissecting microscope in the following manner: a metal cap was filled with paraffin, and a depression approximately the size of a planarian was cut into the paraffin. The metal cap was placed in a Petri dish and the latter was placed in a container of dry ice. A planarian was placed in the depression. Within 1 min the low temperature resulted in the planarian becoming quiescent. When this was so, 20 μl. of the appropriate solution was injected into the body cavity by way of the pharynx. A 100-μl. No. 710 Hamilton syringe with a 1·3 cm 30-gauge needle was used, and a finely drawn-out capillary tube was glued to the needle. The capillary tube was replaced as necessary during the injections. Like the extractions, the injections were conducted in 'blind' fashion; that is, the research worker carrying out the injections did not know to which group the planarian which he was treating belonged.

Twenty-four hours after the injections had been completed, the 75 injected worms were tested individually. Each worm received twenty-five presentations of light alone, spaced 20 sec apart, and responses occurring during the first 2 sec of the light were recorded. No shock was ever given during testing. Again, all testing was conducted 'blind'.

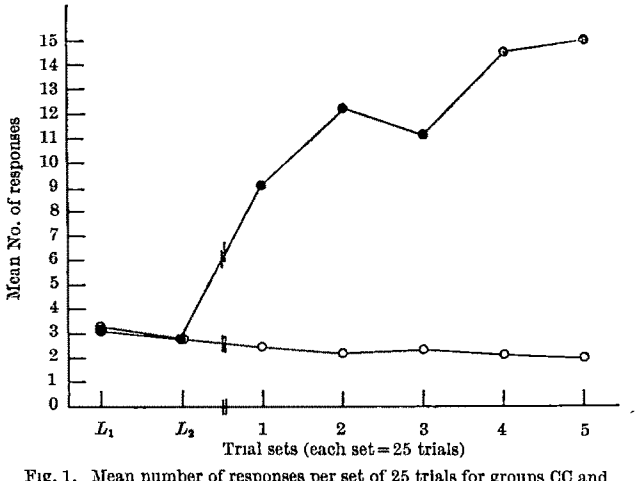


Fig. 1. Mean number of responses per set of 25 trials for groups CC and PC. On the abscissa, L_1 and L_2 are sets in which light alone was presented, and 1–5 represent training sets. ●, CC (N = 20), ○, PC (N = 20)

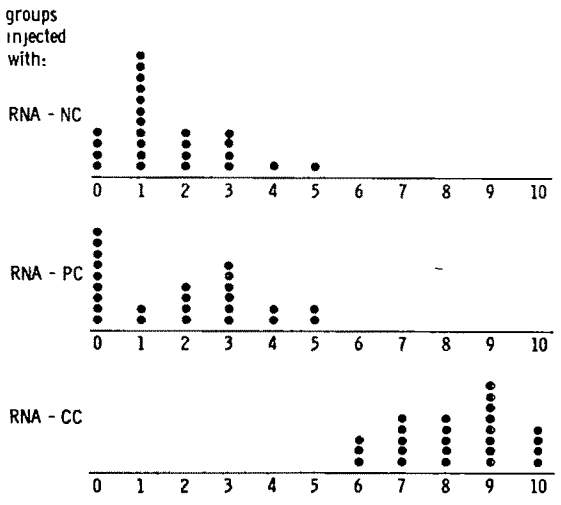


Fig. 2. Distribution of scores (responses per 25 trials) for injected planarians

Fig. 2 presents the number of responses made by each worm in the test series. The mean for planarians injected with RNA from group CC was 8·2, that for the 'PC-injected' group was 1·8, and that for the NC-injected group was 1·6. By a Mann–Whitney U test[7], the group injected with RNA from classically conditioned worms was superior in performance to either of the other injected groups ($P < 0·001$). As Fig. 2 shows, the ranges of the two control groups, on one hand, and of the experimental group, on the other, did not overlap.

Several conclusions can be drawn from this experiment: First, classical conditioning in planarians is a distinct and valid phenomenon, and pseudo-conditioning effects, at least under the present conditions, are negligible. This finding corroborates that of Baxter and Kimmel[8], but in the present experiment the differences between groups were much more pronounced. Secondly, injection of the RNA preparation from group CC was sufficient to induce responsiveness to the light which could not be accounted for on the basis of nutrition, handling, or stimulation received by the donor group. Rather, the critical feature for both the conditioning effect and the RNA transfer effect was apparently the contiguity between the stimuli (light and shock). Since such contiguity is generally considered to be the defining characteristic of 'true' conditioning, the present data would seem to suggest that a specific learned response was transferred by way of the injection of the RNA preparation. This conclusion is strengthened by the fact that no shock was used during testing, and thus no new learning could have occurred.

We have conducted two supplementary experiments designed to ascertain whether RNA was in fact the effective transfer agent: (1) RNA extracted from CC and PC groups by the phenol method as previously described was further purified by 'Sephadex' chromatography (*G*-50 coarse beaded form; 1 mM magnesium acetate 5 mM *tris*-acetate, *p*H 7·2) and methylated albumin chromatography (step-by-step elution with 2·0 M sodium chloride, 0·05 sodium phosphate, *p*H 6·7). A significant difference in the levels of response was found between the two groups injected with these purified preparations (mean of experimentals, 7·8; mean of controls, 2·9; $P < 0·001$). (2) RNA from CC animals was degraded by treatment with 0·3 N sodium hydroxide (16 h at 37° C). Recipients (group 2) of this degraded RNA performed at about the same level as did recipients (group 3) of RNA from PC animals, and both were significantly lower in performance than recipients (group 1) of RNA from CC animals (mean of group 1, 8·4; mean of group 2, 4·7; mean of group 3,

5·7; group 1 versus group 3: $P < 0.01$; group 1 versus group 2: $P < 0.001$).

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II. The Influence of Prior Extinction on the Ribonucleic Acid Transfer Effect

A NUMBER of experiments, including the preceding article, appear to implicate RNA in memory functions. The most prevalent approaches attempt either to correlate RNA changes with a learning experience[1], or to transfer behavioural tendencies from one organism to another by injection of RNA[2]. These experiments and the relevant theory generally concern the acquisition (learning) of new behaviour patterns. Any adequate biochemical concepts of memory must also, however, account for the diminution or loss of learned behaviours which typically occurs in experimental extinction. Proponents of the RNA hypothesis might adopt either of two positions on this point: (*A*) experimental extinction acts to modify (either directly or indirectly) the RNA which was (hypothetically) modified during acquisition; or (*B*) experimental extinction results in behavioural decrements by some mechanism which does not involve changes in RNA. The latter position implies that any modifications in RNA produced during acquisition persist during and after extinction.

The present experiment attempts to assess these two hypotheses. As in the investigation described in the preceding article, one group of planarians was classically conditioned and a second group was pseudo-conditioned. A third group was classically conditioned and then experimentally 'extinguished' by repeatedly presenting the conditioned stimulus (CS) alone. RNA extracted from each of these three groups was injected into naïve planarians. Hypothesis *A* would predict that the group receiving RNA from 'extinguished' worms should be comparable in performance to the group receiving RNA from pseudo-conditioned worms; hypothesis *B* would predict that the group receiving RNA from 'extinguished' worms should be comparable with the group receiving RNA from classically conditioned worms.

Initially, 400 worms received classical conditioning trials with paired light and shock, and 200 worms received pseudo-conditioning trials with unpaired light and shock. The procedures followed those described in the preceding article: fifty exposures to light alone were followed by 250 training trials in sets of 25 trials. Four animals randomly selected from each group were observed individually during the last 25 trials: those from the CC group scored 11, 13, 13, 13; those from the PC group scored 0, 1, 3, 4.

The classically conditioned group was then divided into two equal groups. One of these groups (CCE) was given 200 presentations of light alone at 20-sec intervals, and a second session of 50 light-alone trials after a 1-h break. The other conditioned group (CC) and the pseudo-conditioned group (PC) were equated to group CCE in terms of handling and apparatus exposure—that is, these groups were placed in the apparatus but were not given presentations of light. Four animals selected at random from each

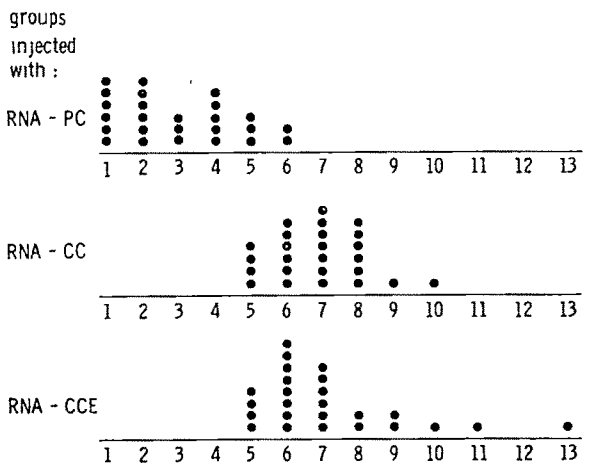


Fig. 1. Distribution of scores (responses per 25 trials) for injected planarians

of the three groups were then tested for 25 trials: CC animals scored 10, 11, 13, 13; PC animals scored 1, 2, 5, 5; and CCE animals scored 1, 2, 2, 5.

RNA was then extracted (in blind fashion) from groups CC, PC and CCE by the phenol method as previously described and was further purified by 'Sephadex' chromatography (*G*-50 coarse beaded form; 1 mM magnesium acetate, 5 mM *tris*-acetate, *p*H 7·2). The RNA extracted from each of the three groups was injected into 25 naive planarians which had previously been given 100 presentations of light alone. Each animal received 25 µl. of the RNA preparation. Injections were performed in blind fashion. Twenty-four hours later, each injected animal was given 25 presentations of light alone and was scored for number of responses (contractions). This testing was 'blind', and animals from the three groups were taken in a randomly interspersed order.

Fig. 1 presents the number of responses made by each worm on the test series. The mean for planarians injected with RNA from group CC was 6·9, that for worms injected with RNA from group CCE was 7·2, and that for worms injected with RNA from group PC was 3·0. The medians were, respectively, 7, 7, and 3. By a Mann-Whitney *U* test[3], the group injected with RNA from PC worms differed markedly from each of the other two injected groups ($P < 0.001$). The latter two groups clearly did not differ.

Thus, the effects on naïve recipients of RNA from extinguished planarians were indistinguishable from the effects produced by RNA from conditioned planarians. One possible interpretation of these data is that spontaneous recovery might account for the level of response of the group injected with RNA from group CCE. Some subsidiary data force us to reject this possibility: five worms from group CCE were put aside following conditioning and subsequent extinction and were then given 25 test trials at the time that testing of the injected animals was conducted. The scores obtained were: 0, 1, 1, 1, 2. Clearly there was no spontaneous recovery of the conditioned response. In addition to substantiating our earlier findings, these results, then, suggest that experimental 'extinction', while abolishing the conditioned response in planarians, did not affect the RNA of the conditioned animals.

This work was supported by University of California research grant 2068. We thank Dr. Robert Ramaley for his assistance in the chemical procedures.

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LETTERS TO THE EDITOR

ASTRONOMY

Transient Lunar Events: Possible Causes

In a recent communication[1], E. J. Flamm and R. E. Lingenfelter discussed the nature of luminescence and of lunar bright spots. They found that of nineteen reported bright spots in the region of Aristarchus, most occurred during years when the sunspot numbers were low, and they stated: "Clearly the 19 events are negatively correlated with solar activity". The Moon possesses only a negligible magnetic field and the particle flux at the Moon is, as pointed out by Flamm and Lingenfelter, too small by orders of magnitude to excite fluorescence, no matter whether flares, the solar wind, or cosmic-ray flux is invoked. A 'negative' correlation with sunspot number, if interpreted as a negative (or inverse) correlation with solar activity generally, implies a connexion with magnetic phenomena just as surely as a positive or direct correlation and would be just as hard to explain.

I analysed the same events with respect to monthly sunspot number, and also the distribution of sunspot numbers from 1749 to 1964 in the groups 0–30, etc., which were selected by Flamm and Lingenfelter for their survey. In addition, reports of a total of 103 events occurring in a number of areas of the Moon between 1749 and 1963 were collected by Mrs. Jayley Burley and me[2] and have also been grouped by sunspot number (see Table 1). The run of the three sets of statistics was quite similar, and my conclusion is that, contrary to Flamm and Lingenfelter's finding, no correlation of lunar events with sunspot numbers exists, and that the excess of lunar events during periods of low sunspot numbers is found because sunspot numbers are generally low. In the present survey, monthly means were used rather than yearly means.

Monthly sunspot relative numbers were available[3] for all months from January 1749 to June 1964. A few events for dates (even one naked-eye observation of 1587) earlier than this were included in Mrs. Burley's and my compilation, but could not be used here because monthly sunspot numbers were not available before 1749. Similarly, five reports within the past year have been omitted because sunspot reports for this period have not yet reached our library. The period 1749–1964 includes 2,586 months, and random fluctuations can be expected to be smoothed out.

When the data in the last two columns of Table 1 are plotted on a histogram, the tops of the columns run very smoothly. The curves for the other two smaller sets of data are not so smooth, as would be expected, but the trend is the same and the deviations from the same mean curve are consistent with the error law, so that no special selection effect or correlation seems to be present. The data for Aristarchus do not appear to be different in kind from those for other locations such as Alphonsus, Plato, Mare Crisium and other lunar features for which records of events recur repeatedly in the literature. There thus does not seem any reason for singling out the Aristarchus events and I have not done so here.

Table 1. Records of Lunar Events according to Monthly Sunspot Relative Numbers

Monthly mean Sunspot No	(Aristarchus E J.F and R.E L.) No. of events	%	(All available, J.B and B M M.) No. of events	%	All months Jan. 1749–June 1964 No of months	%
0 to 30 0	13	59 1	37	35 9	1,064	41 1
30+ to 60·0	4	18·2	26	25 2	691	26·7
60+ to 90 0	1	4 5	20	19 4	423	16·4
90+ to 120 0	1	4·5	8	7·8	220	8·5
120+ to 150·0	3	13·6	3	2 9	109	4·2
150+ to 180 0	0	0	5	4 8	52	2 0
180+ to 210·0	0	0	2	1 9	21	<1
210+ to 240·0	0	0	1	<1	5	<1
240+ to 270 0	0	0	1	<1	1	<1
Total	22	99 9	103	100 0	2,586	100·0

The statistics of Table 1 would seem to represent a random distribution of events superposed on an asymmetric distribution of sunspot numbers, indicating that no correlation exists and that we need to look further for a cause. There are indications that internal lunar causes have been[4] and still may be significant and that lunar events may be initiated by tidal cracking (near perigee—the corresponding time on Earth is high tide) or at periods of maximal crustal relaxation (near apogee, which corresponds to low tide on the Earth) with consequent release of hot or cold gases[5–7].

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PHYSICS

Entropy and Gravitation

The application of the second law of thermodynamics to bodies interacting through gravitational forces has been the subject of much controversy and discussion (see, for example, Kurth's *Mechanics of Stellar Systems*[1]). A purely phenomenological discussion of such a problem is unlikely to provide an answer to this problem and it is therefore important to consider it from the point of view of statistical physics.

The remarkable series of papers by Chandrasekhar on the "Statistics of the Gravitational Field arising from a Random Distribution of Stars"[2a–e] has shown that the main conclusions of the classical theory of Brownian motion, including the approach to equilibrium, may be applied to gravitational interaction. However, as noticed by Chandrasekhar[2], there remained a difficulty due to the slow decay of the force autocorrelation function with time, which leads to a long-distance divergence in the Fokker–Planck type of equation. For plasmas this divergence can easily be eliminated by taking into account screening, but for gravitation the question remains unsolved.

In order to discuss in greater depth the physical implications of this divergence, let us start with the general evolution equation for the velocity distribution function ρ_0 of a classical N-body system (see Prigogine, *Non-Equilibrium Statistical Mechanics*[3]). This equation may be written:

$$\frac{\partial \rho_0}{\partial t} = \int_0^t d\tau G(\tau)\ \rho_0(t-\tau) + F(t) \qquad (1)$$

It has two important features: (*a*) the collision operator $G(\tau)$ has a 'non-Markoffian' character due to the finite duration of the collision; (*b*) the memory of the initial correlations appears through the destruction fragment

$F(t)$. When both effects are neglected we recover, in the limit of weak coupling or small concentration, the usual Boltzmann (or Fokker–Planck) equation. Boltzmann's theorem then follows directly.

It is essential, however, to notice that the H-theorem follows not from the general equation (1) but from its Boltzmann's 'approximation'.

Let us call $\psi(z)$ the Laplace transform of the collision operator $G(\tau)$. The non-Markoffian equation (1) can also be written:

$$\frac{\partial \rho_0}{\partial t} = \psi\left(i\frac{\partial}{\partial t}\right)\rho_0 + F(t) \qquad (2)$$

while its Boltzmannian approximation is:

$$\frac{\partial \rho_0}{\partial t} = \psi(0)\,\rho_0 \qquad (3)$$

The difficulty with gravitational interaction is that $\psi(z)$ has now for $z \to 0$ a logarithmic singularity which can no more be eliminated through a summation of classes of events as in the electromagnetic plasma case (see Balescu, ref. 4).

The study of gravitational plasmas has therefore to start with equations of type (2) and not with their Boltzmannian approximation (3). For rather similar reasons the destruction fragment $F(t)$ can no more be neglected in (2) (see Prigogine and Résibois, ref. 5).

Detailed results will be reported soon by G. Severne and myself in *Physica*. The main conclusion is that the evolution of the system now involves a succession of incomplete collisions: because of their long duration a new collision begins before the preceding one is achieved. The result is not only a randomization of kinetic energy but also a transformation of gravitational energy into thermal energy. A Boltzmann type H quantity can be defined, of course, but its time derivative (even in a coarse-grained sense) has no well-defined sign. It seems, therefore, that the existence of a large number of interacting degrees of freedom is not a sufficient condition for the existence of entropy; conditions on the forces are also necessary (such that $\psi(z)$ for $z \to 0$ is finite). Of course, the canonical distribution is still a time independent solution of the kinetic equation (2), but the system has no mechanism to reach it if it is not imposed at the initial time. Phenomenological thermodynamics seems to be applicable only to short range (or electromagnetic) forces for a given state of gravitational bodies. If, on the other hand, gravitation is included, there seems to be no way to order the states of a galactic system (or *a fortiori* of the universe) according to the value of the entropy.

Most of this work was performed while I was staying at the Research Laboratories, General Motors Corporation, Warren, Michigan. I thank Dr. R. Herman for many stimulating discussions.

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Holography and its Crystallographic Equivalent

Recent advances in holography[1] have made apparent certain formal relationships between optical image synthesis, on one hand, and the heavy atom technique in X-ray crystallography, on the other. Bragg[2] has already qualitatively indicated this analogy. An exact correspondence between the two processes has become clear only since the following two developments: (i) Fourier transform holography for extended sources[3]; (ii) the Ramachandran and Raman[4] α-synthesis to deconvolute the Patterson function when the structure is partially known.

In the case of holography, we perform an interference experiment between the known and unknown extended objects $T_P(\xi)$ and $T_Q(\xi)$, where these symbols define the complex amplitude transmission of the electric field vector distribution on illumination by a wave of unit amplitude. Thus the complex amplitude distribution at point x on the hologram will be $A(x) = t_P(x) + t_Q(x)e^{2\pi i a\,x}$, where $t_P(x)$ and $t_Q(x)$ are the spatial Fourier transforms of $T_P(\xi)$ and $T_Q(\xi)$ and a is the separation of $T_P(\xi)$ and $T_Q(\xi)$, that is:

$$t(x) = \int T(\xi)e^{2\pi i x\,\xi}\,d\xi$$

where the integral is taken over the surface of the source. The intensity $H(x)$ recorded on the hologram at point x will then be:

$$\begin{aligned} H(x) &= A(x).A^*(x) \\ &= |t_P(x)|^2 + |t_Q(x)|^2 + t_P(x).t_Q{}^*(x)e^{-2\pi i a.x} \\ &\qquad + t_P{}^*(x).t_Q(x)e^{2\pi i a.x} \end{aligned}$$

It was shown by Stroke *et al.*[3] that a Fourier transformation would permit the reconstruction of the unknown object, $T_Q(\xi)$, provided that the hologram were illuminated by the same object $T_P(\xi)$ and that the Fourier transform of the product $t_P(x)t_P{}^*(x)$ be a delta function. Further, if it is desired to remove the two centrosymmetrically related images, it is necessary that $t_P(x)t_P(x)$ is not a delta function. It follows that the reconstructed object will be formed by the transform of:

$$\begin{aligned} t_P(x)|t_P(x)|^2 + t_P(x)|t_Q(x)|^2 + t_P(x).t_P(x)\,t_Q{}^*(x)\,e^{-2\pi i a\,x} \\ + t_P(x).t_P{}^*(x)\,t_Q(x)\,e^{2\pi i a.x} \end{aligned} \qquad (1)$$

In the case of a partially known crystallographic structure we observe the interference effect between the known and unknown parts of the structure. The complex amplitude F_H of the resultant wave will then be:

$$F_H = F_P + F_Q$$

where F_P is the structure factor of the known part and F_Q that of the unknown part, at the same reciprocal lattice point. Thus the intensities recorded will be:

$$F_HF_H{}^* = F_PF_P{}^* + F_QF_Q{}^* + F_PF_Q{}^* + F_P{}^*F_Q$$

The Ramachandran and Raman α-synthesis reconstructs the crystal structure by taking the Fourier transform of $F_P|F_H|^2$, that is:

$$F_P|F_P|^2 + F_P|F_Q|^2 + F_P{}^2F_Q{}^* + |F_P|^2F_Q \qquad (2)$$

which is identical in form to (1). If we consider the non-centrosymmetric case we require the condition that $F_PF_P \neq F_PF_P{}^*$. That is, we must have a non-centric configuration in the part of the structure that is known. A measure of this can be given by the Fourier transform of $F_P{}^2$ (ref. 5). The need for this condition arises from the superposition of the enantiomorphic structures in the crystallographic case. In contrast these two images are separated in holography due to the physical separation of T_P and T_Q in the recording of the hologram. In the centro-symmetric crystal structure the two overlapping images coincide.

A second-order improvement on the α-synthesis is produced by the β-synthesis[4] in which $\frac{|F_H|^2}{F_P{}^*}$ replaces $F_P.|F_H|^2$ as coefficients. While this is easy to achieve in crystallography it may present greater difficulties in optics.

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ENGINEERING

Hysteresis in the Flow through an Orifice

In a recent investigation into the behaviour of square-edge circular orifices a well-defined hysteresis in the flow was measured. This was obtained with air for a number of orifices of different diameters but of the same length/diameter ratio, equal to one-half. The experimental measurements were made under carefully controlled conditions and could be reproduced at different times.

It is curious that this phenomenon had not been encountered earlier in the long history of investigation of the flow of compressible fluids.

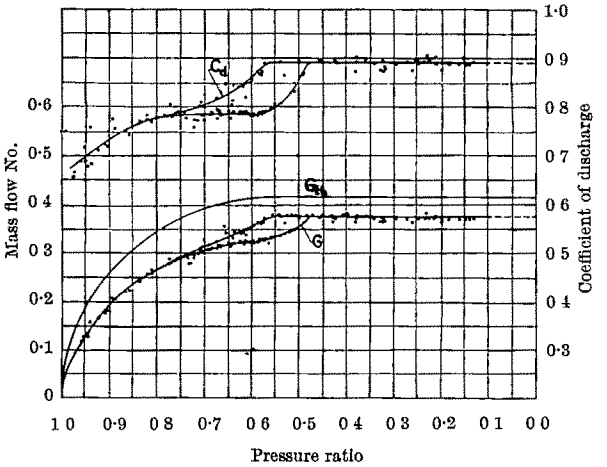


Fig. 1. G, Mass flow number; C_d, coefficient of discharge, G_{th}, theoretical mass flow number based on isentropic flow

The precise nature of the hysteresis is at present under detailed investigation, but it is thought that it is essentially a manifestation of the Coanda effect. It is seen from Fig. 1 that the orifices are choked when, presumably, attachment of the jet is fully secured. The measured mass flows are also the highest among a number of different orifices (thirty-eight in all) with length/diameter ratios ranging from 0·05 to 2·0.

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GEOLOGY

Greenstones from the Central Valley of the Mid-Atlantic Ridge

In a preliminary report[1] of a recent study of the morphology of the Mid-Atlantic Ridge about 22° N. latitude, attention was directed to the dredging of a suite of greenstones from the western face of the central valley. Although similar rocks have been recently reported from the Carlsberg Ridge[2] in the Indian Ocean, rocks of this type have not been previously recorded from the Mid-Atlantic Ridge. Preliminary petrographic examination and chemical analysis of the Mid-Atlantic Ridge greenstones justify the present interim report.

The collection from the upper slope (Dredge 2, 2,670–2,050 m) consists, as noted[1], of fragments of greenstone, basalt, and partly metamorphosed basaltic tuff. The fragments exhibit a thin manganiferous coating, usually on all sides. This observation and the angularity of the fragments suggest that they were collected from a slowly accumulating talus slope. The collection from the base of the slope (Dredge 3, 4,000–3,200 m) consists of greenstones, and rare fragments of diabase. The greenstone fragments exhibit only very thin and spotty manganiferous coatings, rarely on more than two faces. The distribution of these coatings, and the fresh uneroded aspect of the uncoated faces, indicate that these rocks had been only recently exposed. The preservation, unbroken, of thin platey fragments in Dredge 2 we take to indicate that the fresh surfaces of the Dredge 3 fragments are unlikely to have been produced by fracturing within the dredge.

The greenstones consist of actinolite, epidote, chlorite and plagioclase in various proportions. The latter is mainly albite although some specimens contain relics of more calcic plagioclase. Dredge 2 contains greenstones which are essentially free of epidote whereas in those in Dredge 3 epidote is abundant. Although searched for specifically, zeolites and pumpellyite have not been noted in the 13 greenstones which have so far been examined in detail.

Most of the greenstones are not deformed. In some of the specimens shear planes occur but they are widely spaced and give no well-defined fabric. Because of this lack of intense penetrative deformation, the pre-metamorphism textures are preserved. These show that some of the greenstone fragments were derived from submarine basaltic flows and others from basaltic tuffs.

One of the greenstones analysed has unusually high sodium content (Table 1, No. 1). This specimen is textur-

Table 1. Chemical Analyses* of Two Greenstones and a Representative Basalt

	(1) Greenstone Dredge 3	(2) Greenstone Dredge 2	(3) Fresh basalt Dredge 2
SiO_2	50 84	49·71	49 10
Al_2O_3	15 25	15 32	15 27
Fe_2O_3	2 89	2 05	2 54
FeO	4·55	7·31	8·36
FeS_2†	0·19	not sought	not sought
MnO	0 16	0·16	0 20
MgO	9 32	8·92	8·09
CaO	6·44	7·32	10·61
Na_2O	4 48	2·93	2 86
K_2O	0 05	0 05	0 25
H_2O^+	3·26	3 53	0 56
H_2O^-	0 74	0 93	0 25
TiO_2	1 38	1·51	1 73
P_2O_5	0·10	0·17	0 16
	99·65	99·91	99·98

(1) Epidote—actinolite—chlorite—albite.
(2) Quartz—chlorite—actinolite—albite.
(3) Rare plagioclase (An_{32}) and olivine (Fo_{85}) phenocrysts.
* Content as percentage of air-dry ground sample. Analysis by standard wet methods for silicate rocks. Analyst Eugene Jarosewich.
† Calculated assuming all sulphur in pyrite, the only sulphide visible in polished sections.

ally characterized by albite veins, dispersed pyrite and rare relict labradorite. These features suggest that the 'spilitic' composition is a result of hydrothermal albitization during metamorphism. Except with regard to their water content, many of the greenstones (for example, Table 1, No. 2) were evidently derived isochemically from basalts.

The greenstones are associated with basalts in Dredge 2 and with rare fragments of diabase in Dredge 3. Table 1 (No. 3) gives an analysis of a fresh basalt from Dredge 2. This basalt consists of microlites of plagioclase and olivine in a glassy matrix containing rare phenocrysts of plagioclase and olivine. Detailed petrographic and chemical data on these basalts and diabases will be included in the final report on the greenstones.

The occurrence of greenstones raises several questions about the nature of the Mid-Atlantic Ridge. These rocks are not hornfelses and cannot be interpreted as the result of contact metamorphism. The mineral assemblages are in the greenschist metamorphic facies, which is often taken to indicate temperatures of 300°–500° C and water pressures between 3 and 8 kbar (ref. 3). Even in the deep sea, such pressures, along with sustained periods of heating, would require that the original basalts and tuffs were buried under a considerable thickness of subsequent flows or sediments. Because there are very few experimental data available on the actual stability ranges of greenschist assemblages, precise estimates of minimum and maximum depths of burial cannot yet be made. Recovery of the rocks by dredging shows no overburden now to be present; its elimination in recent times by other than tectonic processes presents great difficulties. The burial postulated appears to require a halt in, or a temporary reversal of, the upward movement of material underneath a rift zone, as suggested by Hess[4]. The subsequent raising of buried material to the sea floor, and its exposure there, seems to us perfectly consistent with the suggested tectonic patterns.

Matthews *et al.*[2] have attributed origin of chlorite-rich metamorphosed basalts and gabbros recovered from the Carlsberg Ridge to hydrothermal processes resulting from cross-faulting. Such a suggestion is not admissible as explanation of the fresh greenstone occurrences on the Mid-Atlantic Ridge; no cross-faulting is reflected in the bottom topography within almost 30 min north or south of their location.

If greenstones similar to those from 22° N. prove to be quantitatively important constituents of parts of the Mid-Atlantic Ridge, interpretations of seismic, magnetic and gravity data from the Ridge cannot be based on models which assume the presence only of fresh basaltic rocks, serpentine and unserpentinized ultramafics in a simple layer-cake sequence. The probability that greenstones are of general importance in the structure of the Ridge is increased by our finding of greenstone fragments in the coarse fraction of sediments from the western flank (P.C. 22, South Pond)[1]: in the specimens examined, chlorite is often associated with phillipsite. Careful examination of all undescribed materials from the Mid-Atlantic Ridge is important in assessing the true abundance of greenstones. Because of the fine-grained nature of the rocks from 22° N., only petrographic inspection of thin sections or of grains, combined with X-ray diffractometry, has allowed precise identifications.

Additional petrographic examinations and chemical analyses are now in process, and an extended report of the study will be published elsewhere. We thank Peter L. Sachs and the scientific party and crew of R.V. *Chain* for their part in the dredging, and Eugene Jarosewich for making the chemical analyses. Various aspects of this work were supported by the U.S. Atomic Energy Commission (contract *AT*(30-1)-2174), by the U.S. National Science Foundation (grants *GP*921 and *GP*1599) and by the U.S. Office of Naval Research (contract *Nonr*-2196(00)), at the Woods Hole Oceanographic Institution, and by the U.S. Office of Naval Research under contract *Nonr*-2216(23) with Scripps Institution of Oceanography.

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Isotopic Ages of Minerals from the Pre-Cambrian Complex of the Malverns

THE Malvern Hills, Worcestershire, form one of many Pre-Cambrian inliers of the Welsh Borderland[1]; rocks of Pre-Cambrian age are divided into Malvernian 'schists and gneisses' and the overlying Warren House volcanic group. The analyses reported in the following communication are from minerals occurring within the supposed metamorphic rocks and do not include any from the volcanic group, no suitable material having been obtained from the latter. The 'schists and gneisses' were identified as Malvernian more than a century ago[2], but the origin of their structures remained a puzzle, and it was a matter of some controversy as to whether the rocks were igneous or metamorphic[3,4]. However, most of the rocks seen by us are igneous, usually hydrothermally altered and sheared, but still retaining igneous textures and occasionally igneous mineralogy. Such schistose rocks as have been observed are either in shear zones or in fault blocks, and true gneisses, in the usual sense of high-grade, banded, metamorphic rocks, are absent. The commonest rocks are of intermediate igneous type, classifiable as mafic diorite or tonalite. Amphibolites of various kinds, usually with igneous textures, are another common rock type. Ultramafic rocks are usually rich in tremolite or actinolite, with biotite or chlorite. Granitic rocks are rare, but syenitic veins and pegmatites frequently occur. The usual mineral assemblages are those of the greenschist or epidote–amphibolite metamorphic facies. It is generally agreed that the alteration of the igneous rocks of the Malvernian occurred in the Pre-Cambrian, as no equivalent hydrothermal or metamorphic activity has affected the Cambrian or later strata.

The Pre-Cambrian of the Malverns occurs along a major north–south structural line, regarded as of Late-Carboniferous age[5,6], but which must have been initiated earlier. To the east is Trias, and Cambrian and Silurian lie to the west. The eastern junction is most probably a fault[6], as is much of the western boundary. Groom[5] believed that the entire western boundary was faulted, but recent exposures support Holl's[2] view that both the Cambrian and the Lower Silurian (Upper Llandovery)[7–10] rest unconformably on the Malvernian. There is also discordance between the Cambrian and the Silurian. The conclusion that the Upper Llandovery rests unconformably on the Malvernian, based on the exposure in the Gullet Quarry, and at the Sycamore Tree exposure, has been disputed[11], but having seen the field evidence at the Gullet we strongly support the interpretation of unconformity as stated by Reading and Poole[7]. There were, therefore, three distinct phases of vertical movement, one

Pre-Cambrian, one Post-Cambrian and Pre-Upper Llandoverian, and one Late Carboniferous. A further event in the area is the intrusion of a set of basic sills and stocks into the Cambrian, but not into the Silurian or later strata[12,13]. Whether or not any representatives of these cut the exposed Pre-Cambrian does not appear to be known, but no Pre-Cambrian Malvernian rock in our possession conforms to the descriptions available of the Post-Cambrian intrusions, nor were any unsheared intrusions seen in the Pre-Cambrian in the field.

Our results suggest that it would be of interest to identify the precise stratigraphic position of the Cambrian of the area, in particular that which rests on the Malvernian. An *Olenellus* fauna, indicating a Lower Cambrian age, is known from the Welsh borderland[1], but the Malvern Cambrian has never yielded Lower Cambrian tribolites. It is thought, however, to be Lower Cambrian in its lithological similarity to the proved Lower Cambrian of Comley, Shropshire, its position below the Middle Cambrian, and a fauna of horny brachiopods and *Hyolithus*[14]. As it is the supposed Lower Cambrian which is known to rest on the Malvernian, there would appear to be a good case for further palaeontological investigation into these rocks, in the light of the age data reported and their possible significance for the geological time scale.

The investigation of mineral ages in the Malverns is restricted by the choice of material; perfectly fresh minerals are all but absent (Table 1). The whole rocks so far available to us have such low rubidium–strontium ratios that it was not thought worthwhile to analyse them at this stage of the investigation. Analyses were made for potassium–argon ratio at Oxford by D. Rex, for potassium–argon ratio at Edmonton by R. St. J. Lambert (under the instruction of H. Baadsgaard) and for rubidium–strontium ratio at Oxford by R. St. J. Lambert. Standard techniques were used throughout; the decay constants used are given at the foot of the tables. The potassium–argon ratios, Table 2, and the rubidium–strontium ratios, Table 3, show a concentration of apparent ages at or closely approaching 600 m.y., with a tail of lower ages. Agreement between laboratories, and between potassium–argon ratios on two biotites, one hornblende and one muscovite and rubidium–strontium ratios on one biotite, all at 590 ± 20, suggests that some definite event must have occurred in the history of the Malverns at that time or slightly earlier. The discordant results are perplexing; no obvious explanation is known for the difference between the rubidium–strontium and potassium–argon age of biotite 21038, unless the very subsidiary chloritization (about 5 per cent) of this biotite has occurred late and affected only the rubidium–strontium daughter–parent ratio. The discrepancy between 21038 biotite and hornblende may have a petrographic explanation; the hornblende is sieved with calcite, apparently replacive and associated with late veins of calcite. The other low age, 510 m.y. on 21045 hornblende, is also from a mineral which has suffered alteration, the hornblende being partly replaced by epidote and chlorite.

The concentration of results near 600 m.y. and the agreement of one rubidium–strontium and the potassium–argon results (the rubidium–strontium ages are not sensitive to choice of reasonable initial $^{87}Sr/^{86}Sr$ ratio in mafic igneous minerals with these high rubidium–strontium ratios) suggest that the last major isotopic event in the Malvernian occurred 590 ± 20 m.y. ago, and was associated with the Pre-Cambrian uplift of these rocks. Whether it is a true rock-forming age cannot definitely be stated (590 m.y. is only a minimum for the true age of these rocks), but even if not, it still remains likely that the event recorded is Pre-Cambrian. It is therefore of possible importance in defining the age of the base of the Cambrian, which is only poorly known[15].

The meaning of the lower apparent ages of from 545 to 485 m.y. is less clear. The lowering of the 600 m.y. ages could have been accomplished during the period of Ordovician igneous activity or during the Pre-Upper Llandoverian earth movements, or during the Late Carboniferous movements. The results so far seem to indicate that either of the first two possibilities is more likely than the last.

This work forms part of the age and isotope investigations being carried out in the University of Oxford; we thank S. Moorbath, H. G. Reading and N. J. Snelling for discussions.

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Table 1. Specimen Localities and Descriptions

21038 Grid Ref. 770441. Lower Toll Gate. Mafic hornblende–biotite syenite (pegmatitic). Hornblende very pale green prisms up to 1 cm, containing about 20 per cent calcite; biotite, 1 cm, fresh but deformed, with angular kinks in cleavage flakes, minor marginal chloritization; plagioclase almost completely sericitized and epidotized; microcline fresh; accessory apatite, sphene and hematite.

21039 (As 21038). Ultramafic rock consisting of 30 per cent biotite, pale brown and deformed as in 21038; 65 per cent amphibole, about half pale-green hornblende ('actinolite') and about half colourless cummingtonite. Accessory primary colourless chlorite, oxide and calcite.

21045 Grid Ref. 771496. Clock tower, North Hill Quarry. Hornblende diorite: medium-grained hornblende (50 per cent of rock) mostly fresh, set in turbid plagioclase, strongly epidotized. Hornblende locally altered to epidote plus chlorite.

20633 Grid Ref. 768477. East face of Brewery Quarry, Upper Wyche. Amphibolite, medium-grained, rich in fresh green hornblende, with minor turbid plagioclase. Epidote on joint planes; cut by thin feldspar-quartz veins.

20634 (As 20633). Muscovite–quartz–potassium–feldspar vein cutting 20633-type rock. Muscovite (up to 2 mm) set in a turbid potassium-feldspar and quartz matrix.

Table 2. Potassium–Argon Data

Sample No.	Mineral	Analysis No.	K (%)	^{40}Ar c.c./g S.T.P. radiogenic ($\times 10^{-4}$)	^{40}Ar radiogenic (%)	Age (m.y.)
21038	Biotite	338 Oxf.	7·99	2·171	98	580 ± 20
		558 Edm.	7·96	2·158	99	580 ± 20
		596 Edm.	(7·96)	2·224	97	595 ± 20
21038	Hornblende	353 Oxf.	0·84	0·185	86	485 ± 15
21039	Biotite	559 Edm.	5·58	1·606	98	610 ± 20
21045	Hornblende	551 Oxf.	0·46	0·108	93	510 ± 15
20633	Hornblende	545 Oxf.	1·09	0·292	94	570 ± 20
20634	Muscovite	787 Oxf.	8·16	2·307	99	600 ± 20

Decay constants: $\lambda\beta = 4{\cdot}72 \cdot 10^{-10}$ yr^{-1}
$\lambda e = 0{\cdot}584 \cdot 10^{-10}$ yr^{-1} $^{40}K/K = 0{\cdot}0119$ atom per cent.

Table 3. Rubidium–Strontium Data

Sample No.	Mineral	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Sr/^{86}Sr$ to-day	Age*
21038	Biotite	865 ± 10	6·1 ± 0·2	4·0195 ± 0·0884	545 ± 25
21039	Biotite	470 ± 5	26·5 ± 0·5	1·1604 ± 0·0093	590 ± 20

Decay constant $\lambda = 1{\cdot}47 \cdot 10^{-11}$ yr^{-1}
* Age calculated assuming $^{87}Sr/^{86}Sr$ (initial) = 0·712.

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Structure and Evolution of the Volcanic Cauldrons, Ring Complexes and Associated Granites of the Georgetown Inlier, Queensland

The Georgetown Inlier in North Queensland covers an area of about 37,000 square miles and lies on the western flank of the Palaeozoic Tasman Geosynclinal Zone

(Fig. 1). It consists largely of Pre-Cambrian metamorphic and granitic rocks, and a suite of Upper Palaeozoic acid igneous rocks. The Upper Palaeozoic acid igneous rocks, which occupy an area of about 10,000 square miles, include extensive acid volcanics and associated granitic intrusions, and form a well-defined petrographic province[1]. The volcanic rocks consist predominantly of rhyodacite[2] welded tuff[3] with subordinate rhyolite, trachyandesite, andesite and basalt, belonging to the calc-alkaline rock series. The intrusive rocks are mainly adamellite[2] with subordinate granite and granodiorite.

About 1,000 cubic miles of rhyodacite welded tuff has been preserved in eleven cauldron subsidence areas ranging from 8 to 75 miles long. Six ring complexes occur in association with the cauldrons; five contain both volcanic and intrusive rocks, and the sixth is entirely intrusive. The position of the volcanic cauldrons and the ring complexes appears to have been controlled by pre-existing fractures in the Pre-Cambrian basement. Stocks of medium-grained adamellite which intruded the volcanics in the cauldrons and ring complexes crystallized under a hood of welded tuff sheets only 500–2,000 ft. thick.

In the cauldrons the initial event was the eruption of viscous acid magma from vents aligned along the peripheral faults, accompanied in some cauldrons by lacustrine sedimentation nearby. As the fluidity of the magma increased, vesicular rhyodacite flows and air-fall tuff and breccia were erupted. Interbedded with and overlying these units are thin welded tuff sheets in simple cooling units[3] generally less than 100 ft. thick. The final volcanic event was the eruption of fluidized rhyodacite magma from the peripheral fissures and ring fractures to form voluminous welded tuff sheets in compound cooling units[3] up to 1,500 ft. thick, which filled the cauldrons. Finally, high-level granite stocks were emplaced by underground cauldron subsidence in the base of the downfaulted volcanics.

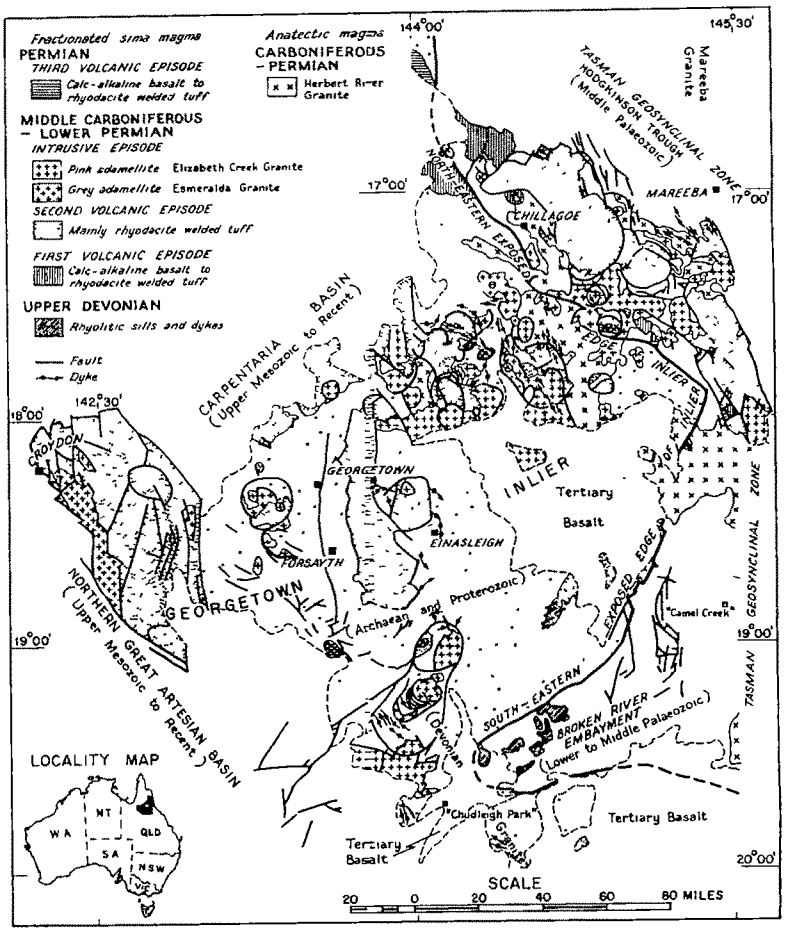


Fig. 1. Major structural and petrological elements of the Upper Palaeozoic Acid Igneous Province, North Queensland

The sequence of events in the ring complexes is similar to that in the cauldrons, and may be grouped into three phases. However, whereas the magma for the volcanic events in the cauldrons was derived from the main, deep-seated magma chamber, the magma for the volcanic phases (1 and 2) in the ring complexes was derived mainly from magma differentiated in a high-level magma chamber formed by underground cauldron subsidence and isolated above the main magma chamber. About the time phase 3 began, the high-level chamber was flushed by magma rising from the main magma chamber, creating a hiatus in the chemical evolution of the complex, and later ring dykes and stocks were derived from this magma.

The structural phases in the ring complexes were: (1) Early volcanic phase, with eruption of acid volcanics from small central vents. (2) Caldera phase, with eruption of andesitic to rhyolitic air-fall pyroclastics and moderately large ash flows from ring dykes filled with fluidized acid magma. (3) Underground cauldron subsidence phase, with the intrusion of ring dykes and stocks.

The formation of acid magma for the comagmatic granites and pyroclastic flows is believed to be an integral phase of geosynclinal development. During the orogenic phase of development of the Palaeozoic geosyncline adjacent to the Pre-Cambrian inlier, acid magma was fractionated from basic rocks in the lower crust under the inlier. The determination of a mantle-strontium ratio ($^{87}Sr/^{86}Sr = 0{\cdot}701$, W. Compston, personal communication) for a rhyodacite welded tuff corroborates this hypothesis. According to radiometric age data, the acid magma accumulated for about 50 million years, but remained *in situ* until the start of the post-orogenic phase, when it was intruded over a period of 30 million years or less. The production of acid magma domed the crust, placing it in a state of tension. Magma rose along pre-existing major faults and, on reaching the surface, caused a drop of pressure in the magma chamber. This allowed the accumulation of a volatile-rich phase which was erupted to the surface as pyroclastic flows. Where the magma was most depleted, subsidence of crustal blocks initiated cauldron subsidence. This was followed by crustal instability and the magma rose by high-level major block stoping. At various stages the volatile-rich part was erupted to produce further pyroclastic flows at the surface. Finally, the magma intruded the pyroclastic flow sheets and crystallized as an adamellite.

A contemporaneous granite crops out along the north-eastern margin of the Georgetown Inlier and within the adjacent Palaeozoic geosyncline, the magma for which is thought to have formed by anatexis at the base of the Palaeozoic geosyncline, in the manner outlined by Tuttle and Bowen[4] and Walton[5].

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CRYSTALLOGRAPHY

Thermal Expansion of Rubidium Dihydrogen Phosphate

A PROGRAMME of investigation into the temperature variation of the lattice parameters of tetragonal crystals, isomorphous with potassium dihydrogen phosphate, in the high-temperature region, has been undertaken in this laboratory. Results on potassium dihydrogen phosphate[1], ammonium dihydrogen phosphate[2] and potassium dihydrogen arsenate[3] have already been published. This communication presents the results of the precision determination of the unit cell dimensions of rubidium dihydrogen phosphate at different temperatures between 29° C and 78° C and also the principal coefficients of its thermal expansion obtained from these data.

Crystals of rubidium dihydrogen phosphate were prepared by slow evaporation of an aqueous solution of rubidium carbonate neutralized with an aqueous solution of hypophosphoric acid. X-ray powder pictures were taken with a high-temperature, back-reflexion camera[4] using copper K_α radiation. Six unambiguously indexed $K_{\alpha_1\alpha_2}$ doublets, with their sine^2 θ values greater than 0·866, were employed in calculating the values of the a and c parameters, using the conventional least-squares technique. An error analysis made for a film taken at 35° C, by the method given by Jette and Foote[5], gave the standard errors in the values of a and c as 0·013 per cent and 0·019 per cent, respectively. Standard errors in the values of the parameters at other temperatures were assumed to be of the same order.

Table 1 gives the values of the two parameters at different temperatures, and the following expressions were obtained for their temperature dependence by the usual statistical methods.

$$a_t = 7{\cdot}6054 + 21{\cdot}11 \times 10^{-5}\,(t\text{-}20)$$
$$c_t = 7{\cdot}2989 + 37{\cdot}95 \times 10^{-5}\,(t\text{-}20)$$

Here a_t and c_t are the values of the two parameters in Ångstroms at t° C. Slight extrapolation of the present data gives the values of the parameters at 20° C as $a = 7{\cdot}6054$ Å and $c = 7{\cdot}2989$ Å and these are found to be in good agreement with the values reported by Magyar[6].

Table 1. VALUES OF THE LATTICE PARAMETERS OF RUBIDIUM DIHYDROGEN PHOSPHATE AT DIFFERENT TEMPERATURES

t° C	a (Å)	c (Å)
29 0	7 6078 ± 0 0009	7 3026 ± 0·0014
35 0	7 6080	7·3040
50 0	7·6114	7 3109
61·5	7·6150	7 3139
74·0	7 6168	7·3196
78 0	7 6174	7·3209

Within the accuracy of the measurements, both the parameters show a linear increase with temperature. The principal coefficients of thermal expansion, defined by the relation $\alpha = 1/L_{20}(dL/dt).(L = a \text{ or } c)$, come out as $\alpha_a = 27{\cdot}75 \times 10^{-6}$/°C and $\alpha_c = 52{\cdot}0 \times 10^{-6}$/°C. It is interesting to note that, like potassium dihydrogen phosphate and potassium dihydrogen arsenate, the coefficient of expansion of rubidium dihydrogen phosphate along the tetragonal axis is greater than that in any direction in a perpendicular plane. This feature is being discussed separately in terms of the structures of these crystals.

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CHEMISTRY

Contact Angles of Mercury on Low-energy Solids

THE recent theory of Fowkes[1-5] concerning the attractive forces at interfaces leads to the following relationship between cosine of contact angle (θ) and liquid surface tension (γ_L):

$$\cos\theta = -1 + 2\sqrt{\gamma_S{}^d}\left(\frac{\sqrt{\gamma_L{}^d}}{\gamma_L}\right) \qquad (1)$$

where $\gamma_S{}^d$, $\gamma_L{}^d$ are the dispersion force contributions to the solid surface tension and liquid surface tension respectively.

A plot of $\cos\theta$ against $\sqrt{\gamma_L{}^d}/\gamma_L$ for the data of Zisman[6,7] for four low-energy surfaces gives good agreement with equation (1). The range of surface tensions investigated was, however, not great. The only liquid investigated with a surface tension greater than water (γ_L = 72 dynes/cm) was mercury (γ_L = 485 dynes/cm), for which values were published only for the following surfaces: polytetrafluoroethylene, 150° (ref. 8); octadecylamine monolayer on platinum, 146° (ref. 9); perfluorodecanoic acid monolayer on platinum, 152° (ref. 10).

Fowkes[5] quotes a value for paraffin wax $\cos\theta = -0{\cdot}9$ (θ = 154°). These points do not fall on Fowkes's plot of $\cos\theta$ against $\sqrt{\gamma_L{}^d}/\gamma_L$. Fowkes[5] comments: "It is possible that some wax spread on mercury resulting in a decrease of surface tension and therefore too high a contact angle".

Few measurements of contact angle of mercury on low-energy solids seem to have been made, and those made by Zisman consisted only of the mean of three measurements with no record of the scatter. The measurements in this communication are put forward in an attempt to increase the available facts.

Fowkes's theory might be expected to be particularly accurate for mercury on low-energy solids and the results should provide a sensitive confirmation of the validity of his theory.

Measurements have been made, therefore, of the contact angle of mercury on polytetrafluoroethylene, paraffin wax, and polyethylene.

Clean mercury was prepared according to the recommendation of Nicholas *et al.*[11]. It was distilled, washed successively with dilute nitric acid and caustic soda solution, distilled again and then shaken, successively, with 50 per cent nitric acid, distilled water, acetone, ether and filter paper before being applied as small drops to the test surface by a clean hypodermic syringe with a polytetrafluoroethylene base. Measurements were made as rapidly as possible—usually within 1 min of application.

Paraffin and polyethylene surfaces were prepared by the methods of Fox and Zisman[12], freshly cut surfaces being melted against cleaned glass slides, which were removed immediately before use. Clean, flat surfaces of polytetrafluoroethylene were prepared by fine polishing on successive grades of powdered emery, final traces of abrasive being removed by lens tissues. Contact angle measurements were made using a horizontal microscope technique as described elsewhere[13]. The surfaces were mounted on the rotating stage when in a vertical plane and the advancing and receding angles obtained by rotating the stage until movement just ceased. A goniometer eyepiece was used to measure angles.

Results are given in Table 1. They include the 95 per cent confidence limits and the standard deviation on $\cos\theta$.

It will be noted that the values for polytetrafluoroethylene (151·7° advancing, 150·2° receding) are very close to the value of 152° reported by Fox and Zisman[8]. The values for paraffin wax, on the other hand (141·1° advancing, 140·9° receding), do not agree with the 154° reported by Fowkes[5].

Table 1. CONTACT ANGLES OF MERCURY ON LOW-ENERGY SURFACES

Surface	Advancing angle					Receding angle				
	No. of observations	θ (degrees)	Mean cos θ	95% confidence limit	Standard deviation	No. of observations	θ (degrees)	Mean cos θ	95% confidence limit	Standard deviation
Paraffin wax	31	141·1	−0·7793	0·0067	0·0188	37	140 9	−0·7765	0·0048	0 0145
Polyethylene	8	148·0	−0·8477	0·0064	0·0076	7	145·5	−0·8231	0 0177	0 0191
Polytetrafluoroethylene	17	151·7	−0·8805	0·0092	0 0187	16	150·2	−0·8678	0·0171	0 0333

It must be admitted that mercury in laboratory air does not retain the surface tension value of 485 dynes/cm assumed by Fox and Zisman[8]. Nicholas *et al.*[11] found 400–380 dynes/cm to be typical.

However, using the foregoing contact angle data one can substitute in equation (1), using Fowkes's values[5] for γ_S^d and γ_L^d, to find the surface tension value for mercury which would satisfy that equation.

Using my mean values for contact angles, the results are as follows:

Polyethylene	γ_L = 1,073 dynes/cm
Paraffin wax	γ_L = 641 dynes/cm
Polytetrafluoroethylene	γ_L = 1,071 dynes/cm

The discrepancy from Fowkes's theory cannot therefore be explained by contamination of the mercury since it is necessary to postulate an increase of surface tension rather than a decrease to comply with his equation.

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Primary Process in the Photolysis of Alkyl Iodides

RECENTLY, Kasper and Pimental[1] have detected stimulated emission from the transition $I(5p^2P_{1/2}) \rightarrow I(5p^2P_{3/2}) + h\nu$ (1·315μ) resulting from the flash photodissociation of methyl and trifluoromethyl iodides. This provided the first direct observation of electronically excited iodine atoms produced from the photolysis of alkyl iodides. A spectral analysis of methyl iodide by Porret and Goodeve[2] had previously indicated that $I(5^2P_{1/2})$ should be predominantly produced on the photolysis of this compound. Furthermore, these authors pointed out the close similarity between the spectra of the lower aliphatic iodides. The work described in this communication, which utilized kinetic spectroscopy in the vacuum ultra-violet region of the spectrum, confirms the work of Kasper and Pimental and extends the observations to ethyl, propyl and butyl iodides. Preliminary observations on the photolysis of hydrogen iodide are also reported here.

We have previously observed both excited ($5^2P_{1/2}$) and ground state ($5^2P_{3/2}$) iodine atoms in absorption resulting from the flash photolysis of molecular iodine in the presence of excess argon[3]. This technique has also been used to monitor the large concentration of excited atoms that accompanied the population inversion on the photolysis of CF_3I, and the relatively long life-time of the excited atoms in this system allowed deactivation by various added gases to be investigated[4].

The spectrum of CF_3I ($P_{CF_3I} < 5$ mm) does not interfere with the strong atomic transitions at 1783 and 1799 Å most convenient for plate photometry. The molecular spectra of the other unsubstituted alkyl iodides investigated, on the other hand, obscured the region below 2000 Å and, for this reason, conditions were sought which allowed the detection of the weaker transition of the excited atom at 2062 Å. The use of high concentrations of CF_3I ($P_{CF_3I} > 5$ mm; $P_{Ar} = 50$ mm; $E = 1{,}024$ Joules) revealed not only the transition at this wave-length but even weaker transitions at 1876 and 1844 Å (Fig. 1). Table 1 lists those transitions that have now been observed in absorption for atomic iodine.

Table 1

λ (Å)	Transition
1783	$6s(^2P_{3/2}) \leftarrow 5p(^2P_{3/2})$
1799	$6s(^2P_{1/2}) \leftarrow 5p(^2P_{1/2})$
1830	$6s(^4P_{5/2}) \leftarrow 5p(^2P_{3/2})$
1844	$6s(^4P_{3/2}) \leftarrow 5p(^2P_{1/2})$
1876	$6s(^4P_{1/2}) \leftarrow 5p(^2P_{1/2})$
2062	$6s(^2P_{3/2}) \leftarrow 5p(^2P_{1/2})$

The transition at 2062 Å could be detected on photolysing ethyl, propyl and butyl iodides but in the case of methyl iodide this is overlain by a strong band in the molecular spectrum[5]. It was found possible by carefully reducing the concentration of CH_3I ($P_{CH_3I} = 0{\cdot}025$ mm; $P_{Ar} = 50$ mm; $E = 1{,}024$ J) to observe the sharp atomic lines at 1799 and 1783 Å at short delay (13·8 μsec) through the relatively diffuse molecular spectrum and that the intensities of these transitions indicated a population inversion. The photolysis of methyl iodide has been studied by time-resolved mass-spectrometry[6], and the rapid production of molecular iodine has been accounted for in terms of the proposed reaction:

$$I(5^2P_{1/2}) + CH_3I \rightarrow I_2 + CH_3 \quad \triangle H = -5{\cdot}2 \text{ kcal}$$

We consider the analogous reaction with CF_3I, where molecular iodine is only observed at long delay, to make a negligible contribution to the decay of the excited iodine atoms[4]. A strong spectrum of the methyl radical was also observed during the first 100 μsec after the flash ($P_{CH_3I} = 0{\cdot}5$ mm; $P_{Ar} = 50$ mm; $E = 1{,}024$ J) previously observed in this system by Herzberg[7].

The decay of $I(5^2P_{1/2})$ resulting from the photolysis of ethyl iodide could be studied by monitoring the transition at 2062 Å by plate photometry using a Joyce–Loebel double-beam microdensitometer (Mark III). The decay roughly exhibits first-order kinetics with respect to both excited atoms and the concentration of ethyl iodide

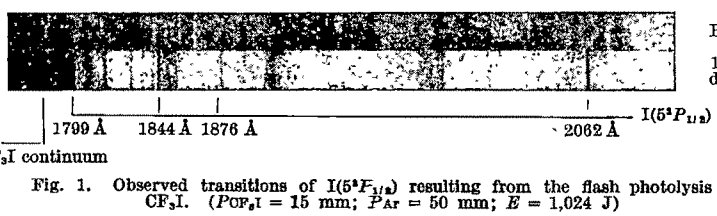


Fig. 1. Observed transitions of $I(5^2P_{1/2})$ resulting from the flash photolysis CF_3I. ($P_{CF_3I} = 15$ mm; $P_{Ar} = 50$ mm; $E = 1{,}024$ J)

itself (Fig. 2). Deactivation by argon is insignificant[3,4]. By comparison with previous experiments on CF_3I, we estimate that about 40–50 per cent of ethyl iodide is photolysed in each case. This allows us to estimate a second-order decay constant for the removal of $I(5^2P_{1/2})$ by EtI from the two slopes in Fig. 2. This yields a value for the rate constant given by $k = 2{\cdot}2 \pm 0{\cdot}4 \times 10^8$ l. m^{-1} sec^{-1}. The only data on spin-orbit relaxation of excited iodine atoms with which we can compare this result is the decay constant for propane ($k = 3{\cdot}5 \times 10^7$ l. m^{-1} sec^{-1}) (ref. 4). The decay of $I(5^2P_{1/2})$ in this system could also, however, be due to deactivation or reaction with the products of the photolysis of ethyl iodide[8] or a chemical reaction with EtI itself, analogous to that already discussed here for methyl iodide. More detailed work would be necessary to resolve this point. HI was not detected in these experiments and we are therefore unable to support the mechanism hitherto suggested[8] in which this molecule is a primary product of photolysis.

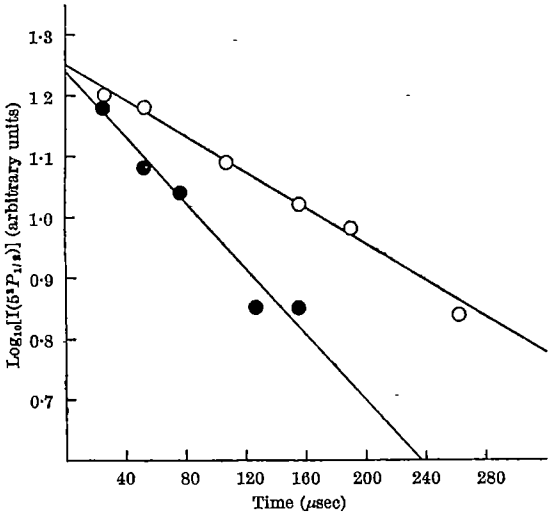


Fig. 2. Decay of electronically excited iodine atoms ($5^2P_{1/2}$). $\log_{10}[I(5^2P_{1/2})]$ (arbitrary units) versus time (μsec). ○, P_{EtI} = 0·5 mm; P_{Ar} = 100 mm; E = 1,024 J. ●, P_{EtI} = 1 mm; P_{Ar} = 100 mm; E = 1,024 J

The 2062 Å transition was observed at short delay on the photolysis of both propyl iodine (P_{PrI} = 0·5 mm; P_{Ar} = 50 mm; E = 1,024 J) and n-butyl iodide (P_{BuI} = 2·0 mm; P_{Ar} = 50 mm; E = 1,024 J). No attempt was made to monitor the decay of the excited atoms as the spectral intensity was weak, that in butyl iodide being weakest. Our observations on the compounds considered are consistent with the general primary process:

$$RI + h\nu \rightarrow R + I(5^2P_{1/2})$$

where $R = CF_3$, CH_3, C_2H_5, C_3H_7 and n-C_4H_9

The growth and decay of the relatively low concentration of $I(5^2P_{1/2})$ that we have observed in the photolysis of HI and DI indicate that the iodine atoms are produced predominantly in the ground state on photolysis. Our experiments further indicate that the yield of excited atoms compared with ground state atoms, resulting from the reaction:

$$H + HI \rightarrow H_2 + I(5^2P_{1/2})$$

is low. The emission from the excited iodine atoms produced from the foregoing reaction has been observed in flow-discharge experiments by Polanyi *et al.*[9].

We thank Prof. R. G. W. Norrish for his encouragement throughout the course of this work and for laboratory facilities. One of us (R. J. D.) thanks the Department of Scientific and Industrial Research for a maintenance grant during the tenure of which this work was carried out.

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BIOPHYSICS

Some Flow Properties of Whole Blood

The purpose of this communication is to outline some observations that have been made on the flow of whole blood when the material has been contained between parallel plates in relative rotation. The top plate was 7·5 cm diameter, while the bottom plate was of the 'reservoir' type to obviate the possibility of the material drying out at the periphery of the (narrow) gap.

The main results obtained were:

(1) If an oscillatory motion was superimposed on steady shearing the maximum torque on the top plate during the combined motion was scarcely more than the torque in steady shearing alone; the minimum was considerably less. The average torque was much less in the combined motion and it is possible that the average rate at which work was absorbed was less in the combined motion than in the steady shearing alone. (A later analysis has shown an increase in these two cases.)

(2) The response of the material to oscillatory motion was non-linear below about 20 c/s in these experiments. For a shear rate of the form:

$$s = s_0 \cos \omega t \qquad (1)$$

The stress was of the form:

$$\tau = \sum_{n=1} A_{2n-1} \cos([2n - 1]\omega t - \varphi_{2n-1}) \qquad (2)$$

it comprised a fundamental and odd harmonics. The phase angles φ_{2n-1} may be due to elasticity or time-dependency of the viscosity or yield criterion[2].

(3) A finite lag occurred after the commencement of steady shearing before the main build up of stress, suggesting that geometric hindrance of suspended particles is important.

(4) After cessation of steady shearing a finite time was required for the torque to decay to its terminal value, which may be due to elasticity or re-orientation of the particles.

(5) In steady forward motion the torque built up to a maximum and then decayed. If the motion was reversed, the torque built up much more slowly and to a higher value than in the forward direction.

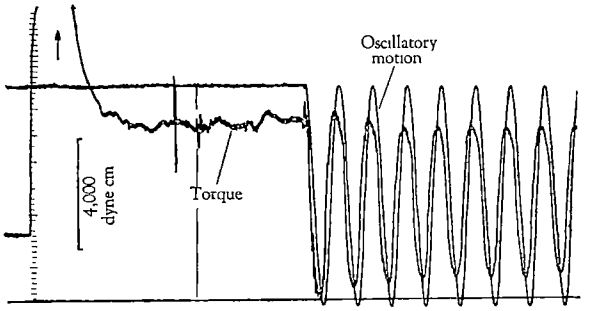


Fig. 1. Steady shearing with subsequent superposition of oscillatory motion. Steady shearing, 18·45 sec^{-1} (periphery), oscillatory strain amplitude 47 (periphery). Frequency, 0·792 c/s gap, 0 001 in. (2×10^{-5} in.)

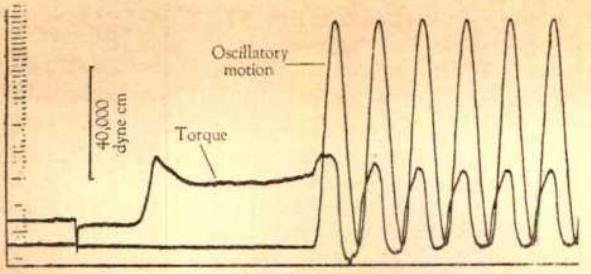


Fig. 2. Steady shearing with subsequent superposition of oscillatory motion. Steady shearing, 12·3 sec^{-1} (periphery), oscillatory strain amplitude 22·8 (periphery), frequency, 2·5 c/s gap, 0·0015 in. (2×10^{-3} in.)

(6) In oscillatory motion alone the torque amplitude would usually decay in time while maintaining a constant strain amplitude.

I have observed all these phenomena in the flow of clay-water systems. Separate complete reports on the clay and blood experiments will be published elsewhere.

Finally it is mentioned that the equation due to Casson[1] which has been applied to blood flow is unsuitable for non-steady flow and would certainly not describe the foregoing phenomena, neither would the well-known power-law which is likewise unsuitable for non-steady flow.

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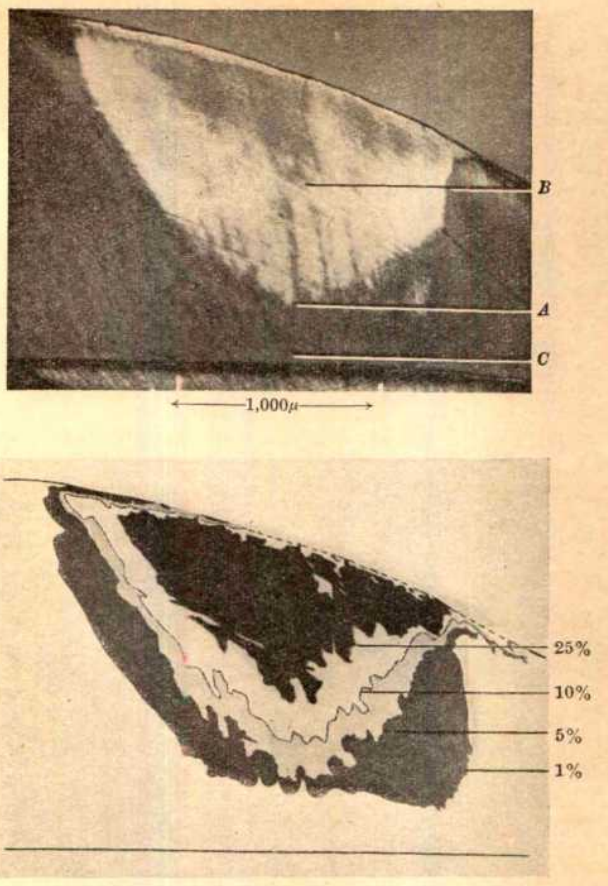
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Dental Caries and Enamel Structure

A GENERALLY accepted feature of the histology of ground sections of carious lesions in human dental enamel is the so called 'dark zone'. This zone, which lies at the border of the lesion, between the main body and the translucent zone or normal enamel, shows positive birefringence when the section is placed in quinoline and viewed by polarized light. It has been demonstrated that the positive birefringence in this zone is related to the presence of small inter-crystallite spaces which are inaccessible to media of large molecular size and contain air or vapour which is of low refractive index. This results in positive form birefringence which reverses the negative intrinsic birefringence of the enamel structure[1].

During examination of multiple sections of carious lesions it has been found that this positively birefringent zone is not always present along the advancing front of the lesion. This zone is usually present in the outer third of the enamel in peripheral sections of advanced lesions but sections cut close to the centre of such lesions frequently show absence of this positive zone, particularly in the inner two-thirds of the enamel. However, in some lesions involving only the outer third of the enamel the positive zone is missing along the deepest part of the advancing front. The positive zone is also more marked on the cervical aspect of a lesion than along its occlusal border and lesions involving the cervical enamel are usually found to have a well-marked positive zone surrounding the lesion.

Contour maps, based on isotropic lines in media of differing refractive indices, serve to provide information about the approximate distribution of spaces throughout the lesion[2]. The relationship of the contours in such maps, from a survey of thirty sections of carious lesions, suggests that the speed of advance and severity of attack relative to the structural front involved determine whether the positive zone is broad, narrow or absent. From experiments with artificial caries *in vitro* it seems that the positive zone is more likely to form under slow than rapid attack. As noted, it is in the central and deepest parts of the lesion, where attack may be expected to be most rapid, that the positive zone is frequently absent.



b

Fig. 1. *a*, Ground section of human permanent enamel showing a carious lesion, mounted in quinoline and viewed by polarized light with quartz sensitive tint. The advancing front of the lesion (*A*) shows little evidence of the positively birefringent 'dark zone'. The body of the lesion (*B*) and the surrounding enamel (*C*) show varying degrees of negative birefringence. *b*, Contour map, relating to the section in *a*, based on isotropic lines seen in air and watery media of refractive indices 1·33, 1·41, and 1·47. The approximate percentage of spaces corresponding to the contours is, respectively, 1, 5, 10 and 25 per cent, as indicated. The contours are well spaced-out towards the advancing front, unlike the variable pattern found when the positive zone is clearly marked

In one group of lesions, mostly in first permanent molars, the positive zone was noticeably absent (Fig. 1*a*) and in maps of such lesions the contours showing space distribution, and thus approximate degree of demineralization, were well spread out throughout the lesion (Fig. 1*b*). This is in contrast to the situation more commonly found where the spacing of the contours varies in direct relation to the width of the positive zone. It was found in these lesions that much of the enamel structure appeared to be defective, showing a marked positive birefringence when viewed in air by polarized light. These appearances could not be attributed wholly to variations in prism orientation. It is possible that the well-spaced contours may indicate a slow diffusion of the carious attack into the enamel, but the absence of the positive zone in quinoline suggests that the attack may have moved rapidly through the defective structure. Such circumstances could account for the deep penetrating lesion which suddenly cavitates without warning.

The first permanent molar in which this type of lesion was found undergoes formation both before and after birth. The deciduous dentition formed under similar circumstances is, therefore, of considerable interest. Such reports as exist in the literature concerning caries of the deciduous dentition make no observations which suggest that it is in any way different to that found in the permanent dentition. It is mostly the early approximal lesion that has been the subject of investigation. However, in an examination of gross caries in 67 teeth of children under the age of four, a group increasingly affected with the

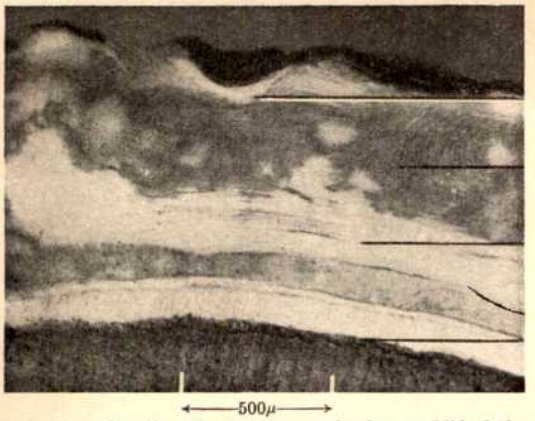


Fig. 2. Ground section of a deciduous molar from a child of 2½ years. The section is mounted in quinoline and viewed by polarized light with quartz sensitive tint. The enamel surface shows irregular pitting and there are prismless 'banded' areas which are negatively birefringent (*A*) in the enamel, interspersed with areas of positively birefringent prismatic structure (*B*); the enamel-dentine junction is shown at (*C*)

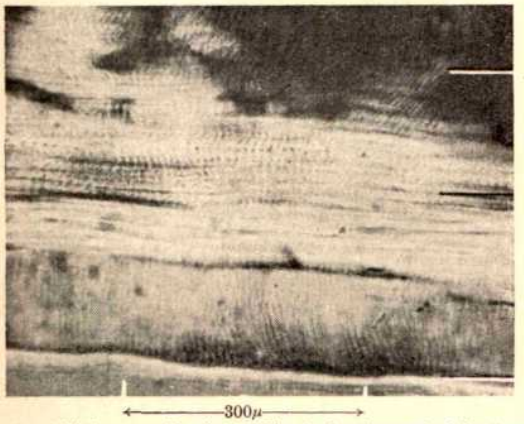


Fig. 3. A higher magnification of Fig. 2, showing part of the 'banded' area (*A*), positively birefringent area (*B*), and enamel-dentine junction (*C*)

ravages of dental caries, several forms of defect in the enamel structure have been noted: (1) Marked positive birefringence of the inner two-thirds of enamel, similar to that seen in the permanent enamel, was present in some of the teeth. Where this occurred the area involved was positively birefringent not only in air but also in water, indicating in all probability 5 per cent or more spaces in the structure. (2) A total lack of prism structure in parts of the enamel, with or without a hypoplastic enamel surface, was present in many of the teeth examined. The regions involved showed an appearance akin to fibrous banding which was highly negatively birefringent with areas of positively birefringent enamel interspersed throughout the structure (Figs. 2 and 3).

These appearances could be found in all the deciduous teeth examined: (*a*) in the prenatal enamel alone; (*b*) in the postnatal and particularly the outer enamel; (*c*) in both prenatal and postnatal enamel.

It would seem that the rapid spread of dental caries may be related to defects which are present in the enamel structure and which facilitate progress of the lesion.

In an attempt to determine whether a common factor exists, such as drug therapy or nutritional deficiency in pregnancy and infancy, which might account for the structural defects in the enamel, the mothers of the affected children and their medical practitioners are being approached for the relevant history.

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BIOCHEMISTRY

Investigations of the Kinetics of Red Cell Pyruvate Kinase in Normal Individuals and in a Patient with Pyruvate Kinase Deficiency

Since the first report by Tanaka *et al.*[1] of non-spherocytic haemolytic anaemia due to pyruvate kinase (PK) deficiency, about twenty further cases have been reported. In patients with this type of haemolytic anaemia, determinations of PK give decreased, but not totally absent, enzyme activities. The remaining activity amounts to 5–20 per cent of the normal. Waller[2] first posed the question as to whether there was any real lack of enzyme or whether the disease was due to a structurally changed enzyme protein with decreased affinity to the substrate. He reported two cases where the Michaelis constant of the PK towards phosphoenolpyruvate (PEP) was significantly higher in erythrocytes of patients than in normal human beings.

In order to verify this hypothesis, we investigated the kinetics of the red cell PK of normal individuals and compared our results with those from a patient with PK deficiency, and his closest relatives.

Erythrocytes of the patient (an 8-year-old boy), of two heterozygous family members, and of healthy controls were washed several times with isotonic sodium chloride solution and haemolysed by distilled water. The haemolysate was partially purified by separating from haemoglobin, using DEAE and 'Sephadex *G*-100' chromatography. The Michaelis constants for PEP and ADP as well as the *p*H-optima were determined in the native haemolysate and in the haemoglobin-free eluate. Furthermore, the specificity of the enzyme was tested against coenzyme-analogues, using an equimolar concentration of inosine diphosphate (IDP), cytidine diphosphate (CDP) and guanidine diphosphate (GDP) instead of adenosine diphosphate (ADP).

The Michaelis constants for PEP and ADP before and after the purification are given in Table 1. These values as well as the determinations of coenzyme specificity of heterozygous carriers did not show any significant difference from the normals (Table 2).

Table 1. Michaelis Constants for PEP and ADP before and after Partial Purification by 'Sephadex *G*-100'

	PK i.u./g Hb	*Km* PEP × 10^{-4}		*Km* ADP × 10^{-4}	
		Native haemolysate	Purified eluate	Native haemolysate	Purified eluate
*p*H	7·5	7·5	7·5 6·5	7·5	7·5
Controls	4·5 ± 2·5	1·9	14 3·7	4·6	3·1
Patient	0·9	0·9	11	4·0	3·2
Father	3·6	0·6	16	2·5	5·0
Mother*	1·0	1·2	15 2·7	3·2	3·0
Brother J. P.	3·1	0·6	20 4·8	6·1	6·3
Brother M.*	1·2	1·0	17 4·0	2·7	6·5

* Probably heterozygotes.

Table 2. Coenzyme Specifity of the Red Cell Pyruvate Kinase

Percentual activities with coenzyme-analogues compared with those with ADP

	Native haemolysate				Purified eluate			
	ADP %	GDP %	IDP %	CDP %	ADP %	GDP %	IDP %	CDP %
Normal	100	65	47	< 5	100	42	36	< 5
Father					100	51	43	< 5
Mother	100	59	56	< 5	100	48	35	< 5
Brother J. P.	100	56	45	< 5	100	38	28	< 5
Brother M.	100	60	54	< 5	100	51	37	< 5

Most striking, however, was the fact that, after partial purification, the affinity towards PEP was lower in the eluate than in the native haemolysate—this was observable from the higher Michaelis constant, both in patient's and in normal erythrocytes.

This paradoxical behaviour was elucidated by the fact that the optimal *p*H after purification was 6·7 instead of 7·2—the *p*H at which all determinations had originally been performed. Repeated determinations of PK at this optimal *p*H in purified eluate resulted in Michaelis constants similar to those in total haemolysate. The

purification, however, had no effect on the Michaelis constant for ADP.

No difference could be demonstrated between the kinetics of PK of normals and of a patient with PK deficiency haemolytic anaemia. It seems, therefore, most unlikely that this disease is caused by an abnormal enzyme protein, but rather by a defect in the gene controlling the synthesis of PK in red cells.

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Binding of Aromatic Compounds to Bovine Serum Albumin

The binding of non-ionic aromatic compounds to bovine serum albumin (BSA) has been approached in terms of the equilibrium between the bound and crystalline states[1]. The binding is quantitated in terms of the number of molecules of an aromatic compound solubilized per molecule BSA, in aqueous solution in equilibrium with the compound in its crystalline state. The measurement of this number, K, may be visualized as tantamount to the determination of the solubility of the compound in a two-dimensional solution on the surface of the protein molecule. This approach is derived from the interpretation of adsorption phenomena as thermodynamically analogous to solution phenomena[2].

Several mechanisms have been suggested for the binding of non-polar compounds to BSA and similar proteins. More than one type of interaction may be involved concurrently for the binding of a given compound; Benhold[3] has invoked two types, or degrees, of binding: (*a*) complete union of bound compound with the protein; (*b*) loose association, to interpret the solubilization of non-water-soluble physiological agents by BSA. Binding to BSA may be principally a function of interactions between a polar, functional group of the bound molecule and some group in the BSA[4]. Other investigators prefer to interpret the solubilization of non-ionic compounds by BSA in terms of hydrophobic bonding, principally[5]. In the light of this multiplicity of speculations regarding the mechanism of binding non-ionic compounds to BSA, the values of K, as defined here, were determined for a number of aromatic compounds. Possibly, the correlation of these values with the structural characteristics and physical properties of the compounds would yield some information on the mechanism or mechanisms of their binding to BSA.

The compounds investigated, their melting points and spectral characteristics are listed in Table 1. The preparation of *p*-phenylacetanilide[6] and *p,p'-bis*(*N,N*-dimethylaminophenyl)methane[7] was effected in this laboratory; the other compounds were obtained from the Eastman Kodak Co., and recrystallized as necessary. The number of molecules of each compound solubilized, that is, bound, per molecule of BSA, K, at 25° C and *p*H 7·5 (phosphate buffer, 0·1 M in total phosphate) was determined in the manner previously reported[1]. The values of K, as log K, are given in Table 2. This table also includes the solubilities of the compounds in the absence of BSA under the same conditions, x in moles per l. as log x.

Table 1. Properties of Compounds

Compound	Melting point (°C)	λ_{max} (mμ)	$\varepsilon \times 10^{-4}$
p-Aminobiphenyl	52–54	271	1·9
Naphthalene	82–83	276	0·54
Biphenyl	69–71	248	1·8
2-Aminofluorene	129–130	278	1·5
p-Phenylacetanilide	177–178	268	2·5
N-Acetyl-2-aminofluorene	203–205	276	2·5
Fluorene	116–118	260	1·8
Phenanthrene	101–102	293	1·4
p-Bromobiphenyl	90–91	260	2·1
p-Phenylacetophenone	122–123	283	2·4
p,p'-bis(*N,N*-Dimethylaminophenyl)methane	94–95	252	1·6
p,p'-bis(*N,N*-Dimethylamino)-benzophenone	178–179	375	3·3
Anthracene	218	252	11
2-Nitrofluorene	160	331	1·8

Table 2. Binding by Bovine Serum Albumin and Solubility of Aromatic Compounds at 25° C and *p*H 7·5

Compound	log K	log x
p-Aminobiphenyl	0·93	−3·2
Naphthalene	0·38	−3·8
Biphenyl	0·25	−4·6
2-Aminofluorene	0·23	−4·0
p-Phenylacetanilide	0·08	−4·2
N-Acetyl-2-aminofluorene	0·04	−4·7
Fluorene	−0·04	−5·0
Phenanthrene	−0·10	−5·4
p-Bromobiphenyl	−0·26	−5·5
p-Phenylacetophenone	−0·32	−4·9
p,p'-bis(*N,N*-Dimethylamino-phenyl)methane	−0·33	−4·9
p,p'-bis(*N,N*-Dimethylamino)-benzophenone	−0·58	−5·4
Anthracene	−0·83	−6·2
2-Nitrofluorene	−0·85	−6·3

Data in Table 2 suggest that the solubilization correlates with solubility; the calculated correlation coefficient for log K and log x is +0·96. A plot of log K as a function of log x is shown in Fig. 1. The regression line drawn according to the method of least squares represents the equation:

$$\log K = 0{\cdot}53 \log x + 2{\cdot}5 \qquad (1)$$

The simplest model for binding involves adsorption of the bound molecule on the surface of the BSA[8]. One surface of the bound molecule (assuming it is planar) is thus exposed to the solvent. In solution, both surfaces of the planar molecule are exposed to solvent, that is, water. There are, therefore, twice as many interactions between the molecule and water in the dissolved state as in the bound state. Thus, the contributions of these interactions to the energy of the molecule will be twice as great in the dissolved state as in the bound state. Since the solubility measurement compares the energy of the dissolved state, E_s, with that of the crystalline state, and K is a measure of the energy of the bound state, E_b, compared with the crystalline state, E_s and E_b should be related (if the entropies of the compounds compared are comparable in each state) according to equation (2):

$$\frac{dE_b}{dE_s} = \frac{d \log K}{d \log x} = \frac{1}{2} \qquad (2)$$

The experimental value of this derivative, obtained by differentiation of equation (1), is 0·53.

In the light of this relationship, it is of interest to note the results obtained in the earliest measurement of the solubilization, the increased apparent solubility, of slightly soluble compounds by serum proteins. The solubilization of anaesthetics in serum, compared with isotonic saline, reported by Moore and Roaf[9] shows this same pattern of increasing solubilization, s in concentration units, with

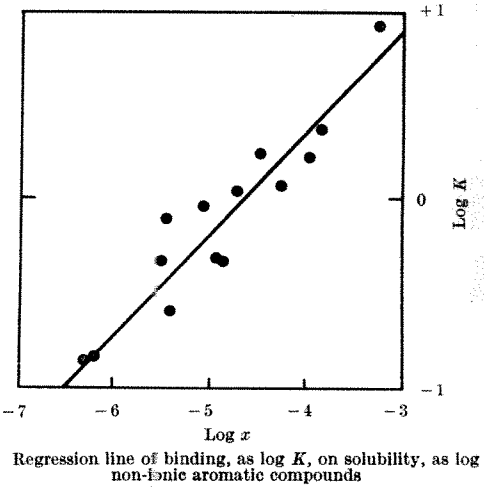


Fig. 1. Regression line of binding, as log K, on solubility, as log x, for non-ionic aromatic compounds

increasing water solubility of the anaesthetics. For these rather primitive data, a correlation coefficient of $c. + 0{\cdot}9$ may be calculated; $d \log s / d \log x$ (corresponding to $d \log K / d \log x$) is, as with the data reported in this communication, c. 0·5. This correlation, however, appears to have escaped the notice of the pioneer investigators.

The invocation of interactions such as dipole–dipole attraction, hydrogen bonding or compound formation between the bound molecule and serum albumin is, therefore, not necessary to the quantitative treatment of the binding behaviour of non-ionic molecules. The success of this simple interpretation is consistent with the visualization of binding as a consequence of hydrophobic bonding[5].

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Tryptic Digestion of Tropomyosin *A*, Tropomyosin *B* and Myosin

Two types of tropomyosins co-exist in lamellibranch adductors and in cephalopod muscle[1]: TM_A and TM_B, TM_A being the protein referred to as invertebrate tropomyosin, or water-insoluble tropomyosin, by Bailey, and TM_B the water-soluble variety.

A prolonged action of trypsin on TM_A from *Pinna nobilis* has been examined[2]; besides dialysable products there remained a non-dialysable fragment which was precipitated by the addition of 3 per cent trichloroacetic acid (TCA). This fragment still contained a high proportion of arginine and lysine; its chemical and physical properties have been investigated[3]. When isolated under neutral conditions it proved to be highly resistant to further tryptic action. The rate and extent of the proteolysis were followed by different methods and the results indicated that trypsin splits approximately half the potentially susceptible bonds present in the whole molecule. Tryptic action on TM_A from other invertebrate species has now been investigated and it has been found to give a similar trypsin-resistant fragment which will be referred to as 'tryptic fragment'. TM_A was prepared from *Pecten maximus* adductor muscle by the ethanol method[1]. When hydrolysed with trypsin for 24 h under similar conditions to those used for *Pinna nobilis* TM_A (ref. 2), much diffusible material was obtained, leaving a non-dialysable fragment which precipitated after the addition of 3 per cent TCA. This fragment was isolated and purified by the same procedure as described for *Pinna* TM_A. The rate and extent of the digestion of *Pecten* TM_A with trypsin for 24 h at *p*H 8·2 and 25° C was followed by determining total nitrogen and α-amino nitrogen in the TCA-soluble fraction obtained after the addition of equal volumes of 3 per cent TCA to a portion of the digest, and by following the alkali uptake in the autotitrator. Table 1 shows the similarity of the effect of trypsin on *Pecten* TM_A and on *Pinna* TM_A, in rate and extent of the reaction. In both, about 50 per cent of the total nitrogen is TCA-soluble at the end of the reaction and the TCA-insoluble material still contains arginine[4]. In this case, as in the tryptic digestion of *Pinna nobilis* TM_A, changes in the enzyme–substrate ratio do not significantly affect the extent of the digestion (Table 1). The kinetics of the proteolysis was followed by alkali uptake in an autotitrator and found to be similar to that observed in the tryptic digestion of *Pinna* TM_A (ref. 4).

Table 1. Action of Trypsin on TM_A from *Pinna* and *Pecten*

	Protein conc. (mg/ml.)	Enzyme–substrate ratio (by wt.)	Conditions *p*H	Temp. (° C)	N in the TCA sol. as % of total N	Bonds split/mole (ninhydrin)	Bonds split/mole (autotitrator)
Pinna	4·3	16 : 100	8·2	25	59·4	144 (17 h)	—
Pecten	4·6	6 : 100	8·2	25	53·4	147 (18 h)	—
Pecten	5·9	0·9 : 100	8·2	25	63·0	—	—
Pecten	1·9	1 : 100	8·2	30	—	—	48 (2 h 30 min)
Pinna	3·4	1 : 100	8·5	30	—	—	52 (2 h 30 min)

The diffusible peptides produced by the digestion, when subjected to ionophoresis at *p*H 6·5, gave a pattern very similar to that obtained with the diffusate of the tryptic digest of *Pinna* TM_A. This is not surprising since the properties of *Pecten maximus* TM_A are in all respects very similar to *Pinna nobilis* TM_A (ref. 5). TM_A was also prepared from the South American mollusc *Aulacomya magellanica*; although it does not show such great similarity of properties with *Pinna* TM_A as *Pecten* TM_A it also leaves a trypsin-resistant fragment[4]. It appears, therefore, that the presence of a resistant tryptic fragment may be a general property of all tropomyosins *A*.

On the other hand, *Pecten maximus* TM_B (prepared by the method of Bailey and Rüegg[5] and kindly supplied by Dr. J. C. Rüegg) does not leave a fragment after digestion with trypsin for 2 h. No precipitate was obtained when the digest mixture was treated with either 3 per cent TCA or 0·1 N sodium acetate, *p*H 5·4 (conditions used for the precipitation of the TM_A tryptic fragment). After paper electrophoresis (0·06 M veronal buffer, *p*H 8·4) of the products of the digestion, staining with bromophenol blue did not show the characteristic blue band of the TM_A fragment. Furthermore, the peptide maps of these two proteins are very different (Fig. 1). Rabbit tropomyosin, which is thought to be similar to TM_B (refs. 5, 6), is also completely digested by trypsin (personal communication from the late K. Bailey).

So far, no TM_A has been found in vertebrate muscle, but molluscan muscle contains quite large amounts. The

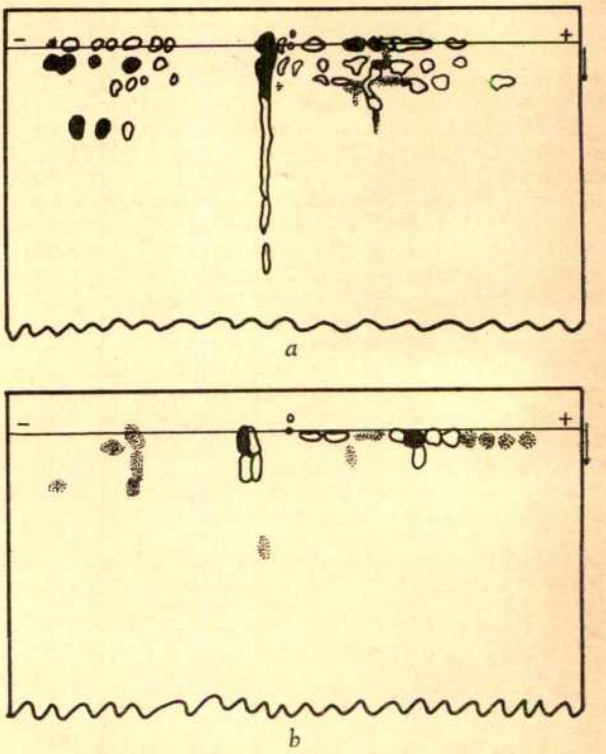


Fig. 1. Fingerprints of tryptic digests obtained by running a portion by descending chromatography in *n*-butanol–acetic acid–water (4 : 1 : 5, by vol.) for 17 h and electrophoresis under toluene in pyridine-acetate *p*H 6·5 for 1 h at a voltage gradient of 40 V/cm. Digestions were carried out for 2 h at 30° C, *p*H 8·2, enzyme–substrate ratio 1 : 100 (by wt.). Decreasing intensities of the ninhydrin colour, ■, □, ▩. (*a*) *Pecten* TM_A; (*b*) *Pecten* TM_B

properties of TM_A and myosin and certain features of their amino-acid composition have suggested[7,8] the possibility that vertebrate muscle contains TM_A as an integral part of the myosin molecule and not in a free form. If so, one would expect to obtain a 'tryptic fragment' similar to that obtained from TM_A, after tryptic hydrolysis of myosin. Experiments designed to test such a hypothesis were performed and it was found that although some TCA-insoluble material was obtained after digestion for 24 h, its solubility properties in ammonium sulphate were quite different from the TM_A 'tryptic fragment'. No material was precipitated at ammonium sulphate saturation exceeding 50 per cent (TM_A 'tryptic fragment' precipitates between 38–50 per cent saturation), suggesting that it is composed of smaller peptides.

The fact that the 'tryptic fragment' has been found in TM_A of three different species but not in TM_B suggests a structural characteristic which differentiates these two molecules. Whether this chemical characteristic is related to its functional properties remains to be seen.

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Distribution of Chlorophylls in Chloroplast Fragments

THE molecular arrangement of the lipid-protein layers in chloroplasts, chloroplast grana, and smaller sub-units, is unknown but is vital to understanding the photophysical and photochemical events that occur there.

Recently, Boardman and Anderson[1] fragmented spinach chloroplasts with digitonin and showed a changing ratio of chlorophyll *a* to chlorophyll *b* in fractions separated by differential high-speed centrifugation. A fraction sedimenting between 50,000 and 144,000*g* had an *a*/*b* ratio of 6 and was interpreted to represent pigment system 1, the long wave-length system of photosynthesis. Fractions sedimenting at 10,000*g* or less had an *a*/*b* ratio of 2 and were interpreted to represent pigment system 2, the short wave-length system.

Murakami *et al.*[2] have also extracted spinach chloroplasts with digitonin and found a progressively increasing chlorophyll *a*/*b* ratio from 2·15 in whole chloroplasts to 12·0 in the supernatant after centrifugation at 105,000*g*. Their investigations showed reduced Hill activity and triphosphopyridine nucleotide reduction in the high-speed fractions suggesting that light-dependent reaction 2 was lost as particle size decreased. This was interpreted to mean that there was loss of an essential component(s), which, they point out, is supported by the concomitantly increasing chlorophyll *a*/*b* ratio, thus signifying loss of chlorophyll *b*.

We have mechanically fragmented spinach chloroplasts by sonication in an aqueous suspension rather than by using digitonin; fragment suspensions were then fractionated by differential centrifugation. The experimental conditions and centrifugation times have been published in detail[3–5]. The fractions (CF) are designated by subscripts indicating the centrifugal force range used to sediment the particular fraction. For example, CF_{1-20} is the fraction that pelleted between 1,000 and 20,000*g*.

Chlorophyll content was assayed in 80 per cent acetone and computed according to Arnon's equations[6]. Boardman and Anderson used Vernon's equations[7]. The method used by Murakami *et al.* is not reported. Thus the differences in absolute values between our results (Table 1) and theirs[1,2] may be due to the method of calculation or perhaps to inherent differences in the spinach. The highest *a*/*b* ratio occurred in CF_{70-145}, which corresponds to Boardman and Anderson's 50,000–144,000*g* fraction. The *a*/*b* ratio of 3 in the lower-speed pellet, CF_{1-20}, containing large fragments, corresponds fairly well to Boardman and Anderson's 1,000–10,000*g* fraction. The 145,000*g* supernatant ($CF_{145\ spt}$) shows a decreased *a*/*b* ratio relative to the CF_{70-145} fraction, which also agrees with the studies of the Australian investigators.

Table 1. CHLOROPHYLL CONTENT OF FRACTIONS ISOLATED FROM SPINACH CHLOROPLASTS

Fraction	Chlorophyll (mg)	Absorbancy ratio†	*a*/*b*
Chloroplast suspension	60·5*	2·56	2·74
CF_{0-1}	3·1		
CF_{1-20}	33·4	2·66	3·01
CF_{20-50}	16·4	2·73	3·25
CF_{50-70}	2·8		
CF_{70-145}	2·3	3·02	4·00
$CF_{145\ pts}$	1·5	2·82	3·32

* Average chlorophyll yield from 800 to 1,000 g wet weight of de-ribbed spinach leaves.
† Ratio of 663 mμ/645 mμ.

Preliminary investigations on small chloroplast particles sedimenting at 173,000*g* from $CF_{145\ spt}$ also showed a differential distribution of chlorophyll content. These small fragments, referred to as polyquantasomes, are photoactive[5,8].

Although the significance of these results is not clear, Boardman and Anderson's assignment of their 50,000–144,000*g* fraction to system 1 and of the remaining fractions to system 2 cannot be accepted unequivocally. The fraction sedimenting at 1,000–10,000*g* (system 2) is probably composed of particles greater than 1500 Å in diameter with an average molecular weight greater than 1×10^8 (refs. 5, 8). The fraction sedimenting at 50,000–144,000*g* is probably composed of particles 600–700 Å in diameter with an average molecular weight of 1×10^7 (refs. 5, 8). The sizes of all these particles are immense in comparison with quantasomes[9] or polyquantasomes[5], and the fractions are unquestionably composed of a considerable number of such sub-units. It is apparent that additional study of small-particle fractions is essential to establish firmly that the chlorophyll of spinach chloroplasts is associated with two different particles representing the two pigment systems.

Nevertheless, regardless of the fragmentation method, the changing chlorophyll ratios when correlated with the size of particles in the centrifuged fractions suggest that natural fault lines exist in the chloroplast lamellar structure along which rupture is most likely to occur when the organelle is subjected to chemical or physical stress.

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Glycogen Synthesis from Pyruvate in Muscle

Under normal conditions glucose is the major source of muscle glycogen. In muscle, pyruvate and lactate make only a minimal contribution to glycogen but the liver can freely convert these compounds into glycogen[1,2]. One of the key enzymes in the process of glycogenesis in liver is fructose-1,6-diphosphatase (FDPase)[3], and the inability of muscle to convert pyruvate to glycogen was attributed to its absence[4,5]. Recently, however, Krebs and Woodford[6] have shown that the enzyme does occur in striated muscle, so that at least one of the energy barriers in the conversion of pyruvate to glycogen is now known to be surmountable.

The possibility that muscle might be able to synthesize glycogen from pyruvate and lactate had to be considered when it was observed that the glycogen content of muscle, obtained from patients suffering from liver glycogen storage disease due to glucose-6-phosphatase (G-6-Pase) deficiency, was moderately but significantly greater than normal (Table 1).

In such patients glycogen is structurally normal and there is no enzymatic hindrance to the breakdown and removal of muscle glycogen. The observation was all the more unexpected because the blood sugar of these patients is low; on the other hand, the blood level of pyruvate and lactate is always high[7,8] (Table 1). It seemed possible, therefore, that under these circumstances these two compounds might become a source of muscle glycogen.

The supposition was supported when it was observed that considerably more radioactivity from labelled pyruvate was incorporated into the patients' muscle glycogen than into normal muscle glycogen (Table 2).

Additional evidence came from the assay of FDPase activity (Table 3) using the method described by McGilvery[9]. Some activity was detected in normal muscle but it was much greater in the muscle obtained from the patients, thus confirming the potential ability of muscle to form new glycogen from 3-carbon precursors.

For comparison, the results of analyses of muscle obtained from 2 patients suffering from a primary muscle glycogen storage disease, due to phosphorylase deficiency[10], are included in the tables. These patients do not have elevated plasma lactate and pyruvate levels and although their muscles contain more than the normal amount of glycogen, they behave like normal muscle with respect to glycogen synthesis from pyruvate.

These investigations confirm the presence of FDPase in human striated muscle. Krebs and Woodford[6] consider that its role in muscle is limited to the conversion of phosphorylated precursors, particularly α-glycerophosphate, because an energy barrier exists for the direct phosphorylation of pyruvate to phospho-enolpyruvate, and that it therefore plays only an insignificant part in the synthesis of glycogen from pyruvate. In the liver, this barrier is circumvented by the enzymes of the 'dicarboxylic acid shuttle'[11]; in this pathway, carbon dioxide combines with pyruvate to form oxalacetate, which is then converted, via malate, to phospho-enolpyruvate. In normal muscle, the activity of the enzymes of the shuttle is less than the sensitivity of the methods at present available for the assay[12]. However, the fact that not only pyruvate but also significant amounts of labelled bicarbonate (Table 2) can be incorporated into muscle glycogen under certain conditions suggests that they are probably also present in normal muscle. These vestigial pathways in muscle may become more active under the influence of high pyruvate and lactate concentrations and play a part in the metabolic homeostasis of the body.

Table 1

Condition	Subject No.	Age	Sex	Muscle glycogen (mg/g wet weight)	Blood lactate (mg/100 ml.)
G-6-Pase deficient		3	M	14	38
G-6-Pase deficient		4	F	18	42
G-6-Pase deficient		19	M	24	58
G-6-Pase deficient		6	M	21	26
Phosphorylase deficient		15	M	52	8
Phosphorylase deficient		38	M	45	9
Normal (4 subjects)		3–12	M and F	1–6	7–12
Normal (24 subjects)		26–58	M and F	2–10	5–12

Table 2. Incorporation of Radioactivity from ^{14}C-labelled Pyruvate or Bicarbonate into Muscle Glycogen

Subjects	No.	C.p.m./100 mg glycogen (range) From pyruvate	From bicarbonate
G-6-Pase deficient	4	2,800–6,400	800–1,460
Phosphorylase deficient	2	700, 1,450	0, 26
Normal adult	6	820–1,600	0–84

About 100 mg muscle were incubated in Krebs–Ringer bicarbonate buffer containing 40 mM pyruvate. Either 0·5 μc. 2-^{14}C pyruvate or 0·5 μc. $NaH^{14}CO_3$ were added to the flasks.

Table 3

Subjects	No.	FDPase activity * (range)
G-6-Pase deficient	4	9–36
Phosphorylase deficient	2	0, 5
Normal adult	4	2–6

FDPase activity determined by McGilvery's method (ref. 9).
* Expressed as μmoles phosphate produced per g fresh tissue in 20 min.

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Conversion of Alcohol to Ethyl Esters of Fatty Acids by the Lactating Goat

Freshly secreted milk contains a lipogenic principle that will incorporate fatty acids into the ester lipids of the milk[1]. While investigating this principle with the aid of palmitic-1-^{14}C acid and thin-layer chromatographic analysis of the resulting labelled lipids, we noted a prominent spot on autoradiograms between the solvent front and the position of the triglycerides (Fig. 1). The position of this unknown substance was very close (following) to that of the cholesterol esters. It occurred to us that this spot might result from ethyl palmitate-1-^{14}C, since the labelled compound was dissolved in ethanol (usually 250 μl.) to facilitate dispersion in the milk. Further investigation confirmed this contention. The radioactive unknown substance exhibited R_F values similar to authentic ethyl palmitate with three different thin-layer chromatographic solvent systems. When the area of a plate containing the unknown was extracted and this extract injected on to an appropriate gas chromatographic column, 45 per cent of the activity measured in the unknown spot was trapped during the time ethyl palmitate would have emerged from the column. Since this recovery value results, in part, from losses in the syringe, in the column, by detector leaks, etc., the major activity is concluded to have moved in correspondence with ethyl palmitate.

Similarly, conversion of stearic acid to its ethyl ester was observed when it was dispersed with ethanol in the milk system, and both stearate and palmitate showed two closely moving spots when the ethyl esters were separated on thin-layer plates by a 60 : 40, hexane–benzene solvent system (data not shown). The capacity of the lipogenic milk system to convert stearate to oleate has been reported[2].

These findings regarding ethyl ester formation led us to consider *in vivo* alcohol metabolism and the problem of alcoholism. Two experiments were performed on each

of two lactating goats. In the first experiment 150 ml. of 30 per cent (v/v) ethanol in water was orally administered to the goats. Closure of the oesophageal groove was promoted by spraying the throat with 1 per cent copper sulphate, thus directing the alcohol to the abomasum and by-passing the rumen. Milk samples were recovered from the milk by extraction with 2 : 1 (v/v) chloroform–methanol. The lipid fractions eluted from silicic acid columns[3] ahead of the triglycerides, those containing the ethyl esters of fatty acids, cholesterol esters and hydrocarbons, were analysed by thin-layer and gas chromatography. Both goats showed evidence of ethyl esters in the milk lipids (Figs. 2 and 3 are representative). The maximum level of ethyl esters was reached in the 3–6 h period and approximated 0·01 per cent of the lipids in the case of one goat and about 0·001 per cent for the other. Milk collected the day following the experiment showed no evidence of ethyl esters. The characteristic pattern of fatty acid ethyl ester composition (Fig. 3), the component identities of which were confirmed on both polar (DEGS) and non-polar ('Apiezon-*L*') column coatings, closely resembles that of C_{16}- and C_{18}-fatty acids of milk fat. Although no short-chain fatty acid components were detected, we presume that ethyl esters were formed in the mammary gland. In the case of the goat showing the highest levels of ethyl esters in the milk, blood samples (25 ml.) taken at the same times as milkings (0, 1/2, 3, 6 and 24 h) showed no evidence of ethyl esters.

Somewhat anomalously the second type of experiment, in which 12 ml. of 20 per cent (v/v) ethanol in water was infused into the mammary gland by way of the teat canal, revealed no formation of ethyl ester by either goat in milk drawn at intervals over 24 h. From the odour of the milk drawn 30 min after infusion, rapid oxidation to acetaldehyde (and then to acetate) might account for the negative findings.

The foregoing results suggest that lactating human beings who consume alcohol might secrete traces of ethyl esters with their milk. Although we could not contrive that situation experimentally, we did analyse adipose tissue removed from eight adult human patients, several of whom admitted to moderate alcohol consumption.

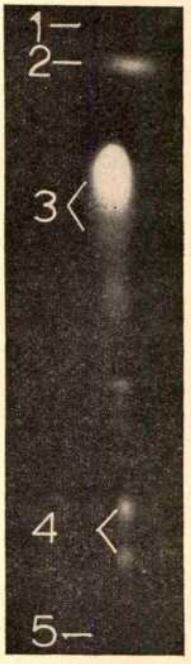


Fig. 1. Thin-layer chromatographic separation (autoradiogram) of neutral lipids from freshly secreted goat milk incubated for 90 min with palmitic acid-1-^{14}C (added in ethanol). 1, Position of solvent front; 2, ethyl palmitate; 3, triglycerides; 4, diglycerides; 5, position of origin. Solvent system 90 : 10 : 1 (v/v) petroleum ether–ethyl ether–acetic acid

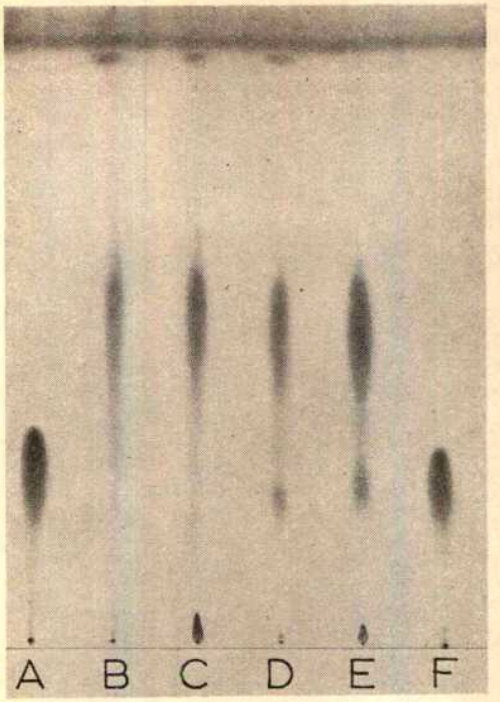


Fig. 2. Thin-layer chromatographic separation of goat milk lipids recovered in milkings 0 (*B*), 1/2 (*C*), 3 (*D*) and 6 (*E*) h after oral administration of a 'cocktail' (150 ml. of 30 per cent, v/v, ethanol in water) to the goat. *A* and *F*, Authentic ethyl oleate. Elongated spots (milk lipid samples) are from cholesterol esters. Solvent system 60 : 40 (v/v) hexane–benzene

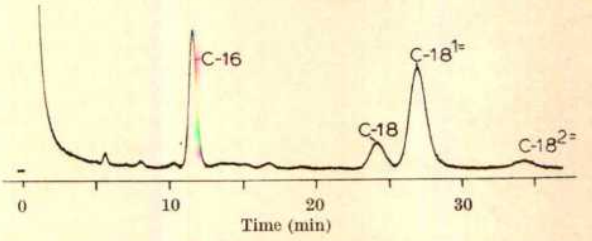


Fig. 3. Gas chromatogram of ethyl esters in goat milk lipids 3 h (see Fig. 2) after oral administration of alcohol to the animal. Peaks corresponding to ethyl palmitate, stearate, oleate and linoleate are designated C_{16}, C_{18}, $C_{18}^{1=}$ and $C_{18}^{2=}$, respectively

The tissue samples (primarily abdominal) were removed at the time of surgery and the lipids recovered and analysed by techniques described, preceding. Ethyl esters could not be detected in any of these samples. These negative results notwithstanding, a tissue by tissue analysis, especially of chronic alcoholics, might be rewarding.

In consequence of our present findings, previous activity data on cholesterol esters may be partly in error when we used ethanol to disperse label[1], but should be free of such error in experiments in which ethanol was not used[4,5].

We thank J. Reed Babcock, surgeon, Center County Hospital, Bellefonte, Pennsylvania, for the adipose tissue samples, and Robert J. Flipse, of our Department, for advice on autoradiography. This work was supported in part by grants *H*3632 and *AM*0677–01 from the U.S. National Institutes of Health.

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PHYSIOLOGY

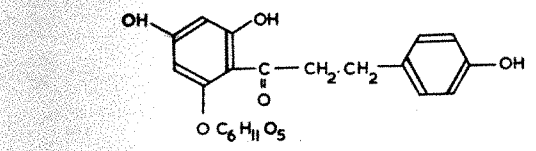
Polyphenolic Compounds and Intestinal Transfer

It is well established that phlorrhizin and its aglycone phloretin affect hexose transfer in a number of tissues. Recently Forsling and Widdas[1] showed that phenolphthalein caused competitive inhibition of facilitated glucose transfer in human erythrocytes. In view of its purgative action, it was of interest to test whether phenolphthalein also affected intestinal transfer mechanisms. At the same time the opportunity was taken to test another polyphenolic compound phenol red (phenolsulphonphthalein), a molecule of similar structure which is known to be transferred by the renal tubule. The formulae of these compounds are shown in Fig. 1.

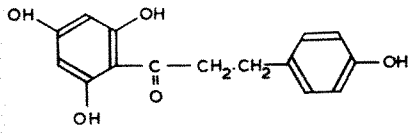
Experiments were carried out with sacs of everted small intestine of the rat, as used by Barry, Matthews and Smyth[2], and the middle fifth of the combined jejunum and ileum was used in all cases. Glucose (28 mM) or methionine (15 mM) was initially present in the mucosal fluid and the transfer of these substances was investigated in the presence and absence of the polyphenols. The results are shown in Table 1.

In confirmation of Parsons, Smyth and Taylor[3], phlorrhizin (5×10^{-5} M) strongly inhibited glucose transfer, and at 5×10^{-4} M nearly abolished glucose transfer. As was found by Jervis, Johnson, Sheff and Smyth[4], phloretin (5×10^{-5} M) caused little inhibition, but we have now found that at 5×10^{-4} M it markedly reduced glucose transfer. Phenolphthalein (5×10^{-5} M) had almost as great an effect as equimolar phlorrhizin on glucose transfer, while phenol red even in a concentration of 10^{-4} M had little effect.

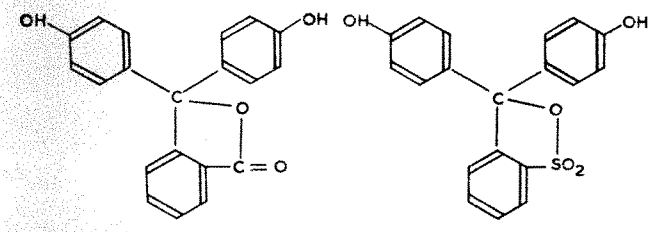
Any substance inhibiting transfer of glucose from the mucosal fluid may produce its effect either on glucose entry or on metabolism. These two activities can be separated by using glucose in the serosal fluid, as the glucose can then be metabolized without going through the entry mechanism. The effect of the inhibitor can be examined either by measuring fluid transfer (which depends on glucose metabolism) or by disappearance of glucose from the system. When glucose was present initially in the serosal fluid, phenolphthalein (5×10^{-5} M) did not affect glucose disappearance, and had only very slight, if any, effect on fluid transfer. Hence its action must be to prevent glucose from entering the cell from the mucosal side. Phloretin, however, reduced fluid transfer from 2·3 ml. to 0·4 ml., and this suggests that glucose metabolism was being impaired.



PHLORRHIZIN



PHLORETIN



PHENOLPHTHALEIN PHENOLSULPHONPHTHALEIN

Fig. 1

Table 1

Polyphenolic compound	Intestinal transfer: Glucose (μM)	Intestinal transfer: Methionine (μM)
None	337 ± 9(12)	61 ± 4 (10)
5×10^{-5} M Phlorrhizin	102 ± 7 (6)	62 ± 1 (7)
5×10^{-4} M Phlorrhizin	18 ± 3 (5)	41 ± 2 (6)
5×10^{-5} M Phloretin	307 ± 10 (6)	53 ± 4 (6)
5×10^{-4} M Phloretin	94 ± 6 (9)	22 ± 1 (6)
5×10^{-5} M Phenolphthalein	131 ± 12 (6)	36 ± 3 (8)
1×10^{-4} M Phenol red	317 ± 19 (6)	66 ± 3 (6)

Values given are the means ± *S.E.* The figures in parentheses indicate the number of experiments performed.

The effect of phenolphthalein is less specific than that of phlorrhizin as it inhibited methionine transfer in a concentration of 5×10^{-5} M, while a much greater concentration of phlorrhizin was required to cause a similar inhibition. Phenol red was also ineffective on methionine transfer in the concentrations tested. Phloretin (5×10^{-4} M) had more effect on methionine transfer than phlorrhizin in the same concentration, and this could be because its greater lipid solubility permitted easier entry into the cell with more effect on metabolism.

The difference between phenol red and phenolphthalein is of great interest on account of the similarity in their structure. A possible explanation is the difference in pK values. At a pH of 7·4 phenolphthalein will be mainly in the undissociated form, while phenol red will be largely dissociated, and the undissociated form may be the effective inhibitor of the entry process.

We thank the Medical Research Council and John Wyeth and Brothers for financial assistance.

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Comparative Study of the Urease in the Rumen Wall and Rumen Content

In the course of the ruminohepatic nitrogen cycle[1,2], or by supplementation in feeding[3], a considerable amount of urea can enter the rumen. The ureolytic activity of the rumen content was investigated and found to be due to some species of ruminal micro-organisms. Attempts to obtain enzyme preparations free from bacteria for a more accurate study, however, were unsuccessful[4]. Further investigations revealed that the urease activity of the ruminal mucosa appeared to be too high to be simply explained by surface contamination by rumen contents[5]. This communication deals with a study of partially purified urease preparations obtained from ruminal bacteria, as compared with partially purified enzyme prepared from the rumen mucous membrane, and the origin of the latter enzyme is also discussed.

Preparation of cell-free extracts. Washed suspensions of ruminal bacteria were prepared from the rumen content of freshly slaughtered cattle[6]. The bacterial cells were broken up using an M.S.E. ultrasonic disintegrator for 15 min. Cell-free extracts were obtained by centrifugation at 75,000*g* for 30 min. The mucous membrane from the rumen of freshly slaughtered cattle was washed thoroughly in a stream of cold water. The villi were cut off, homogenized in two parts of 0·1 M *tris*–HCl buffers (pH 8·5)

and finally centrifuged at 75,000*g* for 30 min to remove the cell fragments.

Preparation of acetone powders. Each cell-free extract was poured, with gentle stirring, into ten volumes of acetone chilled to −20° C. The precipitates were centrifuged down, washed twice by stirring with five parts of cold acetone and re-centrifuging. The washed sediments were suspended again in cold acetone and dried by suction on a Buchner funnel. The acetone powders could be stored in a desiccator at 4° C for several weeks without appreciable loss of activity. All the foregoing operations were conducted at about 4° C.

Preparation of enzyme containing solutions. The enzyme solutions of the acetone powders were prepared by extracting the corresponding powder in buffer by gentle stirring for 15 min. In the case of enzyme activity or *Km* determinations 0·2 M *tris*–HCl buffer (*p*H 8·5) was used. For *p*H-optimum estimations, 0·1 M *tris*-sodium monohydrogen phosphate–HCl buffers were taken. The amount of acetone powder to be extracted was chosen so as to give not less than 0·0015 Sumner units (S.U.)/ml. extract.

Measurement of enzyme activity. Urease activity was measured by incubating the corresponding enzyme solution with excess of substrate (0·04 M urea) in the outer chamber of a Conway unit. The final volume of the reaction mixture was 1 or 2 ml. in most experiments. The incubation period was 15 min at 20° C. The reaction was stopped by the addition of 1 ml. of saturated potassium carbonate. The ammonia produced was determined according to Conway[7]. Each determination was carried out in duplicate or triplicate against a blank. The urease activity measured in the acetone powders varied considerably according to the material used. Acetone powders from the ruminal mucosa contained about 0·0002 S.U./mg. The powders from the ruminal bacteria contained more activity, about 0·0005 S.U./mg.

*Effect of p*H. In Fig. 1 the reaction velocity is shown as a function of *p*H. Maximal velocity for both ruminal preparations is found at *p*H 8·5. The enzyme activity falls sharply on both sides of the maximum. Both curves are similar in shape. For comparison, soya bean urease was also examined. This preparation gave an optimum between *p*H 7·5 and 8·0. Here, the *p*H curve showed a more gradual fall on both sides (Fig. 1).

Effect of substrate concentration. The effect of urea concentration on the reaction velocity for each of the ruminal preparations is shown in Fig. 2. The Michaelis constants (*Km*) calculated according to Lineweaver and Burk were found to be $1{\cdot}5 \times 10^{-3}$ mol for both preparations. No difference could be detected between corresponding activities from purified ruminal bacteria and ruminal mucosa preparations. The *Km* of the soya bean urease preparation was $1{\cdot}57 \times 10^{-3}$ mol.

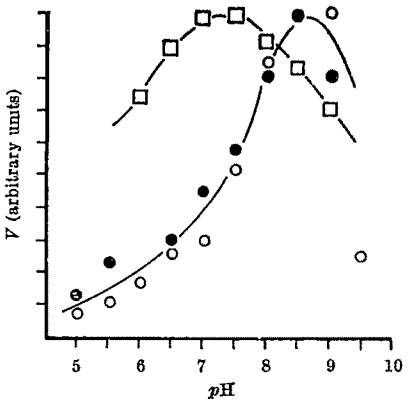


Fig. 1. The effect of *p*H on urease activity. The incubation medium (1 ml.) contained 0·1 mM *tris*, 0 1 mM sodium monohydrogen phosphate, hydrochloric acid added to give a *p*H as indicated, 0 04 mM urea, 0·4 ml. enzyme solution. Incubation period: 15 min at 25° C. ●, Enzyme preparation from ruminal mucous membrane; ○, enzyme preparation from washed bacterial suspension; □, enzyme preparation from soya bean powder

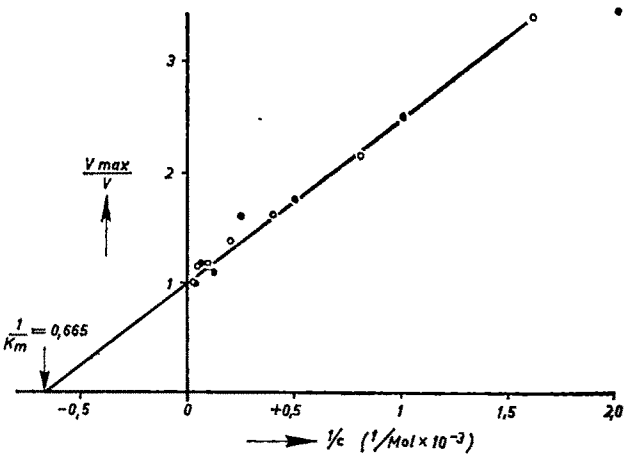


Fig. 2. The effect of substrate concentration on the reaction velocity. The incubation medium (2 ml.) contained 0·4 mM *tris*–HCl buffer *p*H 8·5, 1 ml. enzyme solution, and urea as indicated (*c*). See legend to Fig. 1 for additional explanations

These findings support the assumption that the urease activity of the ruminal mucosa originates from the rumen content. In order to confirm this assumption, experiments were conducted in which the mucous membranes were thoroughly washed with a detergent using a sponge. After this treatment the activity was found to drop to about 40 per cent. The rest of the activity could be detected in the wash solution. These results are in agreement with related experiments on the gastric mucosa of monogastric animals[8,9], in which it was shown that, in monogastric animals, urease activity is due to bacteria in the mucous membrane.

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PHARMACOLOGY

Metabolism of Dimethyl Sulphoxide to Dimethyl Sulphone in the Rat and Man

Dimethyl sulphoxide (DMSO) was first prepared in 1867 (ref. 1), and has been known for some time to be an excellent solvent. However, the first report concerning its metabolic fate in animals appeared only recently, following several papers in which biological and even therapeutic uses for DMSO were suggested[2,3]. Distefano and Borgstedt[4] identified dimethyl sulphide in the expired air of cats given DMSO intravenously.

The present report describes the isolation and identification of dimethyl sulphone in the urine of rats and man given DMSO. (The results for rats were reported at the forty-ninth annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., during April 9–14, 1965 (ref. 5), and while this manuscript

was in preparation, isolation of dimethyl sulphone from the urine of rabbits given DMSO was reported by Williams *et al.*[6].) The metabolite was isolated from rat urine and identified as follows: twenty-four rats were dosed intraperitoneally with 0·5 ml./kg of DMSO. Urine was collected overnight in dry ice-cooled flasks, thawed and filtered through glass wool. The urine was extracted with heptane, which was discarded. (Experiments with urine from rats dosed with ^{35}S-labelled DMSO indicated that only traces of radioactive material were extracted by heptane.) The aqueous residue was then saturated with sodium chloride and extracted with chloroform. The extraction was repeated several times with fresh chloroform, and the combined chloroform phases were evaporated *in vacuo*.

The residue consisted of colourless needles and a pale yellow oil that were separated by filtration. Preliminary evidence indicates that the oil is essentially all DMSO (unpublished data). Recrystallization of the crystals from a mixture of heptane and chloroform yielded needles melting at 110·5–111° C. The melting point was not depressed on admixture with authentic dimethyl sulphone, m.p. 110·5°–111° C. Analysis (in per cent)—calculated for $C_2H_6SO_2$, carbon, 25·51; hydrogen, 6·43; sulphur, 34·07; found, carbon, 25·85; hydrogen, 6·49; sulphur, 33·98. The infra-red spectrum (in potassium bromide pellet) was identical to that of authentic dimethyl sulphone.

The excretion of dimethyl sulphone was quantitated by isotope dilution and 'reverse' isotope dilution in experiments in which rats were given 0·5 ml./kg of DMSO-^{35}S intraperitoneally. The results indicated that approximately 15 per cent of the dose was excreted in the urine as dimethyl sulphone within 24 h. A major portion of the dose of DMSO is excreted in the urine in this time[7]. Rats given dimethyl sulphone-^{35}S (21 mg/kg intraperitoneally) excreted 64 per cent of the dose in the urine in 24 h, apparently as unchanged sulphone, as shown by paper chromatography and thin-layer chromatography on silica gel (ethyl acetate).

Dimethyl sulphone was similarly isolated from the urine of a human subject receiving a daily dose of 21 g of DMSO. Elemental analysis, melting point and the infra-red spectrum were used to establish its identity. Isotope dilution analysis of the 24-h urine showed that the sulphone present represented 3·0 per cent of the daily dose.

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Urinary Excretion of Acetylsalicylic Acid in Man

Mandel, Cambosos and Smith[1] have reported that appreciable amounts of acetylsalicylic acid can be present in human plasma and that it may be detected for up to 2 h after its oral administration. Also, from the work of Davison and Smith[2], the plasma proteins may bind acetylsalicylic acid to a much smaller extent than salicylic acid, thus making a greater proportion available for excretion. It should, therefore, be expected that some acetylsalicylic acid will be excreted in the urine.

The excretion of acetylsalicylic acid in urine after its oral administration has been the subject of a number of reports[3–7]. These have been based on the observed increase in the amount of salicylic acid which can be determined in the urine after mild hydrolysis. However, labile metabolites of salicylic acid which are also present in the urine will be hydrolysed to salicylic acid by the methods used, and the presence of acetylsalicylic acid cannot be conclusively established from such data.

The present investigations were undertaken after preliminary experiments had demonstrated that acetylsalicylic acid could be separated from salicylic acid and its metabolites in urine, by thin-layer chromatography (TLC) on silica gel (Merck *GF* 254). The developing solvent was benzene, ether, acetic acid, methanol (120 : 60 : 18 : 1) (ref. 8), and the following approximate R_F values were obtained for acetylsalicylic acid and its metabolites:

	Approx. R_F
Glucuronides of salicylic acid, etc.	0
Salicyluric acid	0·19
Gentisic acid	0·34
Acetylsalicylic acid	0·50
Salicylic acid	0·57

The acetylsalicylic acid on the chromatogram was detected in ultra-violet light as a dark band on the fluorescent background.

The excretion of acetylsalicylic acid in the urine of healthy young men, who had received an oral dose of the drug, was investigated. Urine collected at 1–2 h after drug administration was applied to the TLC plates as a narrow band. The chromatograms obtained from most of the urine samples examined gave a band corresponding to that obtained with an acetylsalicylic acid standard. To confirm the presence of acetylsalicylic acid, the urines were pooled, acidified to *p*H 2, and 200 ml. was extracted with ether.

Portions of the ether residue were applied to four 20-cm TLC plates and the bands corresponding to acetylsalicylic acid were scraped off into 5-ml. volumes of acetone. The acetone solutions were combined, centrifuged, and the supernatant reduced to a small volume which was applied to a TLC plate and re-developed. The appropriate band was removed and extracted with ether; the ether was evaporated to dryness after the addition of a little potassium bromide. The material isolated had an infra-red spectrum identical with that of acetylsalicylic acid.

A quantitative assessment of the amount excreted was made after the administration of acetylsalicylic acid (12 mg/kg body-weight) to six men. Urine samples were collected at 1·5 h intervals for 6 h after dosage. The 1·5 h urine samples were each diluted to 200 ml., and 0·3 ml. was applied to a 20 × 20-cm TLC plate and developed in the solvent system described. The acetylsalicylic acid band was removed from the plate into 5 ml. of N ammonia, mixed well for 20 min to allow solvation and hydrolysis to salicylic acid, centrifuged and the extinction of the supernatant measured at 295 mμ. Both blank urines and standard solutions of acetylsalicylic acid were subjected to the whole procedure.

The total salicylic acid content of the urine was also determined by the method of Brodie, Udenfriend and Coburn[9], after hydrolysis with 4 N sulphuric acid at 115° C for 3 h.

Table 1. Amount of Acetylsalicylic Acid excreted in the Urine of Six Men after Oral Dosage with Acetylsalicylic Acid (12 mg/kg Body-weight). Urines were collected at 1·5 h Intervals

Subject	Time after administration (h)	Amount of acetylsalicylic acid excreted: μmoles	Percentage of total salicylic acid *	Percentage of dose
A	1·5	72	16	1·9
B	1·5	22	10	0·5
C	1·5	28	11	0·6
D	1·5	111	19	2·6
E	1·5	0	0	0
F	1·5	89	22	1·7
	3	50	9	1·0 } 3·0
	4·5	17	2	0·3

No acetylsalicylic acid was detected in later samples.
* In the same urine sample.

The results (Table 1) show that acetylsalicylic acid was excreted in the urine of five of the six men taking part in this investigation. It was found only in the first 1·5 h specimens of four of the men, but was excreted for up to 4·5 h after its administration in one man. The average amount of acetylsalicylic acid excreted corresponded to only 1·5 per cent of the amount ingested.

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HAEMATOLOGY

Prethrombin as a New Sub-unit of Prothrombin

Prothrombin is one of the important molecules concerned with the clotting of blood. It has been isolated from bovine plasma and characterized as a single molecular entity by means of ultracentrifugation[1], electrophoresis[2], electronmicroscopy[3] and N-terminal amino-acid analysis[4–6]. Under appropriate conditions of activation, this molecule yields two enzymes, namely, thrombin and autoprothrombin *C* (ref. 7). Other sub-units have been obtained by degrading the molecule with autoprothrombin *C* or thrombin, to obtain autoprothrombin I (ref. 8), or autoprothrombin II (ref. 9), respectively. Each of these functions as an accelerator in the conversion of prothrombin to thrombin. Another derivative called autoprothrombin III is inert[10], but is the precursor of the enzyme autoprothrombin *C*, which can be derived from autoprothrombin III with the use of tissue thromboplastin and calcium ions, or by activating with Russell's viper venom ('Stypven')[11].

We have now succeeded in obtaining another sub-unit of prothrombin which is the immediate precursor of thrombin, and we propose to call it 'prethrombin'. Its amino-acid composition as determined by a complete analysis with a Spinco amino-acid analyser was found to be similar to that of purified thrombin. Prethrombin concentration cannot be measured by the original two-stage procedure used for the assay of prothrombin. It becomes thrombin under conditions which we shall describe and then its specific activity is practically equal to the specific activity of purified thrombin. Probably only a small peptide or amino-acid needs to be removed to convert prethrombin to thrombin.

To obtain the prethrombin from the parent prothrombin molecule purified thrombin was used for the degradation. At *p*H 7·0 about 500,000 units of purified prothrombin were incubated with 25,000 U. of purified thrombin in a solvent volume of 40 ml. After 90 min practically no prothrombin remained and the protein was introduced into a DEAE cellulose column and chromatographed according to the procedure of Seegers and Landaburu[12]. The prethrombin was obtained in a separate fraction and autoprothrombin III issued from the column at a much later time as another separate fraction.

Based originally on the discovery of prothrombin by Alexander Schmidt, the view has been held that tissue thromboplastin and calcium ions are needed for the conversion of prothrombin to thrombin. About two decades ago, however, Ac-globulin was also found to be important in the activation. Now we find that prethrombin does not convert to thrombin in the presence of tissue extract thromboplastin, or in the presence of tissue extract thromboplastin plus calcium ions, or even if a concentrate of Ac-globulin is also added. Tissue thromboplastin thus has nothing to do directly with uncovering the active site(s) of the thrombin molecule. Tissue extracts are only concerned with the generation of autoprothrombin *C* activity[13,14]. We shall now describe experiments which show that autoprothrombin *C* is the enzyme that functions in prethrombin activation.

For the conversion of prethrombin to thrombin with autoprothrombin *C* no calcium ions or any other clotting factor was needed (Fig. 1). With the prethrombin substrate in excess thrombin generation depended on enzyme concentration. Zero-order reaction kinetics evidently applied. In each concentration of autoprothrombin *C* all the prethrombin was eventually converted to thrombin. For reasons not understood, there seemed to be an optimum autoprothrombin *C* concentration, and during the first 15 min thrombin generation tended to be retarded. The addition of purified thrombin at zero time did not alter the rate of activation by a given amount of autoprothrombin *C*.

In one of the experiments, 35 μg (160 U./ml.) of autoprothrombin *C* were mixed with 3,600 μg (11,000 U./ml.) of the substrate and 5 h was required for all the thrombin to generate (Fig. 1). This might be regarded as slow activation of the substrate. By adding Ac-globulin (purified according to the method of Aoki and Seegers[15]), cephalin and calcium ions, the activation was accelerated (Fig. 2). Instead of requiring 5 h, all was complete in 30 min with the ratio of enzyme to substrate at about 1 : 100. It was interesting that Ac-globulin did not accelerate the action of autoprothrombin *C* by itself; it only did so in the presence of cephalin and calcium ions. Cephalin and calcium ions, without Ac-globulin, retarded the generation of thrombin by autoprothrombin *C*, presumably because an autoprothrombin–calcium–cephalin adsorption complex formed. Such complexes have been described[16,17].

We conclude that autoprothrombin *C* is the enzyme concerned with uncovering the active sites of thrombin. This is not done by thrombin under the conditions of our experiments, nor by Russell's viper venom, nor by tissue extracts (Fig. 2). Furthermore, we were not able to bring about activation with purified platelet cofactor 1 (factor VIII) plus purified platelet factor 3 plus calcium ions, plus purified Ac-globulin. So autoprothrombin *C* seems to be the sole substance concerned with uncovering the essential thrombin sites of prethrombin. The activation of prethrombin with autoprothrombin *C* was completely inhibited by soybean trypsin inhibitor. Although trypsin is not a clotting factor thrombin activity was readily generated from prethrombin with trypsin; this is

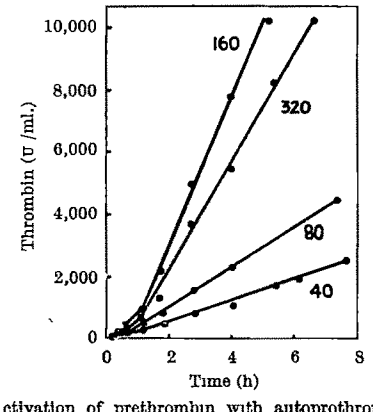


Fig. 1. Activation of prethrombin with autoprothrombin *C*. The prethrombin substrate concentration was 11,000 U /ml. in physiological saline solution buffered with imidazole *p*H 7·2 at 28° C. Numbers on curves indicate units of autoprothrombin *C*/ml. Specific activity of autoprothrombin *C* was 4,700 U./mg protein, that of prethrombin was 3,600 U./mg protein

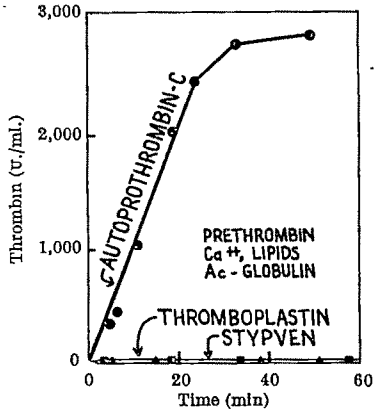


Fig. 2. Activation of purified prethrombin with purified autoprothrombin *C* in presence of calcium ions, lipids, and purified Ac-globulin. In all the activation mixtures the prethrombin substrate concentration was 3,000 U /ml. in 0 025 M calcium solution buffered with imidazole at *p*H 7·2. Concentration of purified Ac-globulin was 65 U./ml., and the cephalin 0·07 per cent w/v. Addition of 'Stypven' or tissue extract thromboplastin did not yield thrombin, but 50 U./ml. of purified autoprothrombin *C* produced rapid thrombin generation

of interest because it hydrolyses only peptide bonds the carbonyl group of which is associated with a positively charged side group (arginine or lysine)[18]. With the knowledge that the N-terminal amino-acid of thrombin is isoleucine[19,20] we can expect to find later that autoprothrombin *C* splits a peptide bond that is either arginyl-isoleucine or lysyl-isoleucine.

In 25 per cent sodium citrate solution, prothrombin becomes activated and thrombin and autoprothrombin *C* are formed by way of various intermediates[21]; either thrombin or autoprothrombin *C* can be used to stimulate the activation[7]. Prethrombin also became activated in concentrated sodium citrate solution; however, in this case, only thrombin activity was yielded. The reaction was rapid when primed with autoprothrombin *C*, but thrombin did not accelerate the reaction. Without autoprothrombin *C* the reaction was slow and usually the yields of thrombin were only 40 per cent of those obtained with autoprothrombin *C*.

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IMMUNOLOGY

19S and 7S Antibody Production in the *Cyclo*phosphamide- or Methotrexate-treated Rat

Previously it was demonstrated that in a number of animal systems the primary antibody response is made up of two distinct phases[1–5]. The initial antibody appearing in the serum is a high molecular weight antibody, 19*S*. Later, the lower molecular weight 7*S* antibody appears and, as it rises, the levels of 19*S* antibody decline. Smith[6] and Svehag and Mandel[7] reported that X-ray treatment of normal rabbits resulted in prolonged 19*S* antibody synthesis, but it suppressed 7*S* γ-globulin synthesis. Smith[6] and Sahiar and Schwartz[8,9] reported that 6-mercaptopurine given to normal rabbits, while prolonging 19*S* antibody production, may also abolish 7*S* antibody synthesis. Blinkoff[10] noted a preferential inhibitory effect of methotrexate on 7*S* as opposed to 19*S* antibody production in the mouse.

Previously we found that *cyclo*phosphamide (CY) and methotrexate (MTX) markedly inhibited the ability of the rat to produce measurable antibody after the injection of sheep red cells[11], and that the timing of antigen injection in relation to drug treatment was of major importance in determining the amount of antibody produced. The investigation described here was designed to elucidate the effect of a 5-day course of CY and MTX given at various times in relation to intravenous immunization with sheep red cells.

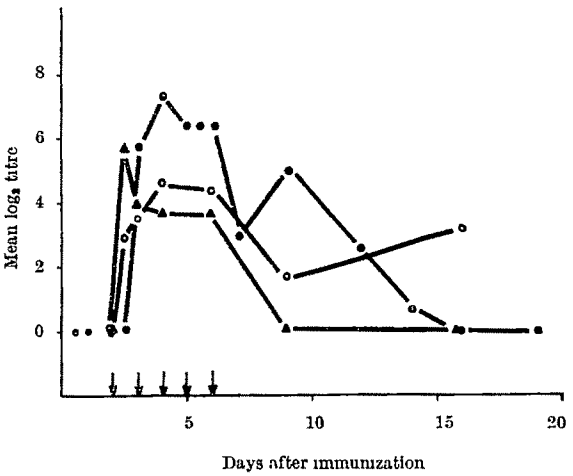


Fig. 1. 19*S* Antibody responses to sheep erythrocytes in rats treated with *cyclo*phosphamide or methotrexate 2 days after immunization. ●, 19*S* controls, ▲, *cyclo*phosphamide, 20 mg/kg × 5, (19*S*); ○, methotrexate, 0 75 mg/kg × 5, arrows indicate drug injections

Female Sprague–Dawley rats, 10–12 weeks old, were injected with 1 ml. of 10 per cent sheep erythrocytes, intravenously. Groups of animals were bled and killed 0·5, 1, 2, 2·5, 3, 4, 5, 5·5, 6, 7, 9, 12, 14, 16 and 19 days after immunization. Two other groups of animals were immunized similarly, but were given five consecutive and equal daily doses of CY (20 mg/kg) or MTX (0·75 mg/kg) intraperitoneally (40–50 per cent of LD_{50} for 30 days), beginning 2 days after immunization[11]. Four animals from each of these groups were bled and killed 2, 2·5, 3, 4, 6, 9 and 16 days after immunization, and the resultant sera titred. Total agglutinin titres were determined by two-fold dilutions in saline, as previously described[12]. Aliquots of each serum were diluted 1 to 2 in saline and incubated with an equal volume of 0·2 M 2-mercaptoethanol (2-ME) for 18 h at room temperature[12]. The remaining, or 2-ME resistant titre, was designated '7*S*' and the 2-ME sensitive

titre '19S'. Representative samples of serum were analysed with the sucrose density gradient centrifugation technique in a Spinco model *L*-2 centrifuge[13]. In all cases, the results of this analysis were in agreement with the 2-ME data. As an additional check on the density gradient technique, the presumed 19*S* and 7*S* fractions were subjected to immunoelectrophoresis with goat anti-rat globulin obtained from Hyland Laboratories, Inc.[14]. The geometric means of the titres for these three experimental groups are shown in Fig. 1. Data were analysed statistically by the method of Duncan and statistical significance accepted at the 1 per cent level[15]. The control animals first demonstrated 19*S* antibody 3 days after immunization. The peak titre was reached at 4 days. At 4 days, the first (see Fig. 2) 7*S* antibody appeared, and rose to its peak by the ninth day. At 14–19 days, the predominant antibody was 7*S*.

Animals treated with CY showed only a short 19*S* response, with an absence of 7*S* antibody. The MTX-treated group also showed no 7*S* response. In this case, however, even though the 19*S* peak was lower than the control, there was a persistence of significant amounts of 19*S* antibody after the control animals had ceased producing this type of antibody.

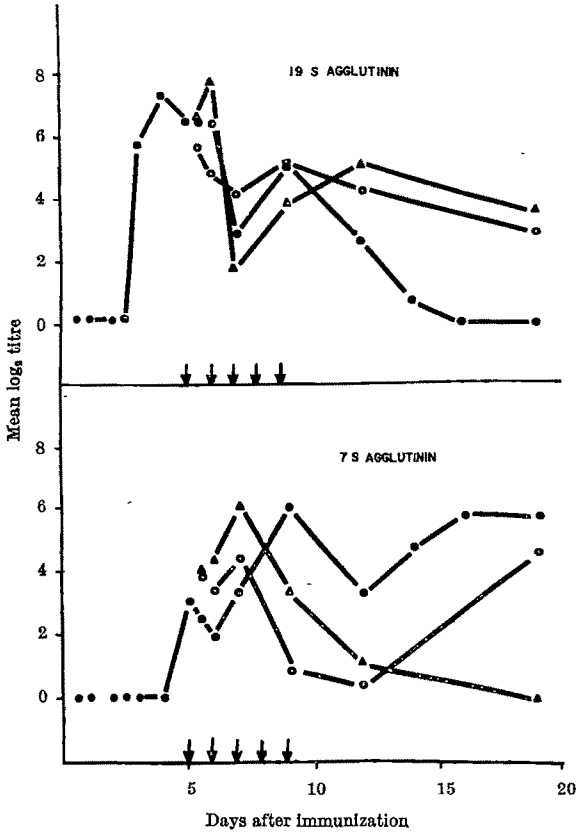


Fig. 2. Antibody responses to sheep erythrocytes in rats treated with *cyclo*phosphamide or methotrexate 5 days after immunization. ●, Controls; ▲, *cyclo*phosphamide, 20 mg/kg × 5; ○, methotrexate, 0·75 mg/kg × 5; arrows indicate drug injections

The effect of treatment with cytotoxic agents, beginning 5 days after immunization, is shown in Fig. 2. Drug-treated animals were bled 5·5, 6, 7, 9, 12 and 19 days after immunization. The data indicate that in CY- and MTX-treated rats 19*S* antibody production was significantly prolonged compared with the control. Production of 7*S* antibody decreased rapidly under the influence of both drugs. The delayed rise of 7*S* antibody after depression to very low levels in the MTX group suggests recovery from the effects of this drug. No such recovery was noted in these experiments for the CY-treated animals. The results of this experiment indicate that both agents can rapidly reduce 7*S* antibody production during its logarithmic phase of production while at the same time effecting a prolongation of 19*S* synthesis.

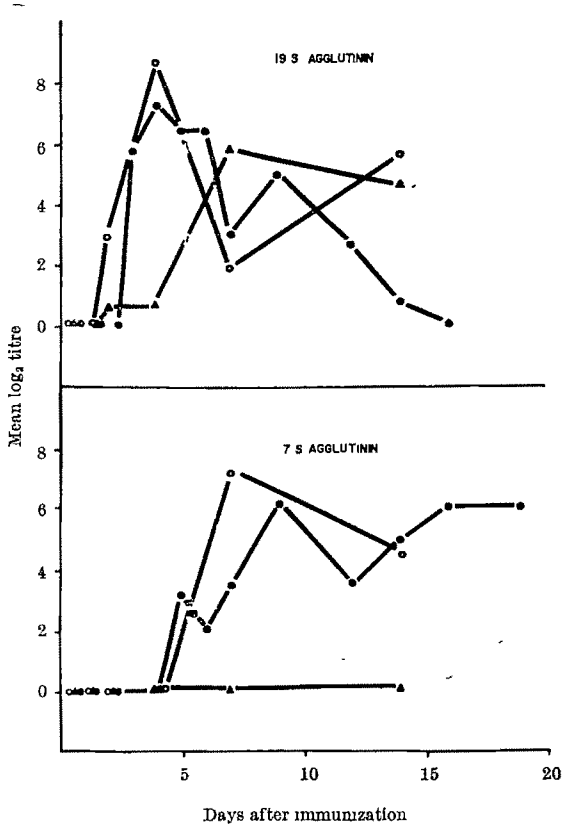


Fig. 3 Antibody responses in rats immunized with sheep erythrocytes 2 days after completion of a 5-day treatment with *cyclo*phosphamide or methotrexate. ●, Controls; ▲, *cyclo*phosphamide, 20 mg/kg × 5; ○, methotrexate, 0·75 mg/kg × 5

Fig. 3 depicts the effect of immunization beginning 2 days after completion of a 5-day course of either CY or MTX. Drug-treated animals were bled 0·5, 1, 2, 4, 7 and 14 days after immunization. The CY-treated animals showed no 7*S* response. The 19*S* production was delayed in onset, but prolonged. In the MTX-treated group, the 19*S* and 7*S* response was similar to that of the control, except for an elevated 19*S* antibody level at 14 days. The results indicate that pretreatment with CY can abolish the 7*S* response and result in a delay in the appearance of the 19*S* response. Pretreatment with MTX, on the other hand, does not produce these effects.

The present experiments, viewed in the light of the previous reports noted already, suggest that 7*S* antibody production in rodents is more susceptible to suppression than 19*S* synthesis by a variety of immuno-suppressive agents. Further generalizations must await the results of additional investigations with other drugs, antigens and species. It is hoped that experiments such as these, correlated with other parameters such as histological response, may lead to a better definition of the type or types of cells involved in 19*S* and 7*S* synthesis. The advantage of using immuno-suppressive drugs in order to study the possible interrelationships of 19*S* and 7*S* synthesis has been already emphasized by Sahiar and Schwartz[9].

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A New Method of detecting Homograft Sensitivity

IN the course of studying the distribution of chromium-51-labelled lymph node cells injected intravenously or intraperitoneally into *A*-strain mice[1], it was found that the uptake of allogeneic and syngeneic cells differed in certain organs. This suggested that it might also be possible to detect differences of uptake between the tissues of control animals and of mice which had previously been sensitized to the donor strain antigens.

Cell suspensions were made from the peripheral and mesenteric lymph nodes of $(A \times CBA)F_1$ or *CBA* mice by the method of Billingham and Brent[2], except that Tyrode's solution, previously gassed with 5 per cent carbon dioxide in air, was used. Sodium chromate (^{51}Cr) injection, B.P. (Radiochemical Centre, Amersham, specific activity 50–150 c./g), was used to label the cells at a dose of 4 μc./10^8 cells by the method of Scalfe and Vittorio[3]. The cells were injected intravenously into mice at a dose of 5×10^6/g body-weight. In the first and second experiments $(A \times CBA)F_1$ cells were used and the recipients were adult *A*-strain animals.

In experiment 1 the recipients were: (*a*) unsensitized mice; (*b*) mice sensitized by grafting with *CBA* skin 4 weeks prior to testing and by intraperitoneal injection of 10^7 spleen cells 3 weeks later.

In experiment 2 the recipients were: (*c*) mice grafted with $(A \times CBA\text{-}T6)F_1$ skin 16 days prior to testing; (*d*) mice injected intraperitoneally with 10^6 or 10^5 $(A \times CBA\text{-}T6)F_1$ spleen cells 7 days before testing; (*e*) unsensitized mice.

In experiment 3 $(A \times CBA)F_1$ or *CBA* cells were injected into two groups of adult *CBA* animals. The recipients were: (*f*) unsensitized mice; (*g*) mice grafted with *A*-strain skin 12 days previously.

At intervals after injection of the test dose recipient animals were killed. Their tissues were removed and weighed; the radioactivity of the specimens was measured in a Packard 'Autogamma' scintillation counter.

In the first experiment (Table 1) unsensitized animals (group *a*) were compared with strongly sensitized mice (group *b*). At 24 and 48 h after injection of the allogeneic test dose the levels of activity in the spleens, livers, lungs, thymuses, grouped peripheral nodes, mesenteric nodes and salivary glands were determined. Table 1 shows the distribution of the dose in the spleens and peripheral nodes: the activity in the spleens was significantly higher in the unsensitized animals (twice that in the sensitized); and in the peripheral nodes it was four- to six-fold greater in the unsensitized mice (or four- to ten-fold when the greater weight of the nodes in the sensitized group was taken into account). A similar pattern was shown by the mesenteric nodes. There was a small, but significant, increase in the level of activity in the livers of sensitized animals. Little difference was observed in other organs.

Table 1. DISTRIBUTION OF $(A \times CBA)F_1$ LYMPH NODE CELLS INJECTED INTRAVENOUSLY INTO SENSITIZED AND UNSENSITIZED *A*-STRAIN MICE

	Dose recovered (%) 24 h	Dose recovered (%) 48 h	Putative No. of cells/g × 10^6 24 h	Putative No. of cells/g × 10^6 48 h
Spleen				
Control	14·35 ± 0·948†	11·17 ± 0·240	135·72 ± 16·31	108·34 ± 12·68
Sensitized	9·26 ± 2·074	7·67 ± 0·808	76·98 ± 13·65	52·36 ± 5·03
P*	0·1%	0·1%	0·1%	0·1%
Peripheral nodes				
Control	7·77 ± 0·573	6·06 ± 0·204	203·81 ± 8·70	150·12 ± 3·55
Sensitized	1·54 ± 0·462	0·84 ± 0·126	27·22 ± 6·66	14·79 ± 2·49
P	0·1%	0·1%	0·1%	0·1%

* Significance of difference. 0·1 per cent > *P*.
† Standard error of mean.

Table 2. DISTRIBUTION OF $(A \times CBA)F_1$ LYMPH NODE CELLS 24 H AFTER INTRAVENOUS INJECTION INTO *A*-STRAIN MICE SENSITIZED BY DIFFERENT REGIMENS

	Sensitizing regime	Dose recovered (%)	Putative No. of cells/g × 10^6
Spleen	Skin graft	5·18 ± 0·136	66·04 ± 6·55
	10^6 cells i.p.	10·23 ± 0·837	157·40 ± 15·36
	10^5 cells i.p.	13·39 ± 1·136	182·31 ± 8·88
	Control	14·42 ± 3·146	182·05 ± 17·24
Peripheral nodes	Skin graft	0·83 ± 0·028	15·64 ± 4·05
	10^6 cells i.p.	2·07 ± 0·070	57·14 ± 8·11
	10^5 cells i.p.	5·98 ± 0·828	124·46 ± 28·06
	Control	9·12 ± 3·792	207·35 ± 68·87

Table 3. DISTRIBUTION OF $(A \times CBA)F_1$ OR *CBA* LYMPH NODE CELLS AT 24 H AFTER INTRAVENOUS INJECTION INTO SENSITIZED AND UNSENSITIZED *CBA* RECIPIENTS

	Dose recovered (%) Allogeneic cells	Dose recovered (%) Syngeneic cells	Putative No. of cells/g × 10^6 Allogeneic cells	Putative No. of cells/g × 10^6 Syngeneic cells
Spleen:				
Control	18·36 ± 0·973	21·54 ± 0·917	318·54 ± 80·67	294 01 ± 13·02
Sensitized	8 74 ± 2·425	25·09 ± 0·729	94·52 ± 24·71	284·64 ± 11 08
Peripheral nodes:				
Control	10·80 ± 1·095	9 35 ± 0·443	247·67 ± 1 93	300·23 ± 28·13
Sensitized	3 26 ± 1·158	12·06 ± 0·216	67 81 ± 27 85	294 09 ± 19 02
Mesenteric nodes:				
Control	3 51 ± 0 205	6·55 ± 0·880	121 98 ± 10 17	195 90 ± 14 29
Sensitized	1·27 ± 0·279	7·34 ± 0·775	33·18 ± 7·31	197·75 ± 13·12

The second experiment (Table 2) was carried out to discover how far the loss of activity in spleens and lymph nodes ran parallel to the state of sensitization of the animal, by comparing mice which had received antigenic stimuli in doses known to elicit different degrees of immunity (groups *c* and *d*), as judged by skin-graft survival[4]. The spleens, peripheral nodes and mesenteric nodes of the various groups of mice were examined at 4 and 24 h after injection of the allogeneic test dose. Table 2 shows the distribution of activity in the spleens and peripheral lymph nodes at 24 h; the results for the mesenteric nodes and at 4 h were essentially the same as these. For both the percentage dose recovered and the putative number of cells per gram of tissue the figures exhibit lower levels of activity in the tissues of more strongly sensitized animals.

The third experiment (Table 3) was designed to establish the specificity or otherwise of the mechanism responsible for these differences. Here, sensitized and unsensitized mice (groups *f* and *g*) were compared in their treatment of allogeneic and syngeneic cells. The spleens, peripheral nodes and mesenteric nodes of the mice were examined at 24 h after the injection of the test doses. The tissues of sensitized and unsensitized mice show the same differences of activity following the injection of allogeneic cells as were observed in the first experiment; but virtually identical amounts are recovered in them following the injection of syngeneic cells. There are in fact slight

increases of activity in the sensitized group, attributable to the greater weight of the tissues.

These experiments thus demonstrate what appears to be immune elimination of allogeneic lymphoid cells (or immune elution of the chromium label) from the lymph nodes and spleens of mice presensitized to their antigens. They establish its specificity and show that the extent of elimination is related to the state of immunity in the recipients. The phenomenon may offer a useful method of measuring homograft sensitivity and is being further investigated.

Note added in proof. The paper of Najarian and Feldman (*J. Exp. Med.*, 121, 521; 1965), which appeared while this communication was in the press, substantially confirms these findings.

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PATHOLOGY

Smell Threshold in Diabetes Mellitus

An elevated taste threshold for dextrose in diabetic patients without evidence of demonstrable diabetic neuropathy has recently been demonstrated[1]. Although it is unlikely that this was due to a non-specific decrease in gustatory acuity, those patients having normal thresholds for sodium chloride, further investigation of this possibility was indicated. The closely related olfactory threshold was, therefore, measured in 56 diabetic patients and 56 control patients, of the same age and sex. The method used was a modification of that of Elsberg and Levy[2], in which increasing quantities of an air–coffee mixture were released into the nose until the subject recognized an odour to be present (minimal perceptible odour). The modifications (to be described elsewhere) were designed to diminish important variables inherent in the original technique. The determination was carried out at the same time of day for each patient in a pair.

When the results were subjected to statistical analysis, the distribution of age and sex was unbalanced. A random sample, therefore, of two male and two female pairs were taken from each age group 20–29 . . . 60–69, and analysis of variance conducted on this sample. No significant difference was found between diabetic and control patients (Table 1). The only significant difference in both sets of patients ($P < 0.05$) was between age groups, although there was no obvious relationship between increasing age and threshold. However, thresholds of more than 30 ml. were not found under the age of 45 years, while thresholds of patients more than 60 years old varied from 5 to more than 80 ml.

Table 1. Mean of Olfactory Thresholds in 20 Pairs of Diabetic and Control Patients randomly selected from 56 Pairs according to Age and Sex

	Diabetic	Control	d	S.E. of d
Mean threshold (ml.)	14·7	16·9	2·2	3·6

This demonstration of normal olfactory thresholds in diabetic patients and the fact that their taste threshold for sodium chloride is normal[1] are against a non-specific decrease in gustatory-olfactory acuity in this disease. A second hypothesis to account for their abnormal taste threshold for dextrose would implicate tolerance to a raised level of blood glucose. This is unlikely, however, in view of the negative correlation which has been found between blood glucose-levels and taste threshold[1,3]. A third possible explanation which requires further investigation is that diabetic patients have an inborn defect of taste for dextrose.

We thank Prof. E. F. Scowen and Dr. K. O. Black for allowing us to study their patients, and the Board of Governors of St. Bartholomew's Hospital for financial assistance to D. S. P. One of us (P. T.) is in receipt of a Wellcome senior research fellowship in clinical science.

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RADIOBIOLOGY

Uptake of Plutonium by the Lobster *Homarus vulgaris*

Because of its long effective half-life and its decay characteristics, and since it is deposited in bone, man-made plutonium is one of the more hazardous of the radioelements. It is released in exceedingly small amounts from nuclear fuel reprocessing plants and in nuclear weapons tests, and, like the long-lived fission products evolved in this way, it may be detected at low concentrations in the marine environment. The concentration of plutonium by marine organisms is, therefore, of some interest. The total α-content of the edible seaweed *Porphyra umbilicalis* and of fillets of the plaice *Pleuronectes platessa*, collected near Windscale Works, are given by Dunster *et al.*[1]. Some data on the contemporary plutonium content of Pacific Ocean sea water and the concentration factors in certain marine plants and animals collected near the southern California coast are given by Pillai *et al.*[2]. The concentration factor is the ratio of the amount of plutonium per g of tissue (wet weight) to the plutonium in the same weight of sea water.

Experiments on the uptake of plutonium-239 from aerated filtered sea water by lobsters were carried out in partitioned glass tanks in a room with a constant temperature of 9–12° C. Every few days the lobsters were transferred to fresh tanks of sea water containing $6{\cdot}5 \times 10^{-2}$ μc./l. of plutonium-239, until the initial rapid uptake of the isotope was completed, when the water was changed less frequently. During the experiment, the lobsters were fed on uncontaminated mussels.

All samples were dried at 110° C to a constant dry weight and ashed at 500° C for 16 h. Weighed amounts of the ashed sample were dissolved in 10 N nitric acid, and the plutonium extracted in the presence of ferric ion into 20 per cent tributyl phosphate/carbon tetrachloride. An aliquot of this extract was slowly evaporated on a large counting tray and its plutonium content determined on an α-scintillation counting unit.

The results show that when a lobster casts its shell, the soft shell, gills, hepatopancreas and flesh contain relatively less plutonium-239 than the normal animal, whereas the content of the cast shell is approximately twice that of the shell of the intermoult lobster (Fig. 1). The plutonium-239 in the cast shell was analysed, following ecdysis, 100 days after a lobster had been placed in the radioactive

water, and the tissues were analysed after 220 days. The results (Fig. 1) show that the calcifying shell accumulates plutonium-239 at a rapid rate. A very high proportion (89·5 per cent) of the total plutonium lies in the calcified skeleton which accounts for approximately 43 per cent of the total weight of the lobster[3]. The corresponding proportion present in the hepatopancreas is 4·6 per cent of the total plutonium. The flesh, which comprises approximately 28·7 per cent of the total body weight[3], contains only 1·2 per cent of the plutonium present in the whole body. The experimentally determined concentration factor of 3 for lobster flesh (Fig. 1) corresponds with the concentration factor of 3 for the fish *Sarda lineolata*[2].

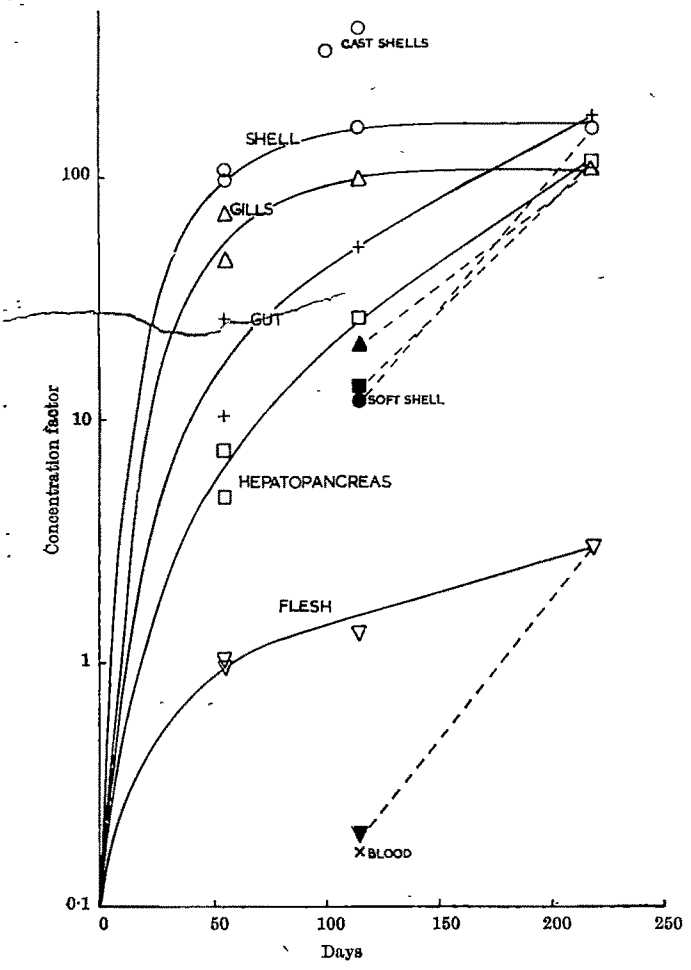


Fig. 1. Uptake of plutonium-239 by lobsters from sea water. Solid symbols represent the same tissues as do open symbols, from a lobster sampled immediately following ecdysis

Since the plutonium content of the flesh and hepatopancreas differs so markedly from that of the shell, it is advisable that edible tissues should be analysed separately if an assessment is to be made of the hazard to man in ingesting molluscs and crustaceans.

I thank the Torry Marine Laboratory, Aberdeen, in arranging for the supply of lobsters from Eyemouth.

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BIOLOGY

Possible Path of Entry of Cations into Plant Cells

In a previous publication[1] some data were presented which were interpreted as indicating that Rb^+ ions move through the plasmalemma of barley root cells on a chain of fixed, negative sites of a macromolecule which probably make up part of a charged pore. The results suggested that this passage of Rb^+ through the membrane could be slowed down by less mobile ions like Na^+ or Ca^{++}. It was suggested, as a corollary, that if such be the case, the movement of a slow ion on such sites could be accelerated by a fast ion. To test this possibility, absorption rates of sodium-24 in the presence of a fast ion, such as K^+, were compared with those in the presence of a slower ion like Na^+.

Excised barley roots (*Hordeum vulgare* L., var. Trebi) were used to follow the uptake of sodium-24 in slightly acid or alkaline solutions of sodium chloride and potassium chloride. *Tris* (10^{-3} M) was used to raise the *p*H of the medium. Roots were excised from four-day-old seedlings grown in darkness at 22° C with calcium sulphate solution of 4×10^{-4} M concentration. They were washed several times with de-ionized water, carefully blotted with filter paper, and 0·5 g samples weighed. For salt concentrations of 10^{-4} M 300 ml. of solution and for higher concentrations 100 ml. of solution were used with each root sample. The radionuclide concentration was the same in all media. Plastic beakers containing the roots and absorption media were placed in a water bath at 22° C and continuously aerated. After an absorption period of 30 min, the roots were washed several times with 2×10^{-3} M sodium chloride and de-ionized water for about 10 min. Roots were then wet-ashed with concentrated nitric acid and perchloric acid. The residue was taken up in 10^{-2} N sulphuric acid and assayed for sodium-24 activity. The experiment was repeated several times. Some typical results from one experiment are presented in Fig. 1 on logarithmic scales and expressed as relative rates of sodium-24 absorption. Rates of total sodium absorption are given in Table 1.

Table 1. Rates of Total Sodium Uptake by Excised Barley Roots in the Presence of Sodium Chloride or Potassium Chloride

NaCl molar concentration	KCl molar concentration	*p*H of solution	Rate of Na uptake (μmoles Na/30 min)
10^{-4}	0	5·6	1·44
10^{-3}	0	5·6	2·53
10^{-2}	0	5·6	4·90
$5{\cdot}4 \times 10^{-6}$	10^{-4}	5·6	0·012
$5{\cdot}4 \times 10^{-6}$	10^{-3}	5·6	0·0059
$5{\cdot}4 \times 10^{-6}$	10^{-2}	5·6	0·0017
10^{-4}	0	8·4	2·09
10^{-3}	0	8·4	3·38
10^{-2}	0	8·4	5·76
$5{\cdot}4 \times 10^{-6}$	10^{-4}	8·4	0·136
$5{\cdot}4 \times 10^{-6}$	10^{-3}	8·4	0·041
$5{\cdot}4 \times 10^{-6}$	10^{-2}	8·4	0·0048

From Fig. 1 it can be seen that, as expected, the uptake rate of the radionuclide is reduced with increasing concentration of Na^+ ions in both acid and alkaline media, but the rates are considerably higher in alkaline media. At *p*H 5·6 and in the presence of K^+ ions, sodium-24 absorption is drastically decreased in comparison with that in the presence of Na^+. At *p*H 8·4, however, the results show that in this alkaline medium the drastic inhibition of K^+ ions is no longer seen. Now, sodium-24 is taken up at a faster rate in the presence of K^+ ions than when Na^+ ions are present. The part played by *tris* is believed to be chiefly in neutralizing H^+ ions. In media made alkaline with sodium hydroxide, results not presented here indicate that the faster rate with K^+ ions is also apparent to some degree.

Although the increased rate of sodium-24 uptake with sodium chloride in an alkaline medium could be due to increased affinity of absorption sites for Na^+ ions, interpretation based on certain considerations discussed in another paper[2] suggests that such is not the case here.

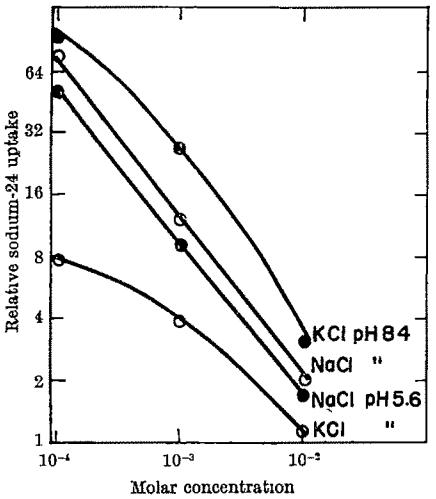


Fig. 1. Relative rate of uptake of sodium-24 by excised barley roots in the presence of sodium chloride or potassium chloride of varying concentrations and at two different *p*H's

Rate changes due to affinity changes should result in significant differences in the slopes of the sodium chloride lines in Fig. 1. Since the two lines do not show significant differences, the increased rate under alkaline conditions must be due to another cause—most probably to an increased accessibility of cation-absorption sites (a result of probable increase in pore size).

In the case of absorption in the presence of K^+ ions, it is suggested that two processes are operating at the same time: one is competition between sodium-24 and K^+ ions for absorption sites, and the other is an acceleration of the movement on a chain of negative sites of the slower sodium-24 ions by the faster K^+ ion. From results presented in the paper[2] previously cited, one would expect the rate of uptake of sodium-24 in the presence of Na^+ ions to fall on a straight line when log rate is plotted against log salt concentration. It can be seen in Fig. 1 that such is the case in both acid and alkaline media for sodium chloride. Now, since the reduction of the rate of uptake of sodium-24 by sodium ions is strictly due to competition for absorption sites, and if K^+ ions decrease the absorption rate of sodium-24 by only that process, the values in the presence of K^+ ions should also fall on a straight line. However, as can be seen in Fig. 1, in neither acid nor alkaline media do the rates fall along a straight line in the presence of K^+ ions. This non-linearity suggests that two processes are operating simultaneously in the presence of K^+ ions. One process is competition resulting in inhibition and the other could be an acceleration of the passage of sodium-24 through the plasma membrane by K^+ ions.

The data in Fig. 1 also illustrate the well-known phenomenon of preference of some plant roots for K^+ against Na^+ (figures for sodium-24 uptake in presence of K^+ ions at *p*H 5·6). Some investigators[4] have interpreted this specificity as indicating two different mechanisms or sites for the entry of K^+ and Na^+ ions into plant cells. One mechanism for both ions could be possible if ions enter plant cells through pores of varying sizes which are formed by a positively-charged protein and a negatively-charged macromolecule. If accessibility to external cation-absorption sites of the pores is determined by the protein, under acid conditions the attraction between the protein and the negatively-charged carrier could result in close juxtaposition of the two and thus limit the accessibility of cation-absorption sites. Under such conditions, a large and slow ion like Na^+ (hydrated) could displace H^+ ions from the cation-absorption site and be taken up fairly rapidly in the absence of any competing ion of lesser size and greater mobility. When such a competing ion is present, however, the absorption of Na^+ is drastically reduced due to the sites being more accessible to the smaller ion and due to the fast movement of the latter into the cell. On the other hand, under alkaline conditions the positive charges on the protein would be reduced, and the resulting diminished attraction for the cation carrier could result in increased pore size making sites more accessible. Under these conditions, a larger ion, especially if it has a strong affinity for this site, would have now a better chance of competing against a smaller ion. Additional evidence that a protein could be involved in accessibility of sites is the very marked effect of –SH inhibitors on the uptake of rubidium by mung bean root tips[5].

In view of the results presented in Fig. 1 and other results published previously[1], there is a strong possibility that cations enter plant cells through pores lined with negative sites. Such a movement of ions can be visualized as a case of exchange-diffusion along a chain of charged sites. The likelihood of a similar mechanism operating for anion uptake has been mentioned before[1]. Needless to say, a concept of a stationary, cation transport system would have advantages over the more popular hypothesis of a moving carrier. Harris[3] has discussed the possibility of movement of ions along such chains of charged sites in biological systems.

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Increase in the Rate of Protein Synthesis with Age in *Drosophila subobscura*

Earlier work[1,2] on the life-span of *Drosophila subobscura* at different temperatures, and in particular of flies transferred from one temperature to another at various ages, led to the suggestion that as flies grow older their capacity to synthesize protein declines, until ultimately they are unable to replace the protein being lost by denaturation. This suggestion has been investigated by measuring the rate at which labelled leucine is incorporated into protein in adult males at different ages and at different temperatures. A parallel investigation is being undertaken of the incorporation of labelled uracil into RNA. These investigations are still being carried out, but some preliminary results on protein synthesis at a constant temperature are reported here; they show that changes in the protein synthetic mechanism are involved in the ageing process, although not in quite the way that had been foreseen.

The flies used were male F_1 hybrids between the *B* and *K* inbred lines of *Drosophila subobscura*, raised and kept throughout at the normal laboratory temperature of 20° C. Groups of five flies were etherized and weighed to the nearest 0·1 mg. As the flies started to recover from the ether, a fine glass pipette containing a solution of sucrose and tritiated leucine (5×10^{-4} molar leucine in 10 per cent sucrose; activity 0·5 mc./ml.) was held to their proboscis. In this condition, flies will readily drink any sweetened solution they are offered. A group of five flies can in 5–10 min be fed from 0·5 to 2·0 mg of solution, amounting to from 10 to 40 per cent of their unfed weight. After feeding, the flies were re-weighed to determine the amount of leucine they had imbibed.

Preliminary tests showed that label can be detected in protein perhaps within 5 and certainly within 10 min of the start of feeding, and that for periods up to several hours the amount incorporated is proportional to the time since feeding. This proportionality shows that the rate of equilibration between leucine in the haemolymph, and

the intracellular pool from which protein is synthesized, must be rapid. It was found convenient to kill and homogenize the flies 1 h after feeding.

The flies were homogenized in 10 per cent trichloroacetic acid and spun for 10 min at 3,000 r.p.m. The precipitate was then washed successively in 10 per cent trichloroacetic acid at 90° C, in ethyl alcohol at 60° C and in two washes of a lipid solvent (2 vols. ether, 2 vols. ethanol, 1 vol. chloroform) at 60° C. Immediately after the lipid solvent was removed, 0·5 ml. of water was added to the precipitate, which was then pipetted on to a ridged planchet; three further washings of the tube, using 1 ml. of water each, were added to the planchet. If this last procedure was carried out immediately after removing the lipid solvent, the precipitate remained finely divided and could be spread evenly on the planchet. The activity was then counted in a gas-flow counter. It is known that this washing procedure is effective in removing any unincorporated leucine; when unfed flies were homogenized and labelled leucine added to the homogenate, the protein extracted later from the mixture had an activity no higher than background

When labelled leucine solution was added direct to the planchet, counting efficiencies of from 23·3 to 28·0 per cent were obtained. But when labelled leucine was added on the planchet to the protein precipitate from five unfed flies, counting efficiencies varying from 6·4 to 12·2 per cent were obtained; a value of 10 per cent has been used in the calculations that follow.

Measurements[3] on an amino-acid analyser have given the following values per fly:

Free leucine	0·56 mμmole/fly
Leucine in small peptides	0·22 mμmole/fly
Leucine in protein hydrolysate	108 mμmole/fly

Since exchange between free leucine and small peptides is rapid, the best estimate of the leucine pool is 0·78 mμmole/fly. The size of the leucine pool was found not to vary significantly with age between 6 and 60 days of age. The amount of leucine fed to each fly was of the order of 10 per cent of the pool. Consequently, it was to be expected that the amount incorporated should be proportional to the amount fed, and this was found to be the case. Comparisons were therefore made in terms of counts per min per mg fed, although care was taken to feed approximately the same amount to all groups.

Protein synthesis in young flies. Since our interest was in the rate of protein synthesis in the non-dividing cells of adults of different ages, our results would be misleading if a significant part of the total protein synthesis in an adult male occurred in the testis—the only organ in which cell division occurs. Therefore, a comparison was made between the rate of incorporation in males lacking testes (the sons of females homozygous for the gene *grandchildless*) and their double first cousins who have testes; the results are shown in Table 1. The reasons for choosing cousins rather than *B/K* males as controls is that the *grandchildless* stock is necessarily highly inbred. It is concluded that the testis does not contribute a major part of the total protein synthesis.

Table 1

Type of male	No. of groups	C.p.m./mg
Lacking testes	6	2,920 ± 270
With testes	5	2,800 ± 160

Table 2

	Experiment 1		Experiment 2	
	No. of groups	C.p.m./mg	No. of groups	C.p.m./mg
1 h after feeding on labelled leucine	6	4,950 ± 350	—	—
25 h after single feed on 0·1 M leucine	6	7,030 ± 590	6	7,280 ± 980
50 h after second feed on 0·1 M leucine	—	—	7	6,250 ± 610

A large number of estimates are available of incorporation in young *B/K* males, since they have been used as controls in most experiments. The mean value is approximately 5,000 c.p.m./mg. If it is assumed that the total protein content of a fly is constant—and, in fact, little if any change occurs in 60 days—it can be calculated, using the values in the preceding section, that the proportion of protein molecules which are replaced in 1 h is approximately 1/3,000; the life-span of a fly at this temperature is approximately 2,000 h. This could mean that all species of protein molecules turn over very slowly, or more probably that most of the protein does not turn over at all, and that a small part turns over rapidly.

To settle this point, we have measured the rate at which label, once incorporated, comes out again once label has been removed from the free leucine pool. Groups of males were fed on labelled leucine solution, and then 1 h later fed approximately 0·4 mg/fly of unlabelled 0·1 M leucine solution. The second feed diluted the label in the pool by a factor of approximately fifty. However, when the flies were homogenized 25 h later, it was found that even after this dilution the rate of new incorporation was greater than the rate of loss of label from protein. Therefore, in a second experiment flies were given a second feed of 0·1 M leucine, 25 h after the first. Since measurements of free leucine have shown that excess leucine is excreted within 24 h, it follows that the two feeds together diluted the pool by a factor of 2,000–3,000. The flies were homogenized after a further 50 h. The results of these two experiments are shown in Table 2.

The drop from 7,280 to 6,250 c.p.m. represents the loss of label from protein in 50 h. The difference is not significant although it is in the expected direction. However, had the level of activity in protein dropped to half its initial value after 50 h, the experimental procedure would almost certainly have been accurate enough to show a statistically significant change. Hence, the experiment enables us to say that the mean survival time of those kinds of protein molecules which turn over most rapidly must be at least 100 h, and may be as great as 3,000 h.

Changes in incorporation rate with age. The most surprising results of these investigations have emerged from comparing incorporation rates in young and old flies. The result of the first comparison of this kind is shown in Table 3; the rate of incorporation is approximately twice as great in old as in young flies. This difference has been confirmed in several subsequent experiments.

Table 3

Age in days	No. of groups	C.p.m./mg
20	6	4,960 ± 610
60	7	9,660 ± 1,000

Measurements of free leucine show no consistent differences between young and old flies. Therefore the difference in incorporation rate demonstrates a difference in total rate of synthesis of protein: old flies synthesize protein at approximately twice the rate of young ones. The rate of incorporation of labelled uracil into RNA is also approximately twice as great in old as in young flies, but in this case we have no measurements of the uracil pool in old flies, so interpretation is less certain.

Two conclusions emerge. First, the mean survival time of even the most rapidly turning-over species of protein molecule is at least 100 h, and may be much greater. Secondly, the rate of protein synthesis is about twice as great in old as in young flies.

Although unexpected, the greater rate of protein synthesis in old flies could be explained if: (1) the rate of synthesis is determined, by way of a feedback loop, by the rate of loss of protein; (2) the rate of loss of protein is greater in old flies.

A greater rate of loss of protein molecules could arise either from a change in the physical and chemical environment in the cell, without a change in the primary structure of the protein molecules themselves, or from damage to the protein synthetic mechanism, leading to the synthesis of proteins with faulty amino-acid sequences. In either

case, our earlier suggestion that in old age flies die because they can no longer synthesize protein rapidly enough to replace losses may still be correct, although the cause of the imbalance may lie in an increased rate of loss rather than in a decrease in the maximum potential rate of synthesis; either process would account equally well for the data on life-span at different temperatures.

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Development of *Boophilus decoloratus* (Koch, 1844) on the Ox

DURING a recent investigation of the post-larval development of the blue tick, *B. decoloratus* (Koch, 1844), using the chief host, the ox, at the Experimental Farm of the Animal Research Institute (that is, Achimota Farm), some interesting peculiarities in the habits and behaviour of this tick species were observed.

Eggs were obtained from engorged and mated females of *B. decoloratus* which were either collected from cattle in the field or bred in the laboratory or at the Achimota Farm. The laboratory-cultured eggs were kept in ordinary glass tubes, stoppered with cotton wool, and maintained over moist sand at a laboratory temperature ranging from 76° F (≏24·4° C) to 88° F (≏31·1° C). On hatching from the eggs, the larvae were allowed to harden at the usual laboratory temperature range before being placed on the host. The hardening time ranged between 2 and 10 days, average 6 days, approximately.

The larvae, contained in specimen glass tubes, were starved for more than 2 weeks after the cuticle had hardened, and were kept in the refrigerator at a temperature ranging from 59° F (≏15°C) to 68° F (≏20° C), during the period of starvation, to render them inactive. Before such larvae were used to infest the calf (No. 219), however, they were removed from the refrigerator and left for about 1 h on the laboratory bench to warm up and move about actively.

To infest the calf a sort of 'belly band' or 'trunk bag' was firmly secured around the trunk of the calf. The 'trunk bag' consisted of a piece of grey-baft cloth provided with a draw-tape at each end. The side seam of the 'belly band' had suitable fasteners for closing and opening it. During each infestation not more than 500 hardened and active larvae were placed direct on the dorsal and dorso-lateral aspects of the body of the calf, through the opening in the seam which was then closed up. The calf was tethered to a stake in the shade to ensure the development of the attached ticks under the prevailing field conditions of temperature and relative humidity (R.H., March, 66·5–91 per cent; April, 74–99 per cent; May, 72–95 per cent). The 'belly band' was removed 3 days after the infestation and any larvae which were found on the 'band' were destroyed.

The 'belly band' did not hinder the movement of the ticks on the host's body and immediately after the larvae were placed on the dorsal and dorso-lateral aspects of the body, under the protection of the 'band', several of them moved to various parts of the host's body, including the neck, head, ears and legs. The tethered calf became extremely excited and tried to rid itself of ticks by licking its legs. Many larvae dropped off the host and were lost, but some of them attached to the host's body within an average time of 1 h (range 30–120 min).

Doubtless many larvae did not complete feeding and could not develop into further parasitic stages because of certain adverse factors including inclement weather (rain) and loss through licking and brushing of the calf's body against the stake, after the 'trunk bag' had been removed. However, every effort was made to collect any fully-fed tick that dropped off the host. In certain cases in which it was expected that ticks which had taken the last blood meal would presently fall off the host, such ticks were specifically attended to until 18.00 hours, in order to collect them. The collecting of ticks, therefore, occurred intermittently between 0700 and 18.00 hours.

The larvae of *B. decoloratus* prefer the dew lap and neck, or else wander to the tip and outer pinna of the ear where they moult into nymphs. The nymphs remain at these sites to feed and moult into adults which migrate to the flanks and belly to feed[1–3].

Although the total number of adult females (36) which were collected was not quite large enough, I found that of this total the greatest number, seventeen, fed at the neck, ten at the back and flanks, five at the belly, one at each of the following sites: axilla, brisket, scrotum and head. Table 1 summarizes the result of the duration of development of the parasitic stages on calf No. 219.

Table 1. 1964 DATA ON DURATION OF DEVELOPMENT OF PARASITIC STAGES OF *B. decoloratus* (KOCH, 1844) ON CALF NO. 219

Mother tick No.	Date infesting with larvae	Adults dropped off	No. dropping off	Field temp. (° F) Range	Mean	Development from larva to adult
268	17 Feb.	9 Mar.	14	78–92	84·4	22 days
		10 Mar.	16	78–92	84·4	23 days
372	16 Mar.	7 Apr.	16	80–88	83·4	23 days
		8 Apr.	15	80–88	83·4	24 days
510	20 Apr.	12 May	15	78–88	83	23 days
		14 May	12	78–88	83	25 days
			Total 88		Average	23·3 days

(1) *Behaviour as a typical one-host tick species on bovine.* It will be seen from Table 1 that *B. decoloratus* showed the normal pattern of behaviour as a typical one-host tick and a total of 88 females developed from the larval through the nymphal to the adult stage on the same single host. These adult females were collected, on completion of development, when they dropped from the host. The average duration of development from larval through nymphal to the adult stage on the single host was found to be 23·3 days (range 22–25 days) at a mean average field temperature of 82·2° F (range 78°–92° F).

(2) *Behaviour atypically as a two-host tick species on bovine.* When some larvae, the progeny of gravid female tick No. 268, were fed on the calf on May 25, 1964, it was observed that as many as 56 dropped off the host after attaining full engorgement as nymphs and were collected within an average developmental period of 9·5 days (range 8–11 days) at a mean average field temperature of 83·1° F (range 78–92° F).

These nymphs were cultured in glass specimen tubes at the usual laboratory temperature ranging between 76° and 86° F (mean 79·9° F). In due course they moulted (*in vitro*) into adult ticks. The average premoulting period for 34 such nymphs was 19·8 days (range 18–20 days). The resulting adults hardened within an average period of 4·6 days (range 2–6 days). Ten of the females derived from this stock of ticks were then placed on the calf for rearing. After 3 days, however, they all detached without becoming even partially engorged[4].

The sites of predilection of the adults of the blue tick were easily noticed when the ticks were about to take the last blood meal, and especially at the stage of full engorgement. Usually the last blood meal was imbibed within 2 or 3 days prior to detachment. In addition to the result already presented on the subject of preferred feeding sites, it is noteworthy that I found 68 females of *B. decoloratus* feeding at various points on the groin and inner parts of the hind legs of four calves (that is, an average of 17 female ticks per calf). I also observed in twenty cases that the male often fed at the same locus as the female, and the ventral aspects of their bodies were in contact. Thus, apart from the flank and the belly, the adult blue tick can also feed at other loci.

Hard ticks show a wide range of adaptability to their hosts, ranging from species which are confined to one host to those which show little or no preference for hosts above and including reptiles[3]. Several workers, notably Hoogstraal[1], Arthur[2,3] and Theiler[4], have stated that the blue tick is a one-host tick. Matthyse[5] has reported that all the parasitic stages of the blue tick occur on cattle throughout the year. Theiler[6] confirmed that the life-cycle of this tick species was completed entirely on this host, except for oviposition. Lewis[7] has also stated that the blue tick completes its life-cycle as a one-host tick. Finally, Loundsbury[8] has recorded that the engorged females detach in 23 days after attaching as larvae. In regard to this particular behaviour of the blue tick as a one-host tick species on the ox, my experience is quite the same as that of the aforementioned authors. However, this communication does seem to report for the first time the occurrence within the genus of a remarkable departure from this typical behaviour: namely, the peculiar behaviour of *B. decoloratus* as a two-host tick species. Hitherto all the three members of the genus (*B. decoloratus*, *B. annulatus* and *B. microplus*) have been described as being characteristically one-host tick species[1–3].

On account of this peculiar behaviour of some individuals of the blue tick, it is inferred that instead of transforming into adults at the sites already selected by the larvae, some engorged nymphs of this tick species detached, wandered about for some time and dropped off eventually to moult into adults. If the nymphs had had opportunity to moult in sheltered areas in the immediate natural environment of the calf, the resulting adults would no doubt have sought the host a second time in order to feed.

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A Method of Isolating and Counting *Nippostrongylus brasiliensis* from Unweaned Rats

Methods for the recovery and counting of adult *Nippostrongylus brasiliensis* from the intestines of adult rats have been described by Jennings *et al.*[1] and Ogilvie[2]. Such methods need to be very accurate, particularly where estimations of the degree of immunity of the host are to be made on the basis of such counts. The techniques described by these authors have been used routinely in this laboratory during experiments on adult rats and have proved quite satisfactory. However, on application to the recovery of worms from the intestines of neonatal or unweaned rats a number of difficulties were encountered.

Because of their small size and delicate structure, opening the intestines of very young rats with scissors is an extremely difficult procedure and can result in a significant loss of worms. The consequent spread of intestinal contents around the site, on instruments, etc., requires repeated rinsing for the recovery of worms, so that they are eventually collected together for counting in an excessive quantity of fluid. Such a method is particularly unsatisfactory during the early stages of an infection when immature forms, not visible to the naked eye, are present. These difficulties are eliminated in the method described here.

The abdomen is opened and the posterior oesophagus cut; the stomach is then loosened from its surrounding attachments and the small intestine gently unravelled, particular care being taken in the region of the pancreas and caecum, where the intestine is most likely to break. It is then threaded on to a glass rod of 5 mm diameter, one end of which has been drawn out and the tip heated to a ball of such size that it easily enters the lumen of the intestine. The whole small intestine splits open during this procedure, and it is then transferred to a 1-l. beaker containing N saline at 37° C. The gloved fingers of the operator and the glass rod are rinsed with saline into the same beaker, until no traces of intestinal contents remain. The contents of the beaker are then drained through a double layer of surgical gauze spread over the top of a second beaker so that the intestine is retained in the gauze. The first beaker is repeatedly rinsed into the second. The gauze is then caught up on a glass rod so that the intestine is suspended in the saline. The beaker is left in a water bath at 37° C for 1 h, by which time the worms have penetrated the gauze and are lying at the bottom of the beaker, in 500 to 1,000 ml. of saline.

The contents of the beaker are filtered through a Büchner funnel using Green's 'Hyduro 904', 18·5 cm diam. filter paper, which has been previously marked off in 8 mm squares on a duplicating machine, with repeated rinsing to ensure that all the worms are transferred from the beaker to the filter paper. By adjusting the flow from the beaker the worms are spread evenly over all but the periphery of the filter paper.

The filter paper is removed and, if the worms are required only for counting, is allowed to dry completely. The worms are easily visible as red coils adhering closely to the paper, and are counted at a magnification of 12·5 × using a binocular microscope. The 8-mm squares on the filter paper act as convenient guidelines.

If the worms are required alive, the filter paper, while still wet, is laid for counting on a sheet of 'Perspex' 20 cm in diameter. Afterwards the filter paper is inverted over a 1/16th in. mesh sieve on top of a filter funnel containing normal saline at 37° C and fitted with a tap. The worms descend through the saline and collect in the narrow stem above the tap, from where they can be run off into a stoppered measuring cylinder to the required dilution.

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A Periodicity of Tritiated-thymidine Incorporation into Cytoplasmic Deoxyribonucleic Acid during the Cell Cycle of *Tetrahymena pyriformis*

Reports indicate that ^{3}H-thymidine is incorporated into a DNase-sensitive material in the cytoplasm of the ciliate *Tetrahymena pyriformis*[1–5]. It is now known that most of this cytoplasmic DNA is localized inside the mitochondria of this species[1]. Furthermore, this cytoplasmic DNA appears to be stable and conserved during cell growth and reproduction[1,4].

The purpose of this communication is to establish whether or not a relationship exists between cytoplasmic DNA replication and the DNA replication in the micro- or macro-nucleus of the cell. *Tetrahymena pyriformis* strain

HSM is known to replicate its micronuclear DNA during a half-hour period at the time of cell division, whereas the macronucleus replicates DNA for about 1 h during the middle of the cell cycle[6]. Thus the two nuclei replicate DNA independent of each other[6,7].

The cells were grown axenically in a defined medium plus 0·04 per cent proteose peptone (see ref. 6 for details). The cells have a generation time of 225 min in this medium. Cultures of these cells, in log growth, were exposed to ^{3}H-thymidine 20 μc./ml. (sp. act. 6·7 c./mmole) for 15 or 30 min, air dried on slides, washed with cold 0·5 N perchloric acid and 3 : 1 ethanol–acetic acid, and prepared for autoradiography[6,8]. Some slides were treated with DNase prior to autoradiography. At least 2 weeks of autoradiographic exposure were necessary to increase cytoplasmic grain counts to a measurable level.

Both cytoplasmic and nuclear radioactivity were removed by DNase digestion. Analysis was performed on cells which were classified into three groups: (1) with a labelled macronucleus; (2) with a labelled micronucleus; (3) without nuclear labelling. An ocular grid was placed over an area of cytoplasm, at some distance from either nucleus, and the silver grains were counted.

The average grain counts per unit area for seven individual experiments, corrected for background, are listed in Table 1. The individual and pooled tests of equality of the following categories of cells are shown in Table 2: (1) without macronucleus labelled versus micronucleus labelled; (2) macronucleus labelled versus micronucleus labelled; (3) without macronucleus labelled versus macronucleus labelled. When the individual t values are pooled, as shown in Table 2, the following conclusions are warranted: The incorporation of ^{3}H-thymidine into cytoplasmic DNA is higher during micronuclear DNA replication than during any other part of the cell cycle. Comparison of ^{3}H-thymidine incorporation into the cytoplasmic DNA of cells without macro- or micronuclear label and cells with a labelled macronucleus show no significant difference. With but few exceptions, the individual tests of equality in the different experiments showed that the cytoplasmic DNA label is significantly above background throughout the cell cycle.

Short exposures of cells to ^{3}H-thymidine were used because longer exposures would allow appreciable numbers of cells to pass from one part of the cell cycle to another and would therefore confuse the classification of cells and subsequent interpretations. An increased ^{3}H-thymidine incorporation into cytoplasmic DNA at the beginning of macronuclear DNA replication has been observed in this laboratory and elsewhere[1]. Apparently, this increased rate of incorporation is of such short duration that it has been missed by my method of analysis, which sums the grain counts during all the macronuclear DNA-synthetic period.

Table 1. Average Grain Count per Unit Area of Cytoplasm

Experiment No.	Labelled macronucleus	Labelled micronucleus	Without nuclear label
1	4·14	9·12	4·17
2	1·32	2·23	−0·59
3	7·30	9·70	4·40
4	1·70	4·40	2·40
5	13·80	15·80	7·20
6	−0·20	5·20	−0·20
7	9·11	11·5	8 5

Table 2. Individual and Pooled Tests of Equality (t Values)*

Experiment No.	No nuclear label vs. micro-	Micro- vs. macro-	No nuclear label vs. macro-
1	3·29†	3·82†	0·017
2	2·88†	1·22	2·24‡
3	2·24‡	1·07	1·22
4	0·79	1·07	0·28
5	1 58	0·37	1·21
6	3·21†	3·21‡	0
7	2·07‡	1·45	0·51
Chi-square with 14 *d.f.*	(χ^2 = 50·2)†	(37·4)†	(14·02)
Pooled experiments§	< 0·0005	< 0·001	≅0·40

* $M_i = M_j$ or $\bar{x}_i = \bar{x}_j$, $t = \dfrac{\bar{x}_i - \bar{x}_j}{\text{s e.}\,(\bar{x}_i - \bar{x}_j)}$

† Significant at 1 per cent.
‡ Significant at 5 per cent.
§ See ref. 9.

Cytoplasmic DNA synthesis occurs during most if not all of the cell cycle. The increased cytoplasmic incorporation of ^{3}H-thymidine which occurs during micronuclear DNA synthesis and which is also observed at the beginning of the macronuclear DNA-synthetic period may reflect an increased rate of cytoplasmic DNA synthesis at these times in the cell cycle. Alternatively, the increased incorporation may reflect a change in the size of deoxyribonucleotide pools which occurs at these two times in the cell cycle. The findings suggest that there is an interdependence between nuclear DNA synthesis and cytoplasmic DNA synthesis.

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Re-examination of the Effect of Daily Rotation in the Horizontal Plane on Growth of Cyclamen Plants and Etiolated *Avena* Seedlings

Jones[1], in New Zealand, has reported that rotating cyclamen and bean plants in a clockwise direction about a vertical axis at one revolution per day resulted in a loss of turgidity and accelerated the death of leaves, whereas normal growth was maintained when the direction of rotation was anti-clockwise. In other experiments it was shown that when etiolated *Avena* seedlings were rotated in darkness in the same manner, clockwise rotation caused a reduction in root and shoot extension growth, while, by contrast, anti-clockwise rotation brought about increased growth. Jones did not interpret these results, which were reported as being distinct and unequivocal, and they do not appear to be gravitational or centrifugal responses. However, Jones's technique of slowly rotating plants about a vertical axis has recently been used[2,3] in experiments to support claims that in some plants, notably cereals, growth and root orientation is affected by the horizontal component of the magnetic field of the Earth. To seek independent confirmation of Jones's findings, similar experiments have been carried out in Griffith, Australia, at latitude 34·18° S., using cyclamen and etiolated *Avena* seedlings. Negative findings are reported here.

Cyclamen. Three single cyclamen plants (*Cyclamen persicum* Mill.), growing in metal cans, were each placed on a horizontal, clock-driven turntable, so that the plants rotated about their own axes in a clockwise direction at a rate of one revolution a day. The three plants, together with stationary controls, were kept in a lightly shaded unheated glasshouse throughout the winter months of July and August, this being the most favourable time for growth of cyclamen in inland Australia. The experiment

was continued for 7 weeks, during which period all the plants flowered.

No symptoms indicating abnormal growth or development could be detected among the rotated plants. The effect of anti-clockwise rotation was not re-examined.

Avena. Husked oats (*Avena sativa*, variety Burke) were soaked overnight in tap-water in darkness at 5° C, and then sown in two shallow trays on moist facial tissue with the embryo facing upwards. Each tray contained 40–50 seedlings. Using a clock-driven turntable, with a vertical axis, one tray was rotated in either a clockwise or anti-clockwise direction at one revolution a day. The non-rotated tray served as a control. The experiments were performed at 25° C and high relative humidity inside a metal incubator, which was kept in a constant-temperature darkroom so that all operations and inspections could be carried out in green light of predominantly 546 mμ. After 3 days the length of the shoot (coleoptile and mesocotyl) and the longest root of each seedling was measured to the nearest millimetre. Seven clockwise replicates, followed by eight anti-clockwise ones, were performed at weekly intervals.

Rotation of the seedlings in either direction produced no visible effects. The percentage mean difference between shoot and root lengths of stationary and rotated plants is shown in Table 1. With one exception, the extension growth of the root and shoot of the rotated plants did not differ significantly from that of the stationary plants, in any of the replicates. These results are in contrast with those of Jones, where in nine replicates the average reductions in coleoptile and root final length in clockwise rotated plants were 6·1 and 8·6 per cent respectively, while the corresponding average increases in anti-clockwise rotated plants were 5·6 and 10·2 per cent. Furthermore, the direction of rotation produced a consistent response in each replicate. Jones used a 5-day rotational period, while the results summarized in Table 1 are for a 3-day period; nevertheless, the plants were growing actively throughout the 3 days, so that an appreciable effect of rotation on the elongation rate of the shoot or root should have been apparent by the third day. Despite the absence of a significant response to rotation in the majority of replicates, examination of Table 1 shows that in each of the first seven, where the plants were rotated clockwise, the shoots grew less than those of the controls. Since the trend of this difference is in agreement with Jones's conclusions, the effect of clockwise rotation on the extension growth of the etiolated shoot of the *Avena* seedling was further examined in a second experiment, in which the experimental technique used by Jones was more closely followed.

Avena seedlings were germinated using the method already described here and kept under the same environmental conditions as in the previous experiment. On the second day, the seedlings were transferred from the shallow trays to two perforated plastic holders each holding thirty seedlings, which were then set up in two glass beakers containing tap-water, so that the roots of the seedlings just touched the surface of the water. One beaker was rotated clockwise about a vertical axis at one revolution a day, while the other was kept stationary. After 2 days, the length of each seedling shoot was measured to the nearest millimetre. The experiment was replicated fifteen times. The results, summarized in Table 2, show that shoots of rotated and stationary plants attained the same mean length. In individual replicates, the mean percentage difference between the length of rotated and stationary shoots ranged from −6·5 per cent to +10·0 per cent; but in no case was this difference statistically significant. From these results, and those for the preceding experiment, it is apparent that the extension growths of the root and shoot of the seedling of the oat variety used were not appreciably affected by daily rotation about a vertical axis in either direction.

Table 1. Effect of Rotation around a Vertical Axis at 1 Rev. a Day on Root and Shoot Extension Growth in *Avena* Seedlings

Replicate No.	Shoot: Percentage difference of root or shoot final length from stationary control	Root: Percentage difference of root or shoot final length from stationary control
	Clockwise rotation	
1	−10·5 ± 4·3*	−5·8 ± 5·8
2	−1·1 ± 3·3†	−1·4 ± 6·0
3	−0·2 ± 9·0	−0·6 ± 6·2
4	−3·4 ± 5·1	−5·1 ± 6·7
5	−3·7 ± 4·0	+9·3 ± 8·4
6	−2·9 ± 5·3	−0·8 ± 8·9
7	−4·4 ± 7·8	−1·8 ± 5·4

	S	*R*	Difference (%)	*S*	*R*	Difference (%)
Mean length for all replicates (mm)	36·8	35·3	4·1	64·0	63·4	0·9

	Anti-clockwise rotation	
8	+2·5 ± 3·1	+7·5 ± 5·6
9	−0·3 ± 3·8	+9·2 ± 6·2
10	+1·5 ± 3·4	+3·8 ± 5·8
11	−2·7 ± 4·4	+5·4 ± 6·6
12	+1·5 ± 4·5	+0·6 ± 7·8
13	+0·6 ± 3·6	+7·7 ± 5·3
14	+1·3 ± 3·8	+0·6 ± 5·9
15	−3·0 ± 4·1	−2·9 ± 8·1

	S	*R*	Difference (%)	*S*	*R*	Difference (%)
Mean length for all replicates	36·4	36·4	0	70·6	73·3	3·8

* Significant at $P < 0.01$.
† Standard error.
S, Stationary control. *R*, Rotated.

Table 2. Effect of Clockwise Rotation around a Vertical Axis at 1 Rev a Day on Shoot Extension Growth in *Avena* Seedlings
Results for 15 replicates

	Mean shoot length (mm)	Mean difference (%)	Total No. shoots per treatment
Stationary	39·7	−0·2	423
Rotated	39·6		435

While these negative results do not rule out the possibility that, under some environmental conditions, cyclamen and *Avena* plants may respond to slow rotation about a vertical axis, it can be concluded that the responses in question, if they exist, are not readily reproducible in the same way as, for example, are responses to darkness and temperature.

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It is pleasing to see a renewal of interest in plant responses to specific rotations. Unfortunately Dr. Palmer's experiments with *Avena* are not strictly comparable with mine of 1960 in at least two features, that of the variety of oat used and in his choice of a 3-day period. I understand that the variety 'Milford' is now unobtainable, but I would mention that with it I was able to experiment with rotating seedlings in a null (magnetic) field, with negative response, that is, the direction of rotation did not affect the growth of shoots; a similar result was obtained with strong magnetic fields.

I would suggest that it might be worth while to carry out experiments with rotations in weak magnetic fields and that this might lead to some explanation of the phenomenon.

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Mutants of *Puccinia graminis avenae* induced by Ethyl Methane Sulphonate

Spontaneous mutations affecting uredospore colour in cereal rusts have been observed by several investigators. The mode of inheritance of the mutant colours in the wheat stem rust fungus *Puccinia graminis tritici* E. and H. and oat stem rust fungus *P. graminis avenae* E. and H. has been shown to follow a simple Mendelian pattern[1,2] and such mutants serve as particularly useful markers in investigations of the role of asexual recombination in this group of fungi. In an attempt to produce uredospore

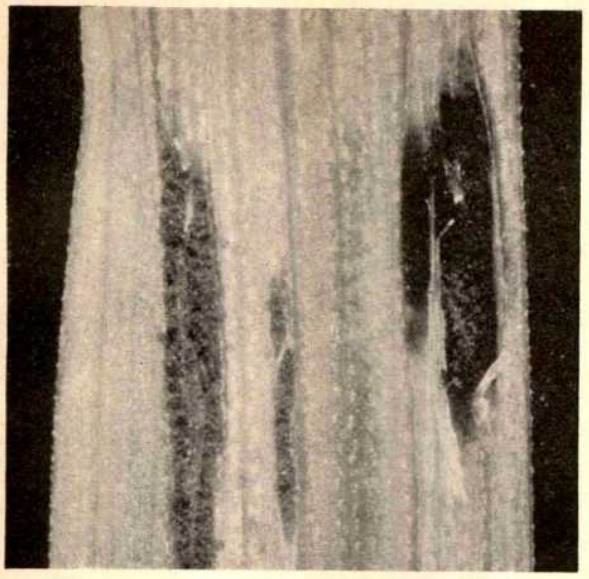


Fig. 1. Uredosori of normal oat stem rust (left) and teleutosori of short-cycle oat stem rust (right) 14 days after inoculation of uredospores on Algerian primary seedling leaf (× 12)

colour mutants in the oat stem rust organism for this purpose, the mutagenic effect of the alkylating agent ethyl methane sulphonate (EMS) was investigated following its success in this respect in such widely diverse organisms as bacteriophage[4] and barley[5].

Freshly collected uredospores of race 12[6] were treated with $0{\cdot}5 \times 10^{-2}$ M, $1{\cdot}0 \times 10^{-2}$ M, $1{\cdot}25 \times 10^{-2}$ M and $1{\cdot}5 \times 10^{-2}$ M solutions of EMS for 2 h with repeated shaking. The spores were then filtered from the solutions, washed several times, and those from each of the four groups were used immediately to inoculate the primary leaf of approximately 400 seedlings of 'Algerian', a variety of oats susceptible to race 12. Samples were retained to assess the effect of the treatments on spore germination.

Germination capacity was determined on 2 per cent non-nutrient agar at 20° C. Untreated spores showed 71 per cent germination; in the case of treated spores germination percentages were reduced from 27 following exposure to $0{\cdot}5 \times 10^{-2}$ EMS to 11 after treatment with $1{\cdot}5 \times 10^{-2}$ EMS.

Two distinctive colour variants were observed among the uredosori following the initial treatment with $1{\cdot}0 \times 10^{-2}$ M EMS. When compared with colour charts in Ridgeway's *Color Standards and Color Nomenclature*[7] the aggregated spores approached most closely 'Mars Yellow' (Plate III) and 'Ochraceous Orange' (Plate XV), and will here be referred to as yellow and orange, respectively. Comparatively, the reddish-brown spores of normal colour were best described as 'Amber Brown' (Plate III). Both mutants were characterized by delayed pustule formation, the uredosori rupturing the epidermis and developing 3–4 days later than the normal colour type. In repeated sub-cultures both mutant types have maintained their distinctive colours and shown delayed pustule development.

Recurrent treatment was then practised, apparently normal uredospores being repeatedly collected after each successive treatment and exposed to the same initial concentration of EMS. Following the fourth treatment with $1{\cdot}5 \times 10^{-2}$ M EMS a third mutant uredosorus, again appearing later than the normal type, was observed in which the massed spores most closely approximated 'Wood Brown' (Plate XL) in Ridgway's nomenclature. This mutant, which will here be referred to as grey-brown, has maintained its characteristics in repeated sub-cultures on the host plant. A control to the various treatments failed to produce any apparent uredospore colour variants.

Normal uredospores contained pigment in both the wall and cytoplasm. The orange mutant lacked pigment in the uredospore wall and that of the cytoplasm was intensely aggregated in the centre of the spore. In some instances macroscopically apparently normal coloured spores appeared in certain uredosori associated with this mutant type. Sub-cultures from mixed pustules with considerable development of reddish-brown areas produced exclusively orange pustules, at least when initially formed. Microscopically these normal coloured uredospores showed some pigment development in the wall, apparently associated with ageing of the spores under certain conditions. However, the characteristic central aggregation of the cytoplasmic pigment component was still exhibited. The yellow mutant lacked colour in the uredospore wall, while in the case of the grey-brown variant no pigment was visible in the cytoplasm. These descriptions agree in general with those of Newton, Johnson and Brown[1] for uredospore colour variants in *P. graminis tritici*.

Two mutant pustules characterized by rapid teleutospore formation were observed following the initial treatment with $1{\cdot}5 \times 10^{-2}$ EMS. In these instances teleutospores appeared in the uredosori 4–5 days after the rupture of the epidermis, and development progressed rapidly from the edge of the pustule towards the centre (Fig. 1). Uredospores were immediately taken from the centre of both pustules and inoculated on to seedlings of 'Algerian'. The characteristic rapid teleutospore formation has been observed in each subsequent generation of sub-culturing and these mutants have been difficult to maintain by uredospore transference on this account. Teleutospore formation was accelerated by increased temperature, and at approximately 27° C daylight temperature frequently no macroscopic formation of uredospores was observed, the pustules appearing immediately as teleutosori. By contrast, normal strains of the fungus do not form teleutosori on leaves under glasshouse conditions and teleutosori are usually restricted in the field to stems of mature plants. The advantages of mutations of this type, which may be designated as short-cycle rusts, in genetic studies involving the sexual stage on the alternate host, the barberry, is obvious. If mutations of this type can be simulated in rusts in general by alkylating agents with the same facility as in the present instance, genetic analyses of this group of fungi will be greatly expedited.

No change in pathogenicity was apparent with any of the mutant characteristics described, the reactions typical of race 12 being maintained on the differential set of varieties in each case. The variety 'Richland' gave a resistant reaction to this race with '1' reaction-type pustules on the conventional system for reaction-type designations[8]. The mutants with rapid teleutospore formation exhibited small teleutosori typical of the small '1' reaction-type uredosorus with no appearance of uredospores in the pustules.

Colour mutants in *P. graminis* have been found to be recessive[1,2]. The mutations of this type described here are at present being analysed genetically, but it is presumed that they have a similar genetical basis. The uredospores are diploid being dicaryotic with haploid nuclei. EMS was apparently an extremely efficient mutagenic agent in the present tests especially as there is no evidence to indicate that the strain of rust used was initially heterozygous at the appropriate loci.

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Calcium Requirements of Higher Plants

The Hoagland nutrient solution for growing plants calls for the use of 0·004 or 0·005 M calcium and 0·002 M magnesium[1]. Plants grown in such solutions contain about the same amount of calcium as plants grown in soil. In the present investigation corn and tobacco plants have been grown with calcium levels 1/50 or less than that of Hoagland solutions. To accomplish this it was necessary to ensure that the copper level of the solutions was 200 p.p.b. or less, that unchelated iron was less than 0·5 p.p.m., that manganese was 0·5 p.p.m. or less, that zinc was 0·1 p.p.m. or less, and that magnesium was 5×10^{-4} M or less. There were some variations due to plant species.

Calcium is required by some algae and other micro-organisms in micro-nutrient quantities only[2] and perhaps also for a gymnosperm[3]. Higher plants seemingly require relatively large quantities of it. The concentration of it in leaves of higher plants generally ranges from 0·2 to more than 5 per cent of dry weight[4]. There are a large number of experiments which indicate that higher plants benefit from the high levels of calcium which they contain.

Calcium-levels in leaves of from 200 to 800 p.p.m. of dry weight appear to be sufficient under some conditions (Tables 1 and 2). The magnesium-level simultaneously must be relatively low since high magnesium-levels can be very toxic under low calcium-levels[5]. The calcium and magnesium assays were made from wet digested samples with an atomic absorption flame spectrophotometer.

There is some indication also that with proper balance the magnesium requirement is also in the same magnitude as that of calcium. The lower limit of sufficient magnesium cannot be less than its need in chlorophyll. This amounts to a need of about 0·03 per cent magnesium on the dry weight basis. The co-factor requirements for magnesium in enzymatic reactions add roughly an additional 0·03 per cent to the magnesium requirement. This is based on a concentration of about 1 mM in the plant liquid. The total magnesium requirement, therefore, would be about 600 p.p.m. of dry weight. Critical magnesium levels for plants generally have been reported as from 0·2 to 0·3 per cent of dry weight for different plant species. This is around three times the postulated level. The difference has never been reconciled and may be related to the calcium-levels, which are usually high. The data in Table 1 indicate that the need for magnesium in tobacco may be from 800 to 1,200 p.p.m. of dry weight.

The plants grown without added calcium as described in Table 1 did have some indications of calcium deficiency as indicated by yields and by appearance of growing tips. When neither calcium nor magnesium was supplied the new leaves had an unnatural curl. The new leaves of these plants contained 80–90 p.p.m. calcium on the dry weight basis, which under the conditions of the experiment was slightly too low for maximum yield. Although these plants were decreased in yields by about 30 per cent they looked reasonably healthy. The relatively high calcium-levels of the old leaves on the plants was due to a pretreatment complete nutrient solution together with no retranslocation of calcium from old to new leaves.

Table 1. Yields and Calcium and Magnesium Contents of Tobacco Plants grown in Complete Nutrient Solutions except for Calcium and Magnesium

Plant part	Differential treatments		
	No calcium, no magnesium	5 mM calcium, no magnesium	5 mM calcium, 4 mM magnesium
	Dry weight/plant (g)		
Shoot	11·6	17·3	15·4
Root	1·7	2·1	2·3
Whole plant	13·3	19·4	17·7
	Calcium (percentage of dry weight)		
New leaves	0·009	0·63	0·44
New mature leaves	0·008	0·57	0·39
Remainder of shoot	0·41	1·26	1·14
Root	0·048	0·44	0·23
	Magnesium (percentage of dry weight)		
New leaves	0·08	0·08	0·22
New mature leaves	0·03	0·03	0·17
Remainder of shoot	0·12	0·10	0·28
Root	0·22	0·12	0·44

Table 2. Yields and Calcium Contents of Corn and Tobacco grown in Nutrient Solutions at Two Calcium Levels

Calcium (p.p.m.)	Yield (g)	Leaf calcium (percentage of dry weight)
		Corn
2	5·1	0·011
5	4·8	0·020
		Tobacco
2	18·6	0·08
5	18·6	0·10

The plant species have been grown in a number of nutrient solution cultures to test their need for calcium. They grew well at the low calcium-levels (Table 2). These data were obtained with 1 mM magnesium, 20 p.p.b. copper and chelated iron and indicate that from 100 to 200 p.p.m. calcium in corn leaves on the dry weight basis is sufficient and that not more than 800 is sufficient for tobacco.

There is considerable evidence that plants do not require all the calcium they ordinarily contain and the present investigation indicates that published critical levels do not reflect the direct need of plants for calcium. They reflect the ability of calcium to prevent injury from excesses of magnesium, copper, iron, manganese, zinc, and possibly other ions.

Corn and tobacco plants grew well in nutrient solutions at calcium-levels lower than 1/50 of that of Hoagland solution provided that levels of copper, iron, manganese, zinc and magnesium were in proper balance. The implication is that beyond ameliorating toxicities of other ions, the role of calcium in higher plants is that, or nearly that, of a micro-nutrient.

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Introduction and Exchange of Coconut Planting Material

The presence of cadang-cadang, lethal yellowing, red ring and other serious diseases in important coconut growing areas, coupled with an awareness of genetic differences in palms in different parts of the world, has stimulated an interest in disease resistance testing. The occurrence of known resistance to lethal yellowing disease in Jamaica has served to emphasize the possibilities.

Pollen introductions greatly speed up the production and testing of hybrids between local and exotic material, but do not eliminate the necessity for seed importations[1]. Coconut seed often has low viability following shipment by sea, and the need for research on collection and dispatch has recently been emphasized[2]. Preparatory to survey and collection of coconut planting material[3], air freight and fumigation tests were therefore undertaken.

Short distance air freight (return trip to Miami from Jamaica) did not affect seed germination, and good germination was also obtained with samples introduced from Wallis Island and with seed exported to West Africa.

Germination of seed nuts with the husk removed is a standard procedure in Jamaica for the production of coconut seedlings for investigations of disease transmission. In a long-distance air freight experiment (Jamaica–England–Jamaica; at high altitude) equal

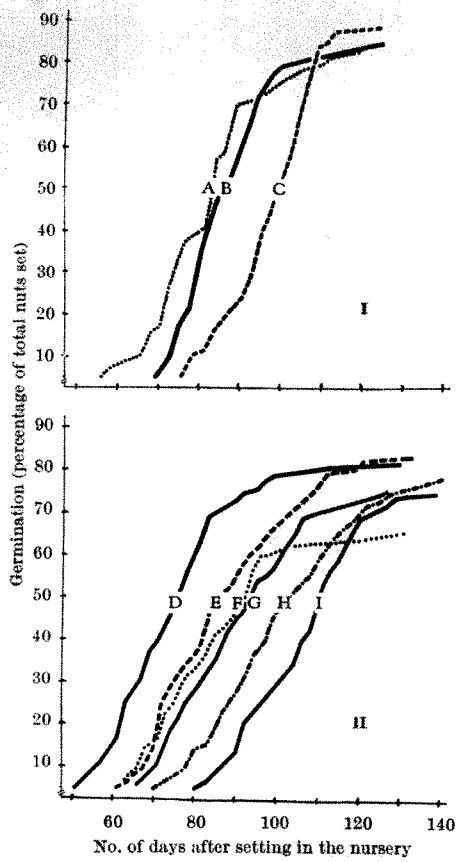


Fig. 1. Germination rates of coconut seed nuts introduced to Jamaica by air freight. I, Completely husked seed nuts; II, partially husked nuts and one sample (*H*) not husked. *A*, Fiji (local talls); *B*, W. Samoa; *C*, Rangiroa (red fruited dwarf); *D*, F.M.S., Solomons; *E*, Bougainville; *F*, Markham Valley, New Guinea; *G*, Karkar Island, New Guinea; *H*, Tahiti; *I*, Rennell Island. Curves for introductions from Fiji ('Niu Leka' dwarfs), Solomon Islands (local talls) and New Hebrides resembled *B*, *D* and *F*, respectively

numbers of husked and unhusked coconuts, packed in hessian bags, were placed both in the forward and rear holds of the aircraft: controls were retained in the laboratory. Results are given in Table 1.

Germination of nuts carried in the forward hold and complete nuts carried in the rear hold was satisfactory. Results for husked nuts carried in the rear hold may be inconclusive as, on this occasion, controls also had poor germination. Germination of nuts with the husk removed normally exceeds 70 per cent and 90 per cent, respectively, for 'Malayan Dwarfs' (as used in the present experiment) and 'Jamaica Talls'. There was no apparent damage during shipment; some breakages in a subsequent consignment served to emphasize the need for adequate packing of 'depericarped' nuts. In the latter shipment 77 per cent of the undamaged nuts germinated.

Since fumigation of seed is often essential, the effect of two commonly used fumigants was tested. Germination and early growth of husked and unhusked seed treated with hydrogen cyanide (1 oz. and 0·5 oz. potassium cyanide per 300 ft. respectively, for 1 h) and methyl bromide (1 lb. per 1,000 ft. for 2 h) were at least comparable to those of untreated controls.

The preliminary trials indicated that, with certain precautions, introductions could be safely made by air freight. With later shipments, mainly from different Pacific Islands, over-dried and immature seed nuts were avoided, and only those with drying husk, and calyx still retaining the 'living' colour, were dispatched. Most seed was partly husked, leaving a thin (1 cm) protecting layer. Husking was complete only when packing in boxes was possible. Insecticide dusting and fumigation conformed with individual territory regulations and all samples were carried in the forward hold of the aircraft. At destination, seed with incomplete husk was planted in a coir and soil mixture contained in bamboo baskets placed in full sunlight. In a preliminary trial, 1 in. depth of medium above each seed prevented nut cracking due to exposure. Most seed nuts were placed in a horizontal position; only the Rennell introductions had the germ pore uppermost. Although there is less mechanical hindrance to growth of seedlings planted in baskets, using the latter method, rate and final percentage germination may be slower[4].

Table 1. Percentage Germination of Coconut Seed after Long-distance Air Freight

	Forward hold	Rear hold	Control
Complete coconuts	70	90	75
Husk removed	81	25	33

Germination rates for the introduced samples are given in Fig. 1. Nut maturity and germination conditions were similar and there are indications of different germination patterns for seed of different origins. However, sample sizes were relatively small (90–200 nuts). Most seed germinated rapidly, unlike that of 'Jamaica Talls'[5], and final percentages were satisfactory. With quick germinating seed it is particularly important to avoid over-maturity and delays in shipping.

The coconut husk protects against desiccation and mechanical injury and, if the husk is removed, careful handling at all stages is essential. Transplanting of seedlings complete with baskets is advisable. Complete or partial husking reduces cost of transport (by 30–50 per cent) but freight costs are relatively very small in view of the possible benefits for areas with serious disease problems. A recent estimate for cadang-cadang, which threatens loss of livelihood for one-third of the population in the Philippines, put cash losses at more than 300 million dollars[6].

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Gibberellin-induced Anther and Pollen Development in a Stamen-less Tomato Mutant

Heslop-Harrison[1] has pointed out that in most investigations flower primordia differentiation has been considered synonymous with flowering as a single indivisible process. Observations on the differentiation and development of individual floral parts (sepals, petals, stamens, pistils) in hermaphroditic flowers thus offer a unique approach in the study of flowering. Certain tomato mutants lacking essential floral parts provide excellent source material for such investigations. This report directs attention to the effects of gibberellin A_3 (GA_3) on anther and pollen development in an otherwise stamen-less tomato mutant[2].

Rooted stem cuttings of the stamen-less mutant were initially exposed to 25 p.p.m. of GA_3 and other chemical stimuli in aerated solution culture root media[3]. Anthers developed in the flowers of plants exposed to GA_3 within 3–4 weeks. Viable pollen was also produced in these GA_3-induced anthers. Progeny grown from seed obtained by

selfing gave 100 per cent stamen-less plants. Such plants must otherwise be propagated from seed with a segregating population of one stamen-less to three normal[4].

In a second and more detailed experiment, uniformly rooted cuttings of the stamen-less tomato were exposed to 0, 10^{-5}, 10^{-4}, or 3×10^{-4} M GA_3 in solution culture root media, and to a 0, 10^{-5}, 10^{-4} or 10^{-3} M GA_3 foliar spray application in all combinations. All solutions constituting the root medium were renewed to the designated GA_3 concentrations at 6-day intervals. After 18 days of continuous exposure to the various gibberellin-levels in the root media the containers were rinsed with water and filled with half-strength Hoagland nutrient solution only.

Concentrations of 10^{-4} or 3×10^{-4} M in the solution culture root medium resulted in essentially all flowers having fully developed anthers (Table 1). Microscopic observations of acetocarmine preparations revealed that these anthers contained normal pollen. The induced anthers, however, did not form normal stamen cones around the style and stigma, but were curled and twisted (Fig. 1). Curling of anthers was most prominent at the highest (3×10^{-4} M) GA_3 concentration. Flower clusters which appeared on plants 3–4 weeks after termination of the GA_3 treatments showed no anther development. Furthermore, flower buds which were visible at the initiation of the GA_3 treatments were not affected. Anther development thus appeared a function of the presence of gibberellin during the flower-forming process.

Table 1. Anther and Pollen Formation in a Stamen-less Tomato induced by Gibberellin A_3 in the Solution Culture Root Media

Concentration of gibberellin A_3 (molar)	Inflorescence 1	2	3	4	5
	(Per cent flowers with anthers and pollen) (Average of 24 plants)				
0	0	0	0	0	0
10^{-5}	0	0	5	7	0
10^{-4}	0	0	98	100	0
3×10^{-4}	0	0	100	100	0

Further investigations revealed that 2-chloroethyl-trimethylammonium chloride (CCC), which inhibits fungal gibberellin synthesis[5], inhibits pollen formation in gibberellin-induced anthers. Vegetatively propagated stamen-less tomato mutants were exposed to all possible combinations of GA_3 at 0 and 10^{-4} M and CCC at 0, 10^{-5} and 10^{-4} M in the solution culture root media. Abnormal pollen was observed in the anthers of plants exposed to a combination of CCC at 10^{-5} M and GA_3 at 10^{-4} M. No pollen developed in the anthers of flowers from plants exposed to 10^{-4} M CCC plus 10^{-4} M GA_3.

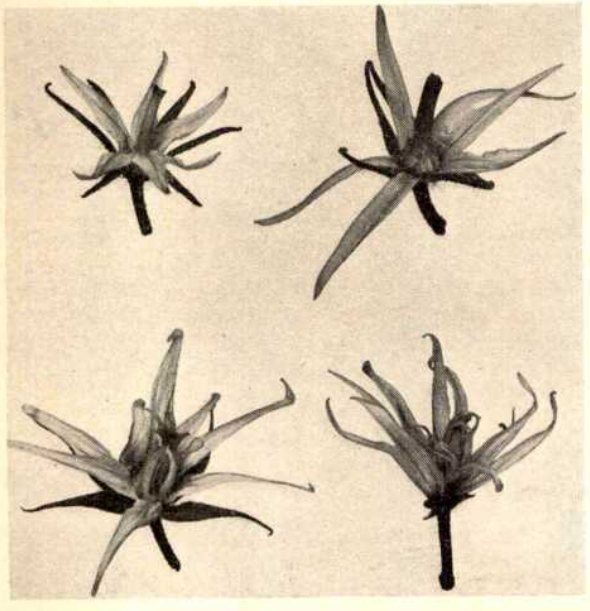


Fig. 1. Effect of various concentrations of gibberellin A_3 applied through the solution culture root medium on the development of stamens in the flowers of a stamen-less (*sls*) tomato mutant. From left to right and top to bottom: 0 (control), 10^{-5}, 10^{-4}, and 3×10^{-4} M

Several reports have suggested that gibberellin and auxin may play a determinative part in the development of male and female gametophytes in monoecious and gynoecious plants[6–10]. The present investigations suggest that gibberellin or gibberellin-like substances are necessary for the development of male gametophytes in the tomato, and that this influence is exerted after flower initiation.

We thank Dr. T. O. Graham, Department of Horticulture, Ontario Agricultural College, Guelph, Ontario, for providing seed, and Sherrill Kocan for assistance in recording data. Gibberellin consisted of potassium gibberellate and was supplied by Merck and Co., Rahway, N.J.

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Comparison of the Spectrum of Amino-acids in the Bleeding Sap of Wheat Maize and Rice

A number of compounds are known to be translocated in the transpiration stream. These include the building blocks needed for protein synthesis and also compounds postulated to play a part in the regulation of protein synthesis in leaves[1,2]. In the present investigation the spectrum of amino-acids translocated in the bleeding sap of three plant species was compared to provide data on the species specificity of the pattern observed.

Wheat variety 'Besostaia 1', maize variety 'Szegedi sarga lofogu' and rice variety 'Dubovskij 129' were included in the experiments. Bleeding sap was collected at the flowering stage during the night. The sterile sap was subjected to one- or two-dimensional paper chromatography (ascending technique, filter paper Whatman No. 1, 2 : 1 : 1 mixture of butanol–acetic acid–water or a 4 : 1 mixture of phenol–water). Hydrolysis with hydrochloric acid was carried out for 24 h at 105° C. Amino-acids supplied by British Drug Houses, Ltd., served as standards.

It can be seen from Fig. 1 that 10–14 amino-acids could be detected in the bleeding sap of cereals. Glutamine and asparagine were the major components in the bleeding sap of all three plants. In contrast to other authors[4,5] reporting on the presence of arginine or alanine in the bleeding sap as major components we have found remarkable quantities of ornithine to be present. Hydrolysis of the exudate resulted in the disappearance of the amides and in the accumulation of the corresponding amino-acids. The amount of ornithine remained unchanged.

As no significant amounts of arginine and citrulline could be detected in the bleeding saps it seems probable that the ornithine observed is not derived from the carbamide cycle. Ornithine present in significant amounts might either represent a translocation amino-acid or a regulatory substance supplied by the root. This suggestion is supported by the finding that no significant amounts of ornithine are present in the shoot.

It is also important to emphasize that of the bleeding saps tested, that of the rice plant exhibited the richest

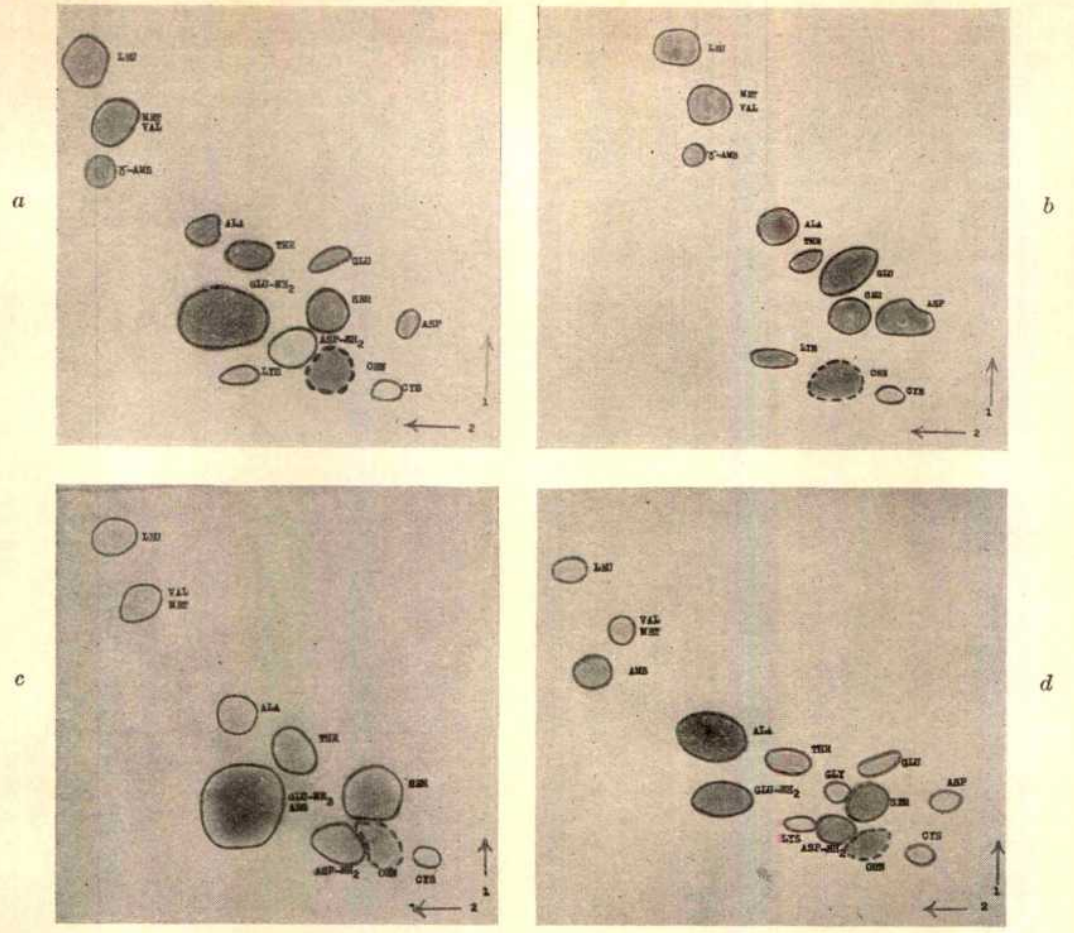


Fig. 1. Amino-acids in: *a*, bleeding sap of wheat; *b*, bleeding sap of wheat after hydrolysis; *c*, bleeding sap of maize; *d*, bleeding sap of rice

amino-acid spectrum. This is probably due to the fact that rice is grown under irrigated conditions and in the flooded soil nitrogen occurs mainly in the reduced form. Ammonium–nitrogen is more readily transformed into organic form than nitrate–nitrogen, which has first to be reduced[5].

The high ornithine of all the bleeding saps investigated might suggest a general role for ornithine as a transport amino-acid.

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MICROBIOLOGY

Possible Importance of Transfer Factors in Bacterial Evolution

Bacterial transfer factors are virus-like agents which mediate the transmission of genetic characters by conjugation in the Enterobacteriaceae. The first to be discovered was the *F* or 'sex' factor of *Escherichia coli* K12 (ref. 1), and for some time this was the only one to be investigated. The demonstration of the transmissibility of colicinogeny[2], and the discovery of transferable drug resistance[3–5], revealed the existence of a multiplicity of transfer factors. Recent work[6–8] suggests that such factors are basically independent of the genetic characters they carry and that they are widely distributed in the Enterobacteriaceae. It is probable that drug resistance and colicinogeny are only two of a range of characters they can transfer.

The transfer factors lack an independent invasion mechanism. They are thus dependent on their host cells to provide the apparatus for their transmission, although the production of this apparatus is probably coded by the transfer factors themselves. The mechanism of transmission is highly efficient, and often leads to infection of almost 100 per cent of cells of recipient strains.

Some bacteria carry genetic determinants which are apparently cytoplasmic in location. The determinants so far subjected to intensive investigation are those concerned with drug resistance and colicinogeny, but there seems to be no reason for doubting that other bacterial characters have the possibility of cytoplasmic determination. The genetic fragments responsible for drug resistance have been designated *R*-determinants. The origin of these determinants is unknown; they may have been initially chromosomal, but the possibility of an intrinsically cytoplasmic origin must also be considered.

Because the *F* factor seems to be concerned mainly with chromosomal transfer, a process which may be relatively unimportant in the distribution of genetic characters in the Enterobacteriaceae, the following discussion is concerned mainly with transfer factors other than *F*.

When a transfer factor infects a cell containing an *R*-determinant, the two elements 'combine' to form a transferable resistance factor (*R*-factor) which can be transmitted by conjugation to other bacterial cells. This transfer occurs within the strain originally containing only the *R*-determinant, and also to strains initially carrying neither the *R*-determinant nor the transfer factor. The *R*-factor also spreads to the strain which originally donated the

transfer factor, so that this strain, too, acquires an advantage in an environment in which the new character is needed for survival.

The transfer factor may be lost at some stage during the spread of an *R*-factor[6]. The resulting organism retains the *R*-determinant which it can transmit to its lineal descendants only and which appears to be stable. Superficially, these organisms resemble drug-resistant mutants.

The transfer factors have a very wide host range. For example, they may be transmitted, together with the resistance determinants they carry, and presumably with other genetic determinants, to the following 'genera' of Gram-negative bacilli: *Escherichia, Salmonella, Shigella, Proteus, Serratia, Pasteurella, Vibrio*, and others. The full range of characters for which the transfer factors can act as vectors is not yet known, but it may prove to be extensive. The same transfer factor will transfer different characters and the same character may be carried by different transfer factors.

The observations presented here suggest that the transfer factors may have more evolutionary importance in the bacterial systems in which they operate than has been hitherto imagined. Although devoid of the property of extra-cellular invasion, they have an immense ecological advantage because of their high communicability and wide host range. They can carry genetic determinants which may ultimately prove to be numerous. By this mechanism these determinants may be widely distributed among different species or genera of Gram-negative bacilli, so that determinants in a given strain may have originated in a bacterial species normally regarded as belonging to a different genus. Whether such genetic determinants can become integrated into the chromosomes of their new carrier cells is not yet known. If they can, it may be supposed that, once integrated, they are indistinguishable in origin from other chromosomal characters and are localizable on the bacterial linkage map. But even if they remain cytoplasmic in location they are stable cellular components. Whether they enter the chromosome or remain cytoplasmic, therefore, they become part of the genome of their host cells.

Extensive exchange of characters between the Gram-negative bacilli need not be expected to lead to genetic uniformity in the different host species, except in relation to the individual characters concerned. On the contrary, it may encourage diversification, because in each case the effect of a new character has to be assessed through its summation with the bacterial genome as it existed up to the time of acquisition of the new character, and the ultimate effect of the now modified genome on the ecology of the recipient organism.

I thought it worth while to voice these speculations because if the long-term activity of transfer factors has influenced bacterial evolution, the evolutionary time-scale may have been telescoped into a shorter span than that envisaged purely in terms of the selection of mutants with survival advantages. The obvious question that arises is whether efficient mechanisms of genetic distribution such as those I have discussed exist only in the Enterobacteriaceae, or indeed whether they are limited to the bacteria. If they operate in other forms of life, some re-thinking may be called for in relation to evolution in other fields of biology.

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Correlation of Temperature-dependent Water Properties and the Growth of Bacteria

In recent years there has been some evidence to suggest that biological activity may be affected by water structure[1,2]. The premise of the above work was that water undergoes subtle structural changes at specific temperatures[3,4] and, if biological growth is affected by water structure, growth should be affected at these temperatures. Since the effect of water structure on biological activity has not been substantiated, an investigation of the effect of temperature on bacterial growth was undertaken.

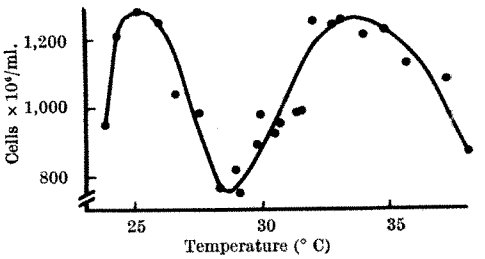


Fig. 1. Number of cells of *Streptococcus faecalis* at different temperatures

Using a polytemperature thermostat, similar to that described elsewhere[2], cultures of *Streptococcus faecalis* were simultaneously grown in broth culture at nineteen temperatures ranging from 20° to 40° C. The culture tubes were prepared and placed in the incubator at least 8 h prior to inoculation so that temperature equilibrium within the medium would be reached. Inoculum consisted of a loop of cells from a 24-h broth culture of the organism. Growth was stopped after a 20-h incubation period by placing the culture tubes in 50° C water. Bacterial multiplication was determined by direct cell counts in a haemocytometer.

Typical results are shown in Fig. 1. Near 30° C there is a marked reduction in number of cells. The temperature of reduced cell multiplication corresponds to a temperature at which a change in water structure takes place. These data support the postulate that biological activity is affected by water structure. This work is being extended to other bacteria and temperatures.

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Staphylococcus aureus Strains in the '52, 52A, 80, 81 Complex'

The phage-typing pattern of a strain of *Staphylococcus aureus* is believed to be determined by two factors: the 'basic' sensitivity of the strain to lysis by the typing phages, modified by interference exerted by the prophage or prophages it carries. The specificity of this interference may be narrow, and prevent lysis only by the phage carried by the strain and by closely related phages, or it may be very broad. Sometimes a single prophage will block the sensitivity to a number of apparently unrelated phages and have a profound effect on the phage-typing pattern[1,2].

Under natural conditions the typing pattern of a strain may be changed by lysogenization, and this may lead to difficulties in the interpretation of long-term epidemiological investigations. It has proved possible, however,

to define by *in vitro* studies some of the changes in typing pattern which might be expected to occur in the field, and these correspond very well with the observed changes in strains endemic in hospitals[3]. The most widely studied group of strains is the '52, 52*A*, 80, 81 complex', which comprises strains with one of the following phage-typing patterns: 80, 81, 80/81, 52/52*A*/80, and 52/52*A*/80/81. The relation between these strains has been clarified by investigations carried out in a number of laboratories[4–10] (see Fig. 1).

(1) Strains of phage-type 80/81 are lysogenic for a completely defective prophage, designated 80′. Sensitivity to the two typing phages, 52 and 52*A*, is blocked almost completely in strains carrying this defective phage[9]. The typing pattern of such strains changes from 80/81 to 52/52*A*/80/81 when prophage 80′ is lost spontaneously[4].

(2) Prophage 80′ may also be lost as a result of prophage substitution during lysogenization with a number of phages carried by various members of the '52, 52*A*, 80, 81 complex'.

(3) Some of these phages have no direct effect on the typing pattern, but the loss of prophage 80′ results in a change of typing pattern from 80/81 to 52/52*A*/80/81 (class 1 phages)[9].

(4) Others (class 2 phages) block the sensitivity to phage 81 on lysogenization. They may also occasionally substitute for prophage 80′. When this substitution occurs the change is from 80/81 to 52/52*A*/80; a change from 80/81 to 80 occurs when substitution does not occur and the strain becomes doubly lysogenic for prophage 80′ and the class 2 phage[5,11].

(5) Members of another group of phages block sensitivity to the three phages 52, 52*A* and 80, and lysogenization of an organism with the typing pattern 80/81 or 52/52*A*/80/81 with one of them causes the pattern to become 81 (class 3 phages)[4,11].

(6) Still other phages (class 4) block the sensitivity to all four phages in the complex and thus change previously 'typable' strains to 'untypable' ones[11,12].

Rountree[4] noted that certain members of phage-type 80/81 carried, in addition to the defective prophage 80′, a fully active phage belonging to serological group *F*. This phage did not seem to play any part in the changes already described, since it was present in all members of the complex that had been investigated. It appeared, therefore, that the 'basic' phage-typing pattern of strains in the complex was 52/52*A*/80/81.

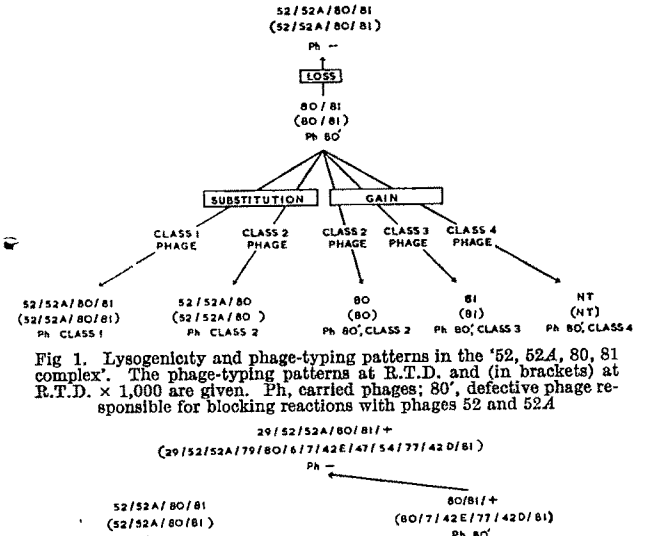


Fig 1. Lysogenicity and phage-typing patterns in the '52, 52*A*, 80, 81 complex'. The phage-typing patterns at R.T.D. and (in brackets) at R.T.D. × 1,000 are given. Ph, carried phages; 80′, defective phage responsible for blocking reactions with phages 52 and 52*A*

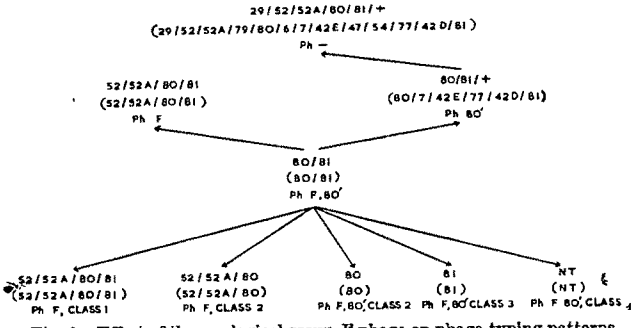


Fig. 2. Effect of the serological group *F* phage on phage-typing patterns in the '52, 52*A*, 80, 81 complex'. The phage-typing patterns at R.T.D. and (in brackets) at R.T.D. × 1,000 are given

Recently, however, Winkler and his colleagues[13] reported that staphylococci of phage-type 80/81 showed a considerable broadening of their typing pattern when they lost the group *F* phage, and were now sensitive to phages 7, 42*E*, 77 and 42*D*. This was confirmed for *P.S.* 80, the propagating strain for phage 80, which has the phage-typing pattern 80/81. This culture was screened for mutants that had lost the group *F* phage spontaneously, using strain 57 as indicator strain for the phage[13]. Having obtained this mutant, a second experiment was carried out to detect a mutant that had also lost the defective prophage 80′. Such a strain has now been obtained, and has the following typing pattern:

at R.T.D. (routine test dilution) : 29/52/52*A*/80/81/+ (weak reactions with 7, 42*E*, 77 and 42*D*)

at R.T.D. × 1,000 : 29/52/52*A*/79/80/6/7/42*E*/47/54/77/42*D*/81

It is, of course, impossible to be certain that this strain is completely non-lysogenic and represents the true 'basic pattern' of members of the '52, 52*A*, 80, 81 complex'. It is noteworthy, however, that such a staphylococcus, which would be classified as a member of phage group I–III, should be the precursor of a series of strains which belong to phage-group I; it might equally be the precursor of typical group III strains if it was lysogenized by phages able to block reactions with the group I phages.

The peculiar lysogenic state of members of phage-type 80/81 from widely separated parts of the world also deserves comment. All are doubly lysogenic for a particular serological group *F* phage and for a completely defective phage of unknown serological group. It is unlikely, therefore, that such strains arose independently in a number of different countries, and more probable that the two lysogenizations occurred as separate events in a single organism which has since spread widely throughout the world.

The relation of the non-lysogenic precursor strain to the members of the '52, 52*A*, 80, 81 complex' is summarized in Fig. 2.

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VIROLOGY

Possible Correlation between Virus Particle Size and Activity of Antiviral Agents

Some success has already been achieved in the chemotherapy of infections caused by the larger viruses, but compounds with an equal degree of activity against the

smaller viruses seem much more difficult to find. It is therefore natural to enquire whether there is any relationship between the size of virus particles and the activity of chemotherapeutic agents. As a working hypothesis one may adopt the postulates that (*a*) a compound is maximally efficient if 1 molecule can inhibit the maturation of 1 virus particle, and (*b*) that to obtain maximal activity each volume of the cytoplasm of the infected cell equivalent to the volume of a virus particle must contain 1 molecule of the compound. From these postulates the minimal inhibitory concentration of a maximally effective compound can be calculated. Thus, 1 molecule must be present in a volume of $\frac{4\pi r^3}{3}$, where r is the radius of the virus particle in mμ. In 1 l. there will be $\frac{3 \times 10^{24}}{4\pi r^3}$ molecules. Since there are $0{\cdot}606 \times 10^{24}$ molecules in a gram molecule, this represents a molarity of $(0{\cdot}808\pi r^3)^{-1}$, whence log molarity $= -0{\cdot}4045 - 3 \log r$.

This theoretical linear relationship between the logarithms of particle size and minimal inhibitory concentration has been plotted in Fig. 1, in which the particle diameter, as being a more familiar measurement, has been substituted for the radius.

Information is available in the literature on the activities of a number of compounds with antiviral activity, and the points corresponding to the minimal concentration causing complete inhibition and the particle diameter of the relevant virus have been inserted in Fig. 1. The compounds and viruses are: methisazone (1-methylisatin 3-thiosemicarbazone) and rabbitpox[1]; 5-fluoro-2′-deoxyuridine and vaccinia[2]; idoxuridine (5-iodo-2′-deoxyuridine) and herpes[3], vaccinia[4] and zoster[5]; *p*-fluorophenylalanine and vaccinia[6]; amantidine hydrochloride and influenza[7], and rubella[8]; 5-chloro-2-(α-hydroxybenzyl)benzimidazole and poliomyelitis[9]. None of these points lies below the line. The hypothesis is therefore not invalidated by any antiviral agent known at present, and with due reservations will be taken as valid for the purpose of further discussion.

The distance of a point from the line can be taken as a measure of the efficiency of the compound, which can be defined in numerical terms as the ratio of the theoretical and actual inhibitory concentrations expressed as a percentage. Thus, the efficiency of methisazone against rabbitpox and of 5-fluoro-2′-deoxyuridine against vaccinia is 15 per cent. Idoxuridine (0·55 per cent) and *p*-fluorophenylalanine (0·01 per cent) are much less efficient. The efficiency of idoxuridine against herpes is 4·6 per cent, and against zoster 0·005 per cent. The efficiency of amantidine hydrochloride against rubella and influenza

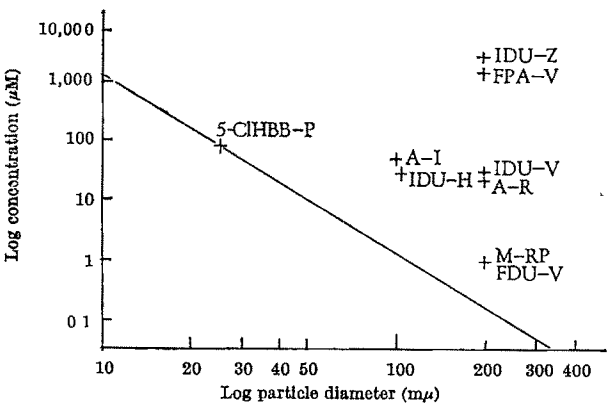


Fig. 1. Relationship between minimal effective concentration of an antiviral compound and virus particle diameter. The line represents the theoretical relationship for a maximally effective compound. The points represent the activities of known antiviral compounds against the relevant viruses. Abbreviations: 5-ClHBB, 5-chloro-2-(α-hydroxybenzyl)benzimidazole; A, amantidine hydrochloride; IDU, idoxuridine, FPA, *p*-fluorophenylalanine; M, methisazone, FDU, 5-fluoro-2′-deoxyuridine; P, poliomyelitis, I, influenza; H, herpes; Z, zoster, V, vaccinia; R, rubella, RP, rabbitpox

Table 1. Relation between Predicted Minimal Inhibitory Concentration of Antiviral Compound and Virus Particle Diameter

Virus	Diameter (mμ)	Minimal inhibitory concentration (mg/l.)	Equivalent adult dose (mg)
Varicella	200	0 03	2
Mumps	140	0·09	6
Measles	140	0 09	6
Rubella	~130	0·11	8
Respiratory syncytial	130	0 11	8
Influenza	~100	0 25	17
Cytomegalovirus	87	0 37	26
Adenovirus	80	0 38	27
Verruca	45	2 7	190
Rhinovirus	31	8·3	580
Coxsackie	28	11 3	790
Tick-borne encephalitis	25	15 8	1,100
ECHO	25	15 8	1,100
Foot-and-mouth disease	22 5	21·7	—
Infective hepatitis	15	73 3	5,100

is low (2·2 per cent and 2·5 per cent, respectively), and it should be possible to find much more active compounds. Against poliomyelitis, however, the efficiency of 5-chloro-2-(α-hydroxybenzyl)benzimidazole is 100 per cent, so that according to the hypothesis a search for more active compounds would be pointless.

The predicted minimal inhibitory concentrations for viruses for which there are as yet no effective antiviral agents are shown in Table 1. As the object of research into antiviral chemotherapy is to find compounds which can be of use in medical and veterinary practice, the concentrations have been transformed into doses in mg/l. (that is, mg/kg) for a compound with a standard molecular weight of 200, and also to the equivalent dose in grams for an adult weighing 70 kg. The dose indicated would provide a concentration in the infected tissue of the desired level only if the compound were perfectly absorbed, uniformly distributed, slowly metabolized and slowly excreted. These requirements will be fulfilled only rarely, and to allow for deficiencies in this respect the theoretical dose would have to be multiplied several-fold. For the viruses from varicella to verruca the revised dose would still be within reasonable limits, but it would follow that treatment or prophylaxis of the common cold would require repeated doses of the order of several grams, and treatment of infective hepatitis might not be practicable. These limitations could only be overcome by the discovery of compounds which had exceptionally favourable pharmacological characteristics, or which had a selective affinity for the infected tissues which would enable the requisite concentration to be obtained with lower doses. Compounds capable of acting against the smaller viruses would elude detection if their solubility in water was less than the predicted minimal inhibitory concentration, and it would seem reasonable to concentrate the search in this case on compounds with high solubility and low molecular weight.

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AGRICULTURE

Occurrence of Sucrose in the Fruit of Some Species of *Lycopersicon*

Two sub-genera are recognized in the genus *Lycopersicon*[1,2]. In one of these the plants are annuals and bear fruits containing carotenoids and lycopene (*Eulycopersicon*). In the other the plants are perennials

with fruits pigmented with chlorophyll and anthocyanins (*Eriopersicon*). The composition of the fruit of the subgenus *Eulycopersicon*, which includes all tomato varieties in commercial production, has been studied extensively, but apart from investigations of their vitamin C and carotene contents[3,4] few data are available for fruit of *Eriopersicon*.

The free sugars of commercial varieties of tomato fruit are predominantly reducing sugars[5,6], the sucrose content rarely exceeding 0·1 per cent on a fresh weight basis[5]. Quantitative chromatographic investigations have shown that the reducing sugars consist almost entirely of glucose and fructose present in approximately equal amounts with, in general, a slight preponderance of the latter[7–11]. Sucrose, when found at all, is present in small amounts only[7,8,10]. Traces of other sugars have been detected, notably a ketoheptose[7], and raffinose, which has been reported in the fruit of an Indian wild variety[12].

Recent investigations of the composition of the expressed juices of the mature fruit of various species of *Lycopersicon* used as sources of genetic material by the Plant Breeding Department have been made at this Institute. Fruit of *Eriopersicon* species, although not unduly acid and having very low reducing-sugar contents, were characterized by abnormally high total solids contents. A preliminary examination by paper chromatography in several solvent mixtures explained this apparent anomaly by revealing, in addition to glucose and fructose, the presence of considerable quantities of a sugar indistinguishable from sucrose. Elution of this sugar from the paper followed by hydrolysis under mildly acid conditions or by incubation with invertase gave a solution containing two sugars which travelled on a paper chromatogram at the same rate as glucose and fructose respectively. Trace amounts of other, as yet unidentified, carbohydrates were also detected in the fruit of some species.

Quantitative determination of the individual sugars in 80 per cent ethanol extracts of the fruit of various species of *Lycopersicon* by paper chromatographic techniques outlined previously[10] gave the results shown in Table 1. Analyses for fruit of the variety 'Moneymaker' (*L. esculentum*) at two stages of ripeness are included in the table for comparison. Sucrose was determined by measuring the increases in glucose and fructose produced following incubation with invertase (B.D.H. concentrate) at 20° C for 1 h after removal of the alcohol.

Table 1. GLUCOSE, FRUCTOSE AND SUCROSE (PERCENTAGE OF FRESH WEIGHT) IN THE FRUIT OF SOME SPECIES OF *Lycopersicon*

Species	Glucose (%)	Fructose (%)	Sucrose (%)	Glucose/fructose ratio
Sub-genus *Eulycopersicon*				
L. pimpinellifolium (B6009)	1·21	1·56	0·16	0·78
L. pimpinellifolium (B34)	1·74	1·95	0·11	0·89
L. esculentum var. *cerasiforme*	1·41	1·80	0·08	0·78
'Moneymaker' (mature-green)	1·25	1·30	—	0·96
'Moneymaker' (orange-red)	1·77	1·86	Trace	0·93
Sub-genus *Eriopersicon*				
L. hirsutum	0·24	0·96	3·30	0·25
L. hirsutum var. *glabratum*	0·44	0·70	4·08	0·37
L. peruvianum	0·14	0·65	3·54	0·22

Fruit of *L. hirsutum*, *L. hirsutum* var. *glabratum* and *L. peruvianum*, all of which are included in the sub-genus *Eriopersicon*, contained considerable quantities of sucrose.

In fruit of the two strains of *L. pimpinellifolium* and *L. esculentum* var. *cerasiforme* only very small amounts of sucrose were present, while in the fruit of variety 'Moneymaker' this disaccharide was virtually absent. The almost complete absence of sucrose in English commercial varieties has been noted previously[10].

Fruit of the species of *Eulycopersicon* examined (including those of variety 'Moneymaker'), while having less than 0·2 per cent sucrose, contained between 1 and 2 per cent of both glucose and fructose, giving glucose/fructose ratios of between approximately 0·8 and 1·0. In contrast, fruit from species included in *Eriopersicon* contained relatively little glucose and fructose. Glucose concentrations were particularly low, giving rise to much lower glucose/fructose ratios than those normally encountered. The results for the fruit of *L. peruvianum*, which contained the lowest glucose and fructose concentrations, are at variance with Luckwill's comment in his investigation of the genus *Lycopersicon*[2]. He states: ". . . the fruits [of *Eriopersicon*] are rich in reducing sugars and in some species (that is, *L. peruvianum*) their taste resembles that of the cultivated tomato. The fruits of other species, however (that is, *L. hirsutum*), have a 'bitter-sweet' flavour due to the presence of solanine . . .". It would appear from the present results that the sweetness of the fruit of these species is due primarily to sucrose and not to reducing sugars.

The sucrose content of the fruit from the progenies of crosses between *L. hirsutum* var. *glabratum* and *L. esculentum* is at present under investigation.

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PSYCHOLOGY

Sex Chromosome Abnormalities in Two State Hospitals for Patients requiring Special Security

THE association between sex chromatin abnormality and mental sub-normality is well established. The incidence of sex chromatin abnormality in the new-born male and female population is about 0·2 per cent and 0·08 per cent respectively; in institutionalized mentally sub-normal male and female populations about 1 per cent and 0·4 per cent respectively. Court Brown[1] reported that a high proportion of sex chromatin positive males recognized in his unit had been committed to a mental defective institution because of anti-social behaviour. He suggested that an abnormal sex chromosome complement might predispose to delinquency. However, a survey by Wegman and Smith[2] on a group of socially disturbed males of relatively normal intelligence failed to show an increase in sex chromatin abnormality.

These observations prompted us to undertake a survey of the sex chromatin pattern in a group of the mentally sub-normal who had been 'institutionalized' because of anti-social behaviour. Since this study was begun a report of a similar survey has appeared[3].

Rampton and Moss Side Hospitals are State hospitals for patients who require special security on account of persistent violent or aggressive behaviour. The patients are mentally sub-normal and have been referred by the Courts or transferred from other mental institutions. Twenty-one sex-chromatin-positive males were detected among 942 male patients, an incidence of 2·2 per cent. Two of the 420 female patients examined had two sex chromatin bodies, an incidence of 0·49 per cent. On examining the chromosomes, twelve of the twenty-one sex-chromatin-positive males had 47 chromosomes and an *XXY* sex chromosome constitution. Seven had 48 chromosomes and an *XXYY* complement. Two male

patients were presumed to have *XXY/XY* mosaicism. The majority of the cells analysed for chromosome complement in both female patients with two sex chromatin bodies had 47 chromosomes. Both female patients had a sufficiently large percentage of cells with 46 chromosomes to indicate the presence of *XXX/XX* mosaicism.

The frequency of sex-chromatin-positive males at Rampton and Moss Side Hospitals is compared with the frequency of similar males in other institutions for the mentally sub-normal in Table 1. There is a statistically significant excess of sex-chromatin-positive males at Rampton and Moss Side when compared with institutionalized mentally sub-normal populations where persistent violent or aggressive behaviour is not a feature ($\chi^2 = 21{\cdot}7$, $n = 1$, $P < 0{\cdot}001$). The incidence at Rampton and Moss Side does not differ significantly from that in a similar population in Sweden (Table 1). The frequency of females with two sex chromatin bodies is not significantly different from that observed in other institutions for the mentally sub-normal (Table 2).

It is thus tempting to refer the increased incidence of sex-chromatin-positive males to violent or aggressive behaviour. It was, however, noted that the patients at Rampton and Moss Side have a higher intelligence quotient (mean $77{\cdot}3 \pm 14{\cdot}07$) than patients in other institutions for the mentally sub-normal. Since it is recognized that the incidence of sex-chromatin-positive males among the institutionalized mentally sub-normal is proportional to the intelligence quotient[4–7], being highest in the range of more than 50, it might be expected that a higher frequency of sex-chromatin-positive males would be found in Rampton and Moss Side than in other institutions for the mentally sub-normal. None the less the unusual distribution of the type of sex chromosome abnormality observed suggests that the observed high incidence is not simply a reflexion of higher intelligence quotient. There is an extraordinary excess of individuals with an *XXYY* chromosome complement (Table 3). Indeed, it may reasonably be suggested that this high incidence at Rampton and Moss Side is attributable entirely to an excess of patients with two *Y* chromosomes.

Table 1. Frequency of Sex-Chromatin-Positive Males in Institutionalized Mentally Sub-normal Populations

Population	Total No of patients	No. of sex-chromatin-positive patients	Incidence %
Institutions for the mentally sub-normal without persistent violent or aggressive behaviour (refs. 4–6, 8–13)	10,685	90	0·84
Rampton and Moss Side. Hospitals for the mentally sub-normal requiring special security on account of persistent violent or aggressive behaviour	942	21	2 2
Criminal or hard to manage males of sub-normal intelligence. Forssman and Hambert (ref. 3)	760	15	2 0

Table 2. Frequency of Females with Two or More Sex Chromatin Bodies in Institutionalized Mentally Sub-normal Populations

Population	Total No. of patients	No. of patients with 2 or more sex chromatin bodies	Incidence (%)
Institutions for the mentally sub-normal without persistent violent or aggressive behaviour (refs. 10–12, 14–16)	6,047	24	0·40
Rampton and Moss Side. Hospitals for the mentally sub-normal requiring special security on account of persistent violent or aggressive behaviour	420	2	0 49

Table 3. Types of Abnormal Sex Chromosome Complement found in Sex-Chromatin-Positive Males

Population	Chromosome complement *XXY*	*XXYY*	Other	Total
Babies				
Maclean *et al.* (ref. 17)	12	1	5	18
Institutionalized mentally sub-normal				
Maclean *et al.* (ref. 10)	16	2	10	28
De la Chapelle (ref. 11)	5	0	1	6
Rampton and Moss Side	12	7	2	21

The nature of offences committed prior to admission by the 21 sex-chromatin-positive male patients at Rampton and Moss Side was compared with the behaviour patterns of a randomly selected group of controls from the remainder of the Rampton population. No statistically significant difference in behaviour pattern between these two groups of patients was observed. However, certain trends are suggested. The sex-chromatin-positive patients tended to abscond more frequently than the controls and they committed less homosexual or heterosexual offences. There were no significant differences in type of behaviour disorder between the *XXY* and *XXYY* patients.

Features of Klinefelter's syndrome were present in each of the patients with a simple *XXY* or *XXYY* chromosome complement. The patients with an *XXYY* sex chromosome complement were taller than those with an *XXY* complement, but the difference is not statistically significant. The difference in height is entirely due to the Pubic-Sole measurement and becomes significant if previously reported cases[18,20] are taken into account. The mean measurement for patients of karyotype *XXY* is 89·4 cm ($\sigma = 5{\cdot}67$, $n = 76$) and for patients of karyotype *XXYY* 94·8 cm ($\sigma = 7{\cdot}7$, $n = 16$). This confirms the suggestion by Barr[18] and Bray and Josephine[19] that the extra *Y* chromosome has some effect on growth. The two patients with *XXY/XY* mosaicism had normal-sized testes and normal secondary sex development.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 7

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. G. M. Berlyne: "The Treatment of Chronic Renal Failure".*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m —Mr. W. S. Metcalf: "Radio Astronomy".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. E. A. Mason: "The Chemical Industry of Japan".

INSTITUT FRANÇAIS DU ROYAUME-UNI (at Queensberry Place, London, S.W.7), at 8.15 p.m.—French Scientific Films.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Peter Beighton: "Easter Island People".

Tuesday, February 8

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. Jane Abercrombie: "Educating for Change".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. A. H. S. Megaw: "Excavations in Euboea, Kythera, and the Cyclades".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m. —Mr. A. G. Strong: "Sealants"; 7 p.m.—Mr. A. Randell: "Surlyn A Ionomer Resins".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir Lawrence Bragg, O.B.E., M.C., F.R.S.: "Properties of Matter II". (Afternoon lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on February 9, 15 and 16.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. C. Rashbass: "The Control of the Direction of Gaze". (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

Wednesday, February 9

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. L. E. Glynn: "The Role of Auto-Immunity in Pathogenesis".*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. F. G. Heath, Mr. M. G. Hartley, Mr. D. Green and Mr. S. Saleeb: "The Manchester Digital Traffic Simulator".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. A. R. Rangabe: "Trutrack—a New Approach to Low Side Thrust Transcription Arms".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. F. E. Duffield: "Transducers for Instrumentation".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6 15 p m —Dr. L. Golberg: "The Toxicology of Food Additives—The Present Position".

CROYDON NATURAL HISTORY AND SCIENTIFIC SOCIETY (in the Maple Room, Fairfield Halls, Croydon), at 7.30 p m.—Dr. G. P. L. Walker: "Some Aspects of Atlantic Geology".

Thursday, February 10

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. L. E. Westrum: "Views of Polynesia".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. R. A. Hinde, F.R.S.: "The Use of Sub-Human Primates for Studying the Effects of the Early Social Environment on Later Behaviour".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. G. Withers and Mr. L. Martland: "The Relationship Between the Oil and Automotive Industries".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. J. Evans: "Actions of Radiations on Chromosomes". (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m —Mr. G. Cox: "The Independent Television News Service".

Friday, February 11

INSTITUTE OF RURAL LIFE AT HOME AND OVERSEAS (at Friends International Centre, Courtauld House, Byng Place, London, W C 1), at 1.15 p.m. —Miss Helen M. Wallis, M.B.E.: "The Women of Sarawak".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. S. Brenner, F.R.S.: "The Genetic Code".

Saturday, February 12

BRITISH MICROCIRCULATION SOCIETY (at the Wolfson School of Nursing, Westminster Hospital, Vincent Square, London, S.W.1), from 10.30 a.m. to 6 p.m.—Papers in the general fields of "Microcirculation and Biorheology".

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mrs. Charmian Biernoff: "Sorkun—a Village of Potters in North West Turkey" *

Monday, February 14

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 5.15 p m.—Prof. Theodor Bücher (University of Munich): "Dynamics of Liver Metabolism". (Further lecture on February 17.)*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. James D. Ebert (Carnegie Institution of Washington): "Interacting Systems in Development: I. Beyond the Ribosome".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

CHAIR OF COMPUTING SCIENCE—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (February 12).

LECTURER (with a good degree in physiology, biochemistry, pharmacology, chemistry, medicine or zoology) IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Deputy Secretary, The University, Southampton (February 12).

LECTURERS (2) (preferably specialized in either urban geography or Latin America) IN GEOGRAPHY—The Secretary of the University Court, The University, Glasgow (February 13).

LECTURER IN THE DEPARTMENT OF PHYSIOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 14).

LECTURERS (2) IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne, 2 (February 14).

RESEARCH ASSISTANT (male or female honours graduate in any of the biological sciences including medicine) IN TISSUE CULTURE IN THE DEPARTMENT OF ANATOMY—The Registrar, University College, Cathays Park, Cardiff (February 14).

SENIOR LECTURER IN SOCIOLOGY—The Registrar, University of Warwick, Coventry, Warwickshire (February 18).

LECTURER IN EDUCATION COMBINED WITH PHYSICAL SCIENCES; and a LECTURER IN EDUCATION COMBINED WITH BIOLOGICAL SCIENCES—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (February 19)

ASSISTANT (preferably with a special interest in human geography) IN GEOGRAPHY—The Secretary and Bursar, University College, Dublin, Republic of Ireland (February 21).

LECTURER IN STEROID BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (February 21).

LECTURER (with a distinguished academic record in pure mathematics, applied mathematics or statistics) IN THE SCHOOL OF MATHEMATICS, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 25).

LECTURER IN MEDICAL PHYSICS—The Registrar, The University, Manchester, 13, quoting Ref. 12/66 (February 26).

LECTURER IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, The University, Aberdeen (February 26).

LECTURER (with a special interest in enzymology) IN BIOCHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea (February 26).

LECTURER (graduate in science, pharmacy or medicine) IN PHARMACOLOGY —The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (February 28)

LECTURER TO SPECIALIZE IN BIOCHEMISTRY—The Registrar (Room 39, O.R.B.), The University, Reading (February 28).

LECTURER (with a good degree, preferably with honours, and well qualified and experienced in the teaching of one or more of the following subjects mathematics, biology or chemistry) IN THE DEPARTMENT OF EDUCATION, University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, February 28).

LECTURER (with either teaching or industrial experience in the physical aspects of pharmaceutics, together with a strong interest in research) IN PHARMACEUTICAL TECHNOLOGY—The Registrar, University of Strathclyde, Glasgow, C.1 (February 28).

LECTURER (with qualifications in approximation theory or related branches of mathematics) IN MATHEMATICS—The Secretary, University of Lancaster, Bailrigg House, Lancaster (February 28).

LECTURERS (2) IN BACTERIOLOGY/IMMUNOLOGY at the Western Infirmary —The Secretary of the University Court, The University, Glasgow (February 28).

CHAIR OF EDUCATION—The Secretary, Trinity College (University of Dublin), Dublin, Republic of Ireland (March 1).

TUTORIAL RESEARCH STUDENT (man or woman) IN PHYSICS (Experimental or Theoretical)—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (March 1).

LIBRARIAN at Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 2)

LECTURER/SENIOR LECTURER (preferably with experience in the control of computer installations carrying out a variety of scientific computations on a service basis) IN THE BASSER COMPUTING DEPARTMENT, School of Physics, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 4).

LECTURERS (2) (with a degree in bacteriology or equivalent qualifications) IN BACTERIOLOGY—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (March 7).

READER and SENIOR LECTURER IN THE DEPARTMENT OF MATHEMATICS, PURE AND APPLIED, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 15).

LECTURER or SENIOR LECTURER IN PLANT PHYSIOLOGY at Lincoln College (University of Canterbury), New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 31).

UNIVERSITY LECTURER and a UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ANIMAL PATHOLOGY in the School of Veterinary Medicine—T. D. Kellaway, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge (March 21).

LECTURER IN PARASITOLOGY (Immunology) at the University of Queensland, Brisbane, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, April 1).

LECTURERS (preferably with research experience in one of the following fields: experimental cytology, developmental biology, comparative physiology or protozoology) IN ZOOLOGY—The Secretary of the University Court, The University, Glasgow (April 1).

UNIVERSITY RESEARCH FELLOW IN THE HISTORY AND PHILOSOPHY OF SCIENCE IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leeds, 2 (April 4).

ASSISTANT LECTURER to assist in the teaching of general geology as part of a scheme of study in earth sciences—The Registrar, University College of North Wales, Bangor, North Wales (April 30).

SECOND CHAIR OF ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (April 30).

CHIEF TECHNICIAN IN THE DEPARTMENT OF ZOOLOGY, to be in charge of the technical staff (25) of the laboratories—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, quoting Ref. No. CT/Z/1/66

MASTER TO TEACH MATHEMATICS WITH SUBSIDIARY PHYSICS; and a MASTER TO TEACH PHYSICS—The Headmaster, Epsom College, Epsom, Surrey.

PHYSICAL CHEMISTS (young graduates) to do research in inorganic thermochemistry and thermodynamics and on vapour phase reactions, partly at high temperature—The Director of Research, Fulmer Research Institute, Stoke Poges, Bucks.

RESEARCH ASSISTANT (biologist) to take part in a programme concerned with embryonic nutrition and its relation to congenital malformations—Dr. E. Beck, Department of Anatomy, University College of South Wales and Monmouthshire, Newport Road, Cardiff.

TECHNICIAN OR JUNIOR TECHNICIAN (preferably with previous experience in sterile techniques and/or tissue culture) IN THE CHROMOSOME UNIT OF THE NUFFIELD UNIT OF MEDICAL GENETICS, for work on human chromosomes—The Registrar, The University, Liverpool, 3, quoting Ref. 412/N

WATER SUPPLY GEOLOGIST (between 30 and 45, national of, and resident in, the United Kingdom or the Republic of Ireland, with an honours degree in geology and at least five years experience in water supply geology, including electric logging of bore-holes) in Nigeria, to supervise water supply exploration drilling projects and to train young Nigerian geologists—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 218/130/02.

REPORTS and other PUBLICATIONS

(not included in the Monthly Books Supplement)

Great Britain and Ireland

Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems. (Cmnd. 2836.) Pp. v+85. (London: H.M. Stationery Office, 1965) 6*s.* 6*d* net. [812

Ministry of Health, Scottish Home and Health Department. Drug Addiction. The Second Report of the Interdepartmental Committee. Pp. 14. (London: H.M. Stationery Office, 1965) 1*s.* 6*d.* net [812

Glasshouse Crops Research Institute. Annual Report 1964. Pp. 168. (Rustington, Littlehampton: Glasshouse Crops Research Institute, 1965.) 12*s.* 6*d.* [812

Ministry of Agriculture, Fisheries and Food. Aids to Management: 7—Arable Crops and Grass. (The Farm as a Business Series.) Pp. iv+41. (London: H.M. Stationery Office, 1965.) 2*s.* 6*d.* net. 812

The Royal Society for the Prevention of Accidents. A Simple Guide to Accident Prevention in the use of Electricity By A. A Beckingsale. (Reprinted from *Industrial Safety*.) Pp. 24. (London. United Trade Press, Ltd., 1965. Distributed by the Royal Society for the Prevention of Accidents.) 3*s.* 6*d.* [812

Agricultural Research Council. Supplementary Report of the Research Committee on Toxic Chemicals. Pp iii+16. (London. Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 2*s.* [812

The Radiochemical Centre, Amersham. Technical Bulletin 65/9· Sources for Interstitial and Intracavitary Radiotherapy. Pp. 4. Technical Bulletin 65/10. Clinical Radiation Sources for Afterloading Methods. Pp. 4. (Amersham: The Radiochemical Centre, 1965.) [812

Dilemmas of Peacemaking. By The Rev. E. Gordon Rupp (The 1965 Alex Wood Memorial Lecture) Pp. 10. (New Malden, Surrey. The Fellowship for Reconciliation, 1965.) 2*s.* [812

Royal Anthropological Institute of Great Britain and Ireland. Occasional Paper No. 22 The Nomenclature of the Hominidae, including a Definitive List of Named Hominid Taxa By Bernard G. Campbell. Pp. v+34. (London: The Royal Anthropological Institute of Great Britain and Ireland, 1965.) 10*s.* [812

Ministry of Power. Safety in Mines Research 1964: Forty-third Annual Report of the Safety in Mines Research Establishment. Pp. 79+7 plates. (London: H.M. Stationery Office, 1965) 7*s.* 6*d.* net. [812

Two Essays on Humanity. By K. W. Monserrat. 1: Man's Thoughts. 2 Man's Health. Pp. 19. (Liverpool: Henry Young and Sons, 1965.) [812

The Empire Cotton Growing Corporation. Progress Reports from Experiment Stations. Seasons 1961–62 and 1962–63. Kenya. By M S. Hastie. Pp. 24. 2*s.* 6*d.* Season 1963–64. Kenya. By M. S. Hastie. Pp. 20. 2*s.* 6*d.* Tanzania—Western Cotton Growing Area. Pp. 26. 2*s.* 6*d* Survey of Reports. By H. E. King. Pp. 10. (London. Empire Cotton Growing Corporation, 1965.) [812

Maps and Metabolism· a Guide to Metabolic Pathways. By Dr. D. E. Nicholson. Fourth edition. Pp 17. 2*s.* 6*d.* Metabolic Pathways Chart. 1*s.* 3*d.* (Colnbrook. Koch-Light Laboratories, Ltd., 1965.) [812

Agricultural Research Council. Recommended Procedures for use in the Measurement of Beef Cattle and Carcasses. Pp 18 (London: Agricultural Research Council, 1965. Obtainable from H.M. Stationery Office.) 3*s.* 6*d.* [812

Other Countries

The Future of Nuclear Power. By Sir John Cockcroft. (The John Murtagh Macrossan Lectures, 1964.) Pp. 15. (St. Lucia, Queensland: University of Queensland Press, 1965.) 4*s.* 6d. [2911

World Health Organization. Technical Report Series, No 313 The Biochemistry and Microbiology of the Female and Male Genital Tracts—Report of a WHO Scientific Group. Pp. 14 (Geneva: World Health Organization; London: H.M. Stationery Office, 1965.) 2 Sw. francs; 3*s.* 6*d*; 0.60 dollars. [2911

Proceedings of the California Academy of Sciences. Fourth Series, Vol. 30, No. 13 (October 29, 1965): Observations on the Type Specimen of the Scorpion *Syntropis macrura* Kraepelin. By Herbert L. Stahnke. Pp. 257–263. (San Francisco: California Academy of Sciences, 1965) [2911

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 26. Instrumented Telemetering Deep Sea Buoys. By H. W. Straub, J. M. Arthaber, A. L. Copeland and D. T. Theodore. Pp. 25. (Washington, D.C.. Government Printing Office, 1965.) 25 cents. [2911

Hebrew University of Jerusalem. Geomorphological Map of the Judean Desert, Scale 1 : 100,000, with Explanatory Notes. By Dov Nir. A New Application of Hypsographic Colours in Map Reproduction. By D. H. K. Amiran and Miriam Karmon. (Scripta Hierosolymitana, Vol. 15.) Pp. 36+11 plates+map. (Jerusalem: At the Magnes Press, The Hebrew University; London: Oxford University Press, 1965.) 18*s.* net. [2911

Food and Agriculture Organization of the United Nations. FAO Agricultural Studies, No. 66: Joint FAO/WHO Expert Committee on Brucellosis—Fourth Report. Pp 65. (Rome· Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1964.) [2911

Pubblicazioni della Università degli Studi de Firenze, Facolta di Scienze Matematiche, Fisiche e Naturali Osservazioni e Memoria dell' Osservatorio Astrofisico di Arcetri. Fascicolo 80: Osservazioni Eseguite dell' Osservatorio Astrofisico di Arcetro Durante l'Anno 1964. Pp. 65. (Firenze: Università Degli Studi di Firenze, 1965.) [2911

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wheat Research Unit, 1964–1965. Pp. 14. (Ryde, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1965) [2911

Commonwealth of Australia. Thirteenth Annual Report of the Australian Atomic Energy Commission for the year ended 30th June, 1965. Pp. 100. (Coogee, N.S.W : Australian Atomic Energy Commission, 1965.) [2911

Publikationer fra Det Danske Meteorologiske Institut Arbøger. Isforholdene i de Grønlandske Farvande, 1961. (The Ice-Conditions in the Greenland Waters, 1961.) Pp. 35+63 ice-charts (Charlottenlund: Det Danske Meteorologiske Institut, 1965) [2911

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo–Lunda–Angola. Museu do Dundo. Subsidios para o Estudo da Biologia na Lunda. Publicacoes Culturais, No. 69: Estudos Diversos (28). Pp. 138. (Lisboa Compania de Diamantes de Angola, 1965.) [2911

The Exploration of the Moon. By I. L. Thomsen. (Thomas Cawthron Memorial Lecture, No. 39.) Pp. 17. (Nelson, New Zealand: Cawthron Institute, 1965.) [2911

Transactions of the Royal Society of New Zealand. Botany. Vol. 3, No. 5 (16 September 1965): The Liverwort Genus *Asterella* (Marchantiales) in New Zealand. By Ella O. Campbell. Pp. 99–110. Geology Vol. 3, No. 7 (July 21, 1965): New Species of Brachiopoda from the Torlesse Group of Kaiwara Valley, North Canterbury. By J D. Campbell. Pp. 95–97+1 plate. Vol. 3, No. 8 (July 21, 1965). Fossil Localities of the Torlesse Group in the South Island. By J. D. Campbell and G. Warren. Pp. 99–137+5 plates. Vol. 3, No. 9 (August 6, 1965): Lower Pleistocene Mollusca from Devil's Elbow, Hawke's Bay. By A. G. Beu. Pp. 139–149+2 plates. Vol. 3, No. 10 (August 6, 1965): Capricorn Seamount, South-West Pacific Ocean. By J. W. Brodie. Pp. 151–158+2 plates. Vol. 3, No. 12 (16 September, 1965). The Permian Spiriferoid Genus *Ambikella* Sahni and Srivastava (1956) and Its Relationship to *Ingelarella* Campbell (1959) and *Martiniopsis* Waagen (1883). By J. B. Waterhouse. Pp. 159–170. (Wellington: The Royal Society of New Zealand, 1965.) [812

Transactions of the Royal Society of New Zealand. General. Vol. 1, No. 11 (August 6, 1965): Genetics in Biological Teaching and Research. By J. B. Hair. Pp. 127–138. Vol. 1, No. 12 (August 6, 1965): Why Science? Why Poetry? By Sir Gilbert Archey. Pp. 139–142. Vol. 1, No. 13 (August 6, 1965): Rutherford in 19th Century Cambridge. By Sir George Thomson. (The Rutherford Lecture given in New Zealand in 1964 at Christchurch and Auckland.) Pp. 143–151. Vol. 1, No. 14 (August 6, 1965): Exploration of the Moon. By W. H. Pickering. Pp. 153–160+15 plates. Vol. 1, No. 15 (16 September, 1965). The Rise of the Concept of a Physical Model. By D. F. Lawden. Pp. 161–174. Vol. 1, No. 16 (16 September, 1965): Conscious Experience and the Human Brain. By J. C. Eccles. Pp. 175–182. (Wellington: The Royal Society of New Zealand, 1965.) [812

Transactions of the Royal Society of New Zealand. Zoology. Vol. 6, No. 12 (August 6, 1965): The Adaptation and Increase in the Paradise Shelduck (*Tadorna variegata*) Within a Man-Modified Environment. By H. J. F. McAllum. Pp. 115–125. Vol. 7, No. 1 (August 6, 1965): Siphonaptera of New Zealand. By F. G. A. M. Smit. Pp. 1–50. Vol. 7, No. 2 (July 21, 1965): Descriptive and Taxonomic Notes on *Grahamichthys radiatus* (Valenciennes), Eleotridae. By R. M. McDowall. Pp. 51–56. Vol. 7, No. 3 (August 6, 1965): Muraenid Leptocephali in Australasian Waters. By P. H. J. Castle. Pp. 57–84. Vol. 7, No. 4 (16 September, 1965): A New Species of *Paromola* (Crustacea, Decapoda, Thelxiopidae) from New Zealand: By D. J. G. Griffin Pp. 85–91+2 plates. Vol. 7, No. 5 (16 September, 1965): Ecological Variation of *Chlamys dieffenbachi* (Reeve) (Mollusca, Lamellibranchiata). By A. G. Beu. Pp. 93–96+1 plate. Vol. 7, No. 6 (16 September, 1965): Ophichthid Leptocephali in Australasian Waters. By P. H. J. Castle. Pp. 97–123. Vol. 7, No. 7 (16 September, 1965): Moringuid Leptocephali in Australasian Waters. By P. H. J. Castle. Pp. 125–133. Vol. 7, No. 8 (16 September, 1965): Revision of the Rhaphidophoridae (Orthoptera) of New Zealand. Part 12: A New Species of *Pallidoplectron* Richards. Pp. 135–139 (Wellington: Royal Society of New Zealand, 1965) [812

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada Memoir 334· Geology of the Rocky Mountain Foothills, Alberta (between latitudes 53° 15′ and 54° 15′). By E. J. W. Irish. Pp. 241 (7 plates). 4.50 dollars. Paper 64–49: Construction and Calibration of an Apparatus for the Measurement of Surface Areas of Powdered Samples. By R. A. Washington. Pp. v+13. 75 cents. Paper 65–26: K–Ar Age of Igneous Pebbles in the McDougall-Segue Conglomerate, Southeastern Canadian Cordillera. By D. K. Norris, R. D Stevens and R. K. Wanless. Pp. v+11 (1 plate). 75 cents. Paper 65–19. The Mineral Industry of Yukon Territory and Southwestern District of Mackenzie 1964. By L. H. Green. Pp. v+94. 75 cents. (Ottawa: Queen's Printer, 1965.) [812

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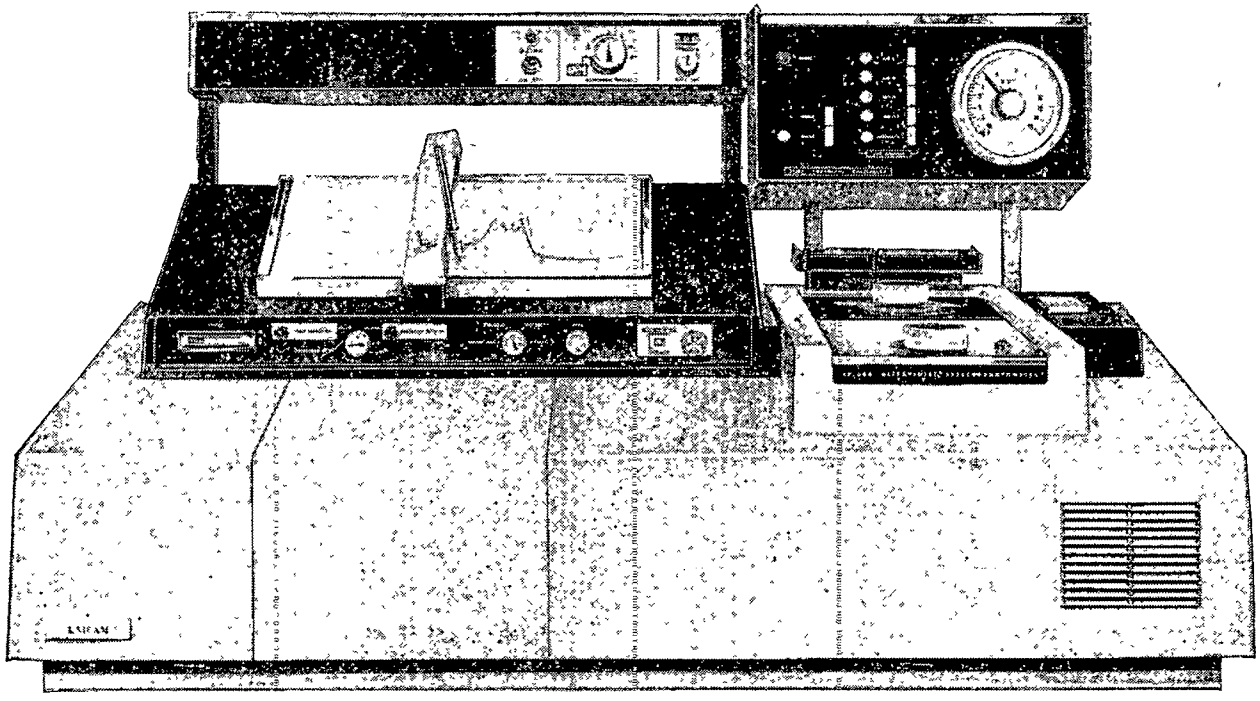
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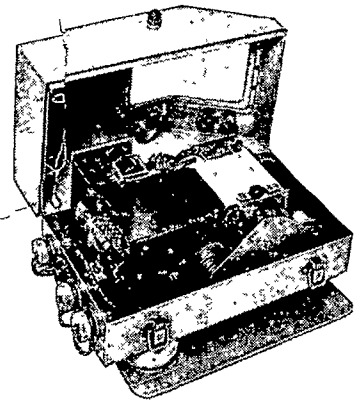
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ELECTRICAL AND ELECTRONIC EQUIPMENT FOR THE SERVICES

THE seventh report from the Estimates Committee for the session 1964–65*, dealing with electrical and electronic equipment, is of far wider scientific interest than its formal subject might suggest. The Sub-Committee which examined the Defence Estimates and relevant sections of the Civil Estimates, more particularly the Votes for the Ministry of Aviation, was told by one witness that electronics was a technique, a modern technology in the same sense as hydraulics or mechanics. Moreover, while equipment such as radar sets can be clearly defined as electronics, other equipment which may embody electronic techniques cannot be regarded solely as electronic. It was difficult for the Sub-Committee to determine with any accuracy how much was spent by or for the Services on electrical and electronic equipment; furthermore, much of the expenditure on research and development, borne largely by the Ministry of Aviation, was regarded as classified and could not be published.

In encountering this problem of security the Estimates Committee touches on one of the aspects of its enquiry that is of major scientific interest. There is indeed nothing new about the practical difficulties of security which confronted the Committee, and its approach is eminently sensible and reasonable. In its fourth report, for the session 1946–47, the Select Committee on Estimates noted the reluctance of departments to supply information on defence matters, and the present Committee recognizes that there has since been some improvement. It also recognizes that, from the point of view of security, electrical and electronic equipment is a highly sensitive subject: it would be wrong to seek to disclose, for example, details of research in progress at the research establishments of the Ministry of Aviation. The Committee did not find it easy to determine in all cases what was or was not secret or confidential, and respect for or deference to the views of the department concerned has necessarily restrained some of the Committee's comments. Inevitably, this has to some extent diminished the impact of those comments.

The Committee itself is not entirely satisfied with this situation and is far from convinced of the necessity for secrecy on, for example, levels of stockholding or siting of equipment. Furthermore, a department was, on occasion, forced to admit the absence of any real reason for its request that certain evidence should not be published. The enquiry also brought to light the existence of clear inconsistencies between the defence departments.

The Committee was not, however, so much concerned about the problems caused by such unnecessary security. Its real anxiety arises from the danger to national security which this practice presents. As the Estimates Committee rightly emphasizes, regular classification of information which can safely be disclosed causes the system to become meaningless and brings it into disregard. As a result, information of a really secret nature ceases to have adequate protection. The importance of this point was made in the Radcliffe Report on Security Procedures in the Public Service and more recently, by implication, in the report of the Standing Security Commission.

The Estimates Committee is content to leave the question of security procedures with the remark that a realistic approach to security classification in the defence departments and the Ministry of Aviation is essential for proper control of really secret information. Too often there is a general tendency for departments to classify certain information and to refuse to disclose it, not because disclosure would prejudice national security, but because it might involve departmental inconvenience. The Committee might have added that security depends not so much on an appropriate mechanical system as on intelligence and continued hard critical thinking which, as already noted, restricts the classified material to the essential minimum.

Scientists and technologists, who are well aware of the vital need for informed public discussion of so many of the issues raised by the invention and development of scientific equipment, should be grateful to the Estimates Committee for the clarity and firmness with which this vital issue is presented. The view of the Committee that it is entitled to as much information as possible, consistent with national security, is reasonable; the request is made, moreover, in a context which indicates full awareness that this information will form the basis of public discussion. In the field of electronics, informed discussion in Britain has been handicapped by information which is inadequate when compared, for example, with that given to the Congress of the United States. As a Parthian shot, the Committee points out that, with rapidly evolving techniques, a piece of electronic equipment may be highly secret one year and almost obsolete the next. The first of the Committee's twenty-one recommendations is that a thorough-going review should be undertaken of the security classification of electrical and electronic equipment for the Services, including the desirability of regular re-classification after a lapse of time. This recommendation calls for immediate implementation by any Government professing to be concerned with economic efficiency as well as security. It is no party issue and the Parliamentary and Scientific Committee might well press the matter firmly.

The next issue raised in this report of prime concern to the scientist and technologist is that of research. This is considered at some length in the section of the report which deals with the system for obtaining equipment, and behind the Committee's approach is a concern with the failure of present procedures to overcome the difficulties caused by constantly increasing costs which may be one cause of delay in bringing a project to fruition. The history and cost of any project begin with research, and for electrical and electronic equipment the cost and responsibility for research are borne almost entirely by the Ministry of Aviation. The function of the research programme of the Ministry is briefly described, in a memorandum submitted in evidence, as consisting of research in support of projects to meet operational requirements and general research with the object of increasing the Ministry's knowledge and authority to

* Seventh Report from the Estimates Committee, together with part of the Minutes of the Evidence taken before Sub-Committee C, Appendixes and Index, Session 1964–65. *Electrical and Electronic Equipment for the Services*. Pp. liii+275. (London: H.M.S.O., 1965) 27*s*. 6*d*. net.

assist in formulating future requirements and in supervising development work carried out by industry.

The research work is undertaken largely within the Ministry's own establishments, although contracts are placed with industry for the investigation of particular aspects. The establishments concerned with electronic research and development are the Royal Radar Establishment at Malvern, the Signals Research and Development Establishment at Christchurch and the Radio Department at the Royal Aircraft Establishment, Farnborough. The Estimates Committee was concerned mainly with the direction of this effort, and its division between industry and these establishments of the Ministry of Aviation. It admits that secrecy considerations hindered the discussion of specific questions, since neither the nature nor the cost of certain research could be disclosed.

The Ministry's research and development programme is controlled within the department by the Research and Development Board, of which the Permanent Secretary is Chairman and on which the Central Department of the Ministry of Defence is represented, while representatives of the Service Departments attend as necessary. Other members of the board are the Chief Scientist, the Deputy Secretaries, the Controller of Aircraft and the Controller of Guided Weapons and Electronics. The two Controllers are executively responsible for the research, development and production programmes, while the Chief Scientist, advised by the Directors-General of Research and Development and the various Directors of Research, is responsible for co-ordinating the broad research programme of the Ministry as a whole. There is close day-to-day liaison between the Ministry of Defence Departments and the Ministry of Aviation in formulating requirements, and this close association continues throughout the development programmes. Altogether, nearly eleven hundred professional and technical staff are engaged at the headquarters and at other establishments of the Ministry in supervising aimed research, development and production of electrical and electronic equipment.

Each Director-General reviews his technical area at half-yearly intervals, the Chief Scientist dealing with all his establishments on an annual basis. There is also the Electronics Research Council, which includes eminent scientists in the field of electronics, as well as representatives from the Ministry of Aviation and the Ministry of Technology. Formally, the Electronics Research Council reports to the Minister of Aviation, but in practice its work is seen essentially as feeding in new ideas for research.

Expenditure on research in the establishments of the Ministry of Aviation increased during 1964–65 and is greater than that on research contracts with industry, although representatives of the Electronic Engineering Association suggested in evidence that industry, from its own funds, was spending between £3 million and £4 million a year on research. This sum is distinct from that expended by the Ministry on research contracts with industry, which is described as an "adjunct of the research programme of the establishment which sponsors the contracts". Expenditure on research contracts with industry has not varied greatly in recent years, although it was stated in evidence to the Committee that it was the policy of the Ministry of Aviation to increase the amount of research undertaken in industry and that this was being carried out.

The Estimates Committee recognizes the importance of ensuring that there is no wasteful overlapping in the research carried out by the Ministry's establishments and by industry, and that the Ministry is fully aware of what is happening in industry, not only with regard to research contracts sponsored by the Ministry but also in the general programme of industrial research. Some duplication of effort between individual firms may be desirable: a witness from the Electronic Engineering Association thought that some duplication of research before a problem was solved might be beneficial because, on occasion, one of several teams might arrive at a solution which would be missed by another team. Duplication of effort between the Ministry of Aviation and industry in the field of specific research contracts is another matter, but neither the Ministry nor the industry considered that such undesirable duplication existed. The flow of information from the research establishments to industry was regarded as good and the research establishments of the Ministry were said to have a remarkably wide knowledge of what was going on in industry. Rightly, the Estimates Committee insists that this awareness must continue to be maintained and it regards a regular series of progress reports from all electronics firms, given in close confidence, to be an essential part of the research planning of the Ministry of Aviation.

The extent to which Government-sponsored research should be undertaken by industry is a difficult question. The Electronic Engineering Association accepted that some research must be carried out in Government establishments but, at the same time, thought that industry should continue to conduct a fair proportion of research, particularly in materials and where the techniques, the technology and the knowledge lay close to the production process and the knowledge of industry. Here industry is generally better equipped to visualize the commercial prospects arising from particular lines of research and to exploit them. On the other hand, in general research it is essential for Government establishments to possess systems experts who are familiar with all the broad complex of new equipment and its techniques, and who are competent to consider how such equipment can be brought together into a coherent whole. Nor, again, can the Ministry of Aviation's establishments supervise the technical work on development of equipment by industry unless they possess the knowledge and authority derived from participation in such research.

The Estimates Committee recognizes the force of these arguments but, without lessening the control of expenditure exercised by the Ministry of Aviation, it suggests that the number of individual research contracts given to industry might be increased with advantage. It is also possible that research in a given field may enable a firm, which in any event may have to carry out the development programme for the project, to increase its proficiency and thus lessen the eventual cost of development. The second recommendation of the Committee is, accordingly, that the Ministry of Aviation should review the programme for individual research projects with the view of increasing the proportion of contracts placed with industry.

The most crucial phase in the procurement of equipment is development, and the next section of the report deals in some detail with the three main phases towards full development. Before considering recommendations of the Committee here, it may be as well to note some questions regarding organization on which, for all their restraint, it was apparent that the Estimates Committee had some reservations, if not misgivings. Its enquiry into the organization of research failed, for example, to elucidate the position or function of the Chief Scientific Adviser. It would seem from the Minutes of Evidence that the

relations of the Ministry of Aviation and the Ministry of Defence may well have prevented him from conducting just such an enquiry as the Estimates Committee was led to make.

The whole picture of ministerial relations which the report presents is one of confusion. This extends to the Ministry of Technology also and to the Ministry of Education and Science, embracing besides the Electronics Research Council, the Science Research Council and the Advisory Council for Technology. There is evidence of a lack of clear thinking on organization which almost certainly must handicap effective management and control, and not even the Estimates Committee could withhold comment as to the nebulous function of the Ministry of Technology as the sponsor of the electronics industry. It points out, indeed, that the Treasury and the Stationery Office are also involved with computers but it maintains firmly that removal of this function of sponsorship from the Ministry of Aviation enlarges the gap between military and civil uses of electronics. This is the opposite of what is desirable, and such weighty criticisms will scarcely strengthen confidence in the wisdom of extending the sponsorship responsibilities of the Ministry of Technology to the electrical industry as a whole.

The Estimates Committee was convinced that the present division of departmental responsibility does not lend itself to the bold decisions which are necessary. The Committee felt that extensive reorganization of the structure of the departments is essential if they are to cope with the ever-increasing complexities of one of the most rapidly evolving technologies. The structure of Britain's research effort in civil science cannot yet be regarded as settled, perhaps even less its relation with Britain's effort in defence research. The most effective means of control and of efficient use of trained manpower has still to be established, and in the light of this enquiry the value of the changes made in the past two years is still unproven.

In this context it should not escape notice that in the December issue of *State Service* the Institution of Professional Civil Servants makes strong representations to the Ministry of Technology that, unless urgent consideration is given to an alternative role in the civilian field, resources and staff at Aldermaston are in danger of being frittered away. In this article, Mr. J. Lyons advances the idea that Aldermaston might become a major regional laboratory for engineering technology, basing the proposal on Aldermaston's ability to undertake prototype work for industry, specializing particularly in metallurgical, mechanical, electronic and computer applications. It could undertake contract work which industry is not equipped to handle and in this way Aldermaston could be developed into a centre available not only to industry but also to universities, colleges of technology and Government departments.

Mr. Lyons presents a reasoned argument, and the way in which the Minister of Technology handles this problem of an alternative role for Aldermaston may well be a touchstone of his own competence. Neither the Ministry of Defence nor the Ministry of Technology is shown in a good light by the Estimates Committee's examination of development in the present report, although the Committee recognizes that the adopted procedures do, in fact, follow the ideas laid down in the Zuckerman Report on the Management and Control of Research and Development. The main object of the Zuckerman Report was to emphasize the importance of the project investigation as a basis of decision whether or not to proceed to full development. The Committee admits that these procedures have inherent difficulties in the correct estimation of cost and in the time factor involved, speed being essential if rising costs are to be avoided.

The Electronic Engineering Association suggested that the Zuckerman procedures might be accelerated by more research in techniques in advance of a project programme, by close co-operation between the Ministry and industry, and by an effort, during the feasibility study or project study, to resolve the major points towards the end of the time allotted for the study. The Ministry of Aviation accepted the first suggestion, with a minor qualification, and the Estimates Committee believes that their recommendation for increasing the number of research contracts in industry will also help. In spite of reservations by the Ministry of Aviation and the Ministry of Defence, the Estimates Committee was unconvinced that the decisions could not begin to be taken at a rather earlier stage, as proposed in the third suggestion. Accordingly, it recommends that, wherever possible, interim reports of feasibility studies and project studies should be made so as to facilitate preliminary decisions whether to proceed to the next phase.

The Estimates Committee was not entirely satisfied with the amount of control exercised by the Ministry of Aviation over the development contract. This is of the highest importance not only because of the expense involved in development and the need to ascertain costs accurately as soon as possible but also because of the very close link between development costs and costs of production. The Committee recommends, therefore, that the Ministry of Aviation should, in consultation with the Treasury, ensure that the Contracts Division is so staffed that a technical cost estimator can be attached to and remain with each major contract throughout its development and production phases. It also recommends that the Ministry should seriously consider the further reorganization of the staffing of this Division.

These are probably the major recommendations of the Estimates Committee, as they are those of the greatest scientific and technical interest, though the principle of the research and development levy, fixed as a percentage of the selling price on any goods sold to third parties, is questioned, and a review recommended in relation to the exports of electrical and electronic equipment. For the rest, the Estimates Committee's report touches on production and inspection, reviews the system in practice and the control and handling of electrical and electronic equipment in service, more especially the use of automation in storekeeping and the future programme for computers for the Services. It touches on the difficult problem of the interdependence of the purchase of foreign equipment and exports and most of its recommendations relate to these matters.

Nevertheless, the main value and interest of this report from the Estimates Committee lies first in its searching comments on secrecy and security procedures, and secondly in the questions which it raises regarding the organization of research and its relation to development. It is here that the critical issues lie: here also are tested the competence as well as the organization of departments. What stands out above all is the need for some clear and unprejudiced thinking on all these issues, for constructive criticism as well as open-mindedness, for a readiness to reconsider in fact whether the independent existence of particular Ministries like those of Technology and of Aviation is really justified, and whether the functions of these Ministries would not be more appropriately redistributed in the Ministry of Defence and elsewhere.

RECENT METALLURGICAL ADVANCES

The Theory of Transformations in Metals and Alloys

An Advanced Textbook in Physical Metallurgy. By J. W. Christian. (International Series of Monographs in Metal Physics and Physical Metallurgy, Vol. 7.) Pp. xvi+973. (London and New York: Pergamon Press, 1965.) 190*s*. net.

Lattice Defects in Quenched Metals

Edited by R. M. J. Cotterill, M. Doyama, J. J. Jackson and M. Meshii. (An International Conference held at the Argonne National Laboratory, June 15–17, 1964.) Pp. xxii+807. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 176*s*.

Proceedings of the Joint International Conference on Creep

New York, 25–29th August 1963; London, 30th September–4th October 1963. (Sponsored by the American Society of Mechanical Engineers; the American Society for Testing and Materials; and the Institution of Mechanical Engineers.) Pp. xviii+972. (London: The Institution of Mechanical Engineers, 1965.) 160*s*.

DR. J. W. CHRISTIAN has produced a magnificent work of scholarship which must have occupied many years of investigation. *The Theory of Transformations in Metals and Alloys* is at once, at an advanced level, both a text-book for those interested in transformations, and a reference book for a wide range of research workers. The approach is totally different from those of past authors. Classification and definition of transformations occupy a chapter, since the author includes many solid-state changes and not only phase reactions. His preface claims, rightly, to include "every major kinetic effect in physical metallurgy and solid state physics, with the notable exception of plastic deformation by slip". Twinning is included, and lattice defects, since they are involved in martensitic transformations and diffusion.

The discussion is exceptionally well done in the standard text-book topics of lattice geometry, point defects, boundaries, and diffusion. The author has used vector analysis and matrix algebra, particularly the latter, but has also illustrated the main points to give a corresponding physical picture whenever possible. As the author suggests, undergraduates in honours schools should be capable of coping with the text, but graduate students are likely to be the main readers.

To take one example from this book, the classical theories of nucleation and growth processes are followed by a chapter on transformation kinetics, and a gentle follow through to growth of crystals from the vapour phase and on to solidification and melting. This section forms perhaps the most complete treatment of this particular subject ever to appear. References up to 1962 are included, with one or two up to 1964 added in proof. Where little experimental work has been carried out on a particular problem, the author wastes no time on pure supposition, as for example in dealing with the peritectic reaction. Where the reverse applies, as for Guinier–Preston zones in aluminium–copper alloys, a full treatment is given.

Many readers may well consider the book should have been split into two volumes, partly for content, and partly for ease of handling. Already my colleagues are devouring it.

The other two volumes constitute the formal records of international conferences, suitably edited. In the case of *Lattice Defects in Quenched Metals*, the topics centred around the use of a now well-established technique (the increase in lattice defect concentration by quenching from a high temperature) to explore numerous aspects in both metals and alloys. As with any similar technique, where advantage is taken of metastability to explore a material not in equilibrium under the conditions of experiment, the value of the results lies in what can be interpreted by interpolation or extrapolation to different conditions. The papers presented in this volume achieve this end with varying degrees of success, but consolidate the technique as a means of exploring migration and binding energies, distinction between types of defect, and the role of impurity atoms.

Experimental methods have largely overcome the most serious drawbacks, namely quenching strains and contamination of the specimen surface. The majority utilize electrical heating with immersion in liquid nitrogen or helium, quenching resulting from interruption of the heating current. Electrical resistivity remains the most favoured property for investigation of quenched-in defects, with density and stored energy close behind. Perhaps the most rapidly advancing techniques are those of internal friction and transmission electron microscopy of thin foils. Low angle scattering of X-rays is invaluable in work on agglomeration of vacancies or dislocation loops, but presents problems with certain materials. Magnetic measurements, particularly with ferromagnetics, are of value in cold worked and irradiated metals, but less so after quenching.

The majority of papers recorded in this volume dealt with complex vacancy arrangements, that is, clusters associated with dislocations and stacking faults. The simpler forms, with more than two but less than one thousand vacancies involved, are less understood, particularly in their crystallographic character. Body-centred cubic metals were examined in two of the papers but the majority of attention, as usual, revolved around face-centred structures, since these are more amenable to this type of treatment. This volume is a valuable stepping stone to those workers concerned with lattice defects, but will probably be out of date in a few years' time.

The joint international conference on "Creep" attracted three hundred delegates from twenty countries when held in New York, and four hundred delegates from twenty-two countries when repeated in London. Three prestige lectures, two sponsored by the two American institutes (with British speakers) and one sponsored by the Institution of Mechanical Engineers (with an American lecturer), were included in the *Proceedings*.

The subject of creep is of tremendous practical significance in view of the rapid technological advances of the space age, and therefore attracts a wealth of more fundamental work into the background science. This conference covered a very wide range of topics, from the relationships between metallurgical structure and deformation, through plasticity theory, thermal cycling effects, and design data, to the correlation of service data with laboratory tests and the effects of environment. Steels featured prominently as the most common material for this type of service, particularly in power generating stations, but magnesium, aluminium, and nickel-based alloys appeared in the more basic science papers.

Such a variety of materials, methods of approach, and objectives, whether basic or technological, creates a mess of mental pottage from which any attempt to extract a reasonably lucid précis would be both meaningless and impossible. The outstanding differences between the fundamental work and the service problems are such that intermediaries become necessary, to bridge the gap which alternately widens and narrows with succeeding conferences of this kind. Such organizations as the Central Electricity Generating Board and the Atomic Energy Authority in Britain, and the Atomic Energy Commission in the United States, must, and do, provide such liaison. The interpretation of creep parameters in terms of activation energies and dislocation mechanisms requires careful experimentation with near perfect specimens, in order to achieve precision. The use of bulk quantities of steels in power generating equipment involves economic stringency, preventing any close approach to perfection of chemical composition, metallurgical structure or homo-

geneity, quite apart from strict controls or evaluation of stress and temperature. The only parameter which can be measured with the same degree of certainty on both sides is time.

It would not be unfair to say that nothing new came out of this conference in the scientific sense, but in the correlation of creep data with service life, a considerable step forward must result from the additional evidence supplied here.

C. R. TOTTLE

ROCK MAGNETISM TO-DAY

Rock Magnetism

By Prof. Takesi Nagata. Revised edition. Pp. viii + 350. (Tokyo: Maruzen Company, Ltd.; New York: Plenum Press, 1961.) 9.50 dollars.

ROCK magnetism is that branch of geophysics that deals with the origin of magnetization in rocks and its stability. Workers in rock magnetism are also interested in the phenomenon of self-reversal, that is, a rock acquiring a magnetic moment exactly opposed to the ambient magnetic field. It is necessary to point out that almost all the minerals that cause rocks to be magnetic are also electric insulators and often of very high coercivity. This makes them of interest to solid-state physicists as possible permanent magnet materials. It was the work on rock magnetism that first drew Néel's attention to the possible existence of spontaneous reversal of magnetic moments in ferrites. Again, it was the work on the highly unstable red rocks containing haematite (α-Fe_2O_3) that led Néel to the idea of 'Superantiferromagnetism'. In spite of these occasions, when rock magnetism has been responsible for fundamental advances in pure magnetism, it has mostly lagged behind the advancing front of our knowledge of the magnetism of solids. An example of this can be found in the statement that if, when dropped or struck, bar magnets can lose their magnetization, how can rocks retain their remanent magnetization over geological time-scales? To pose this question is to display ignorance of the concept of magnetocrystalline and shape anisotropy in solid-state physics. *Rock Magnetism* is admirable in this and other respects.

This new edition includes references to almost all the new theories and discoveries in the field of magnetism of solids. Even though these references must, of necessity, be brief, they are there to guide the interested and enthusiastic reader. It appears that, compared with the previous edition, the present one has been increased in volume by only a third. This, however, is not the case. There are twelve chapters now instead of the six in the previous edition. Also, some old chapters have doubled or more than trebled in size. This is the case for the chapter on "Outline of Ferromagnetism" and that on "The Natural Remanent Magnetism of Sedimentary Rocks". This is a direct result of the increased application of the new techniques of solid-state physics to rock magnetism, and this trend will continue. Because it is such a fruitful and controversial field of research, many will query some statements made in Chapter 6 on chemical remanent magnetization. The phrase 'chemical remanence' is in itself a misnomer, because, as has been shown by the excellent work done in Prof. Nagata's own laboratory, the new remanence in minerals undergoing a chemical change is produced not as a direct result of the chemical action or ionic migration but due to the growth of new crystallites through critical sizes. To describe it as 'crystallization remanence' would be more appropriate. On page 213 it is claimed that "there is no magnetite in any normal red sandstone". On the contrary, the very extensive work of Van Houten on the sedimentary petrology of these rocks shows the unambiguous presence of magnetite in red beds. Van Houten also shows a curious correlation between the Triassic and post-Triassic rocks, and the abundance of magnetite.

For the benefit of the uninitiated reader it must be mentioned that it can no longer 'safely' be said that all haematite in red beds "has been produced *in situ* by post depositional chemical changes". Similar criticisms also apply to misdirected use of the word 'titanomaghemite'. If this is to be taken to mean, by analogy with titanohaematites, maghemite containing variable amounts of titanium, the titanium reducing the ferric ions but leaving the vacancies alone, it is one thing. But if it is also claimed that titanium fills the vacancies and stabilizes the metastable maghemite, the end-member of such a solid solution of titanomaghemites is a titanomagnetite! If expressed as a solid solution (x Fe_2TiO_4 $(1-x)$ Fe_3O_4) the previously mentioned end-member of the titanomaghemite series will have $x = 0{\cdot}33$. There are also a few minor errors and omissions. On page 64, electromagnets are said to be used to produce strong alternating magnetic fields, and on page 272, Stacey's main conclusion from compression experiments is omitted. This was that as long as the rock has no pronounced anisotropy to begin with, thermoremanence cannot be deflected by pressure applied during geological time.

Such criticisms apart, the volume is sure to prove a valuable source-book of information for geophysicists, geologists and physicists in that order. There are two very satisfactory chapters which deal with the application of rock magnetism to geology, pure and applied. The final chapter, on the history of rock magnetism, is bound to be of great help to future historians of science. In short, this book brings rock magnetism up from the level of a 'stamp collecting' science to the present day. It is up to the geophysicists and physicists to-day to proceed further, to a complete 'take-over' by solid-state physics of rock magnetism. This, indeed, would be an admirable thing.

SUBIR K. BANERJEE

PHYSICAL GEOGRAPHY OF THE OCEANS

The Physical Geography of the Oceans

By Charles H. Cotter. Pp. 317. (London: Hollis and Carter, 1965.) 35*s*. net.

THE publication of *The Physical Geography of the Oceans* is a welcome sign of the increasing awareness of oceanography as an important branch of study, from many points of view. It aims at providing a course in the physical geography of the oceans for students in the sixth forms of schools or in the first year at university. A good deal of basic physical oceanography is covered as well as a more detailed treatment of some aspects of particular interest to the geographer.

The first chapter starts off by taking a global view, describing the distribution of oceans and continents, and going on to give a general account of the bottom topography of the oceans. This is done clearly and adequately for the most part, but it is rather surprising to see that the chart given of the Arctic Basin shows no sign of the Lomonosov Ridge, which divides this region into two distinct sub-basins. The origin of the continents and ocean basins is treated in the next chapter, which leads on from a historical approach to more recent ideas on this subject. There are several points, however, in which the treatment has not been brought up to date. One diagram, for example, shows the earth's crust as being thicker under the ocean than under the continent, contrary to the view now accepted. The boundary regions of the oceans: the coastlines and shores, islands and the ocean bed, including its deposits, are dealt with in several chapters. A separate chapter is devoted to coral reefs and islands, the origin of which provided one of the famous scientific disputes of the nineteenth century and which is a subject of renewed interest to-day.

The weather and climate over the oceans are treated thoroughly, bringing out the interaction between the oceans and the atmosphere and its great importance to both. Ocean currents are treated quite fully, beginning with an account of the physical principles which influence currents of various types and going on to a description of the general circulation of the oceans. This is well done and includes references to some recent discoveries, such as the Cromwell Current in the equatorial Pacific. The chapter on tides, on the other hand, is the least satisfactory in the book. The concept of the equilibrium tide is wrongly defined and the misleading statement is made that the equilibrium tide is so close to the actual tide that tidal predictions are still largely based on the equilibrium theory. It is sad to see that old chestnut, Whewell's 1833 cotidal chart, reproduced as if it had any relevance to modern views of tidal oscillations in the Atlantic. There is a chapter on instruments used in oceanography and the book ends with a rapid historical survey, from Captain Cook to the International Geophysical Year. A few mis-statements occur in various places throughout the book, which may mislead the unwary. Thus the sperm whale is not one of the whalebone whales and it is surprising to see it implied that nitrogen is needed for the formation of carbohydrates. It also seems a mistake to say that metallic sodium is extracted from the sea on a commercial scale. These are minor criticisms, however, of a book which should succeed in interesting many of the younger generation in the scientific study of the seas.

K. F. Bowden

MEDICINE FOR ALL

Medicine in Modern Society

Medical Planning Based on Evaluation of Medical Achievement. By Prof. Thomas McKeown. Pp. 234. (London: George Allen and Unwin, Ltd., 1965.) 35*s*. net.

So much of modern medicine has grown up in a fragmentary and empirical manner that it is more than refreshing to find a book where the concern is only to promote a total medical service which will most efficiently satisfy the needs of society as a whole. To evaluate the existing overall position and to lay down guide-lines for the future, Prof. McKeown examines, in *Medicine and Modern Society*, the way in which our medical services have developed over the past three hundred years and the contributions they have made to the improvement of health. The main influences responsible for the advance of health have undoubtedly been the rising standard of living since 1770, associated with the industrial revolution, as well as the control of the physical environment (water, sewage and housing) from about the 1870's. These influences have been accelerated in the present century and have been assisted by specific medical measures applied to the individual, most of them arising from control of infectious diseases and from the discovery of new forms of chemotherapy. Except as a means of spreading knowledge, the contribution of the medical practitioner to improvement of health has been relatively slight; his main concern has been with the alleviation of pain and suffering.

To-day, the predominant medical issues which face technically advanced countries like Britain are those determined at the time of birth (abortions, foetal and early post-natal deaths, congenital malformations and disabilities), mental illness at all ages, and disease and disability associated with ageing. Since the control of inheritance has contributed little or nothing of practical value in the advance of health, the present-day medical problems are likely to yield most to the manipulation of the physical environment and the prevention and treatment of disease in the individual. As McKeown so rightly points out, further advance is anticipated in both these directions and from modifications of personal habits injurious to the health of the individual (excessive smoking, consumption of alcohol, faulty nutrition) or of social behaviour which adversely affects the health of others (atmospheric pollution, excessive noise, job monotony). Despite these advances, however, many individuals (those handicapped from birth or mentally ill and the aged) will be left with periodical illnesses, and their continued treatment will need extensive medical care and treatment.

To cope with all these demands, the expansion of medical services must be considerable. They should not be allowed to develop in the fixed pattern of earlier years, which, as this careful analysis shows, has led to "the separating of general, chronic and mental hospitals, the isolation of domiciliary from institutional medical care (due mainly to the relationship established between general practitioner and consultant) and the separation of preventive medicine under a public health service from the treatment services provided by general practitioner and hospitals".

McKeown's long-term proposals for medical services are not unexpected. He recommends a balanced hospital community where all facets of hospital work are brought to the same site and where there is a common medical and nursing staff assigned to patients whose hospital care is not completed in an acute unit (particularly psychiatric and geriatric patients). Health centres should be staffed by groups of doctors and related health workers providing facilities complementary to the out-patient department of hospitals, which they would relieve of patients not requiring the hospital's special facilities. The relationship of consultant to general practitioner should be considerably modified; 'general' practitioner should disappear and be replaced by 'personal' doctors, who would provide care in patients' homes, health centres or hospitals and who would specialize either in obstetrics, paediatrics, as general physicians, or in geriatrics. A new type of administration of medical services is needed although the local authority 'population' approach to medical services and due emphasis on preventive measures should be retained. Prof. McKeown also recommends a complete re-orientation of medical education so that medical students are able to see all types of patients and not the 'special' cases with which, by tradition, the teaching hospitals concern themselves. McKeown also discusses the administrative and financial implications of his sweeping proposals and makes appropriate recommendations.

This is the burden of one of the most important medical books to be written for many years. Its purpose is not how to improve medical services for their own sake, but how to treat disease; not how to improve the lot of doctors but how the general health of the community can be improved in all physical, mental and emotional aspects. It has relevance in underdeveloped as well as other countries.

The book may be dismissed as being impractical and even absurd. Others may fairly say that, in its concept of medical planning, it does not go far enough. In his proposal for a balanced hospital community, for example, McKeown pays little regard to the transport problems of getting patients to and from a few very large hospital groups; his proposals would need to be carefully worked out with town and country planning consultants, who should pay as much regard to rural as well as urban needs. Nor would he satisfy many that he is rightly aware of how a hospital should be designed to meet patients' true needs over the next thirty years; here much help would have to be obtained from specialists in method study, operational research and ergonomics. He would also have to exercise much more persuasion to convince many pregnant mothers—who have emotional as well as medical needs—that their babies should not be born at home. His difficulties with consultants and medical practitioners

and local authorities need not be underlined. However much these protest, and despite all the lamentations about cost, the proposals put forward in this most readable work are, in the end, largely bound to prevail. The inadequacies of modern medical services are so glaringly revealed in a book which is logically presented, skilfully written, and supported by periodic summaries to facilitate reading and understanding that it could well become a springboard to national remedial action.

T. H. HAWKINS

CINEMICROGRAPHY IN RESEARCH

Cinemicrography in Cell Biology

Edited by George G. Rose. Pp. 500. (New York and London: Academic Press Inc., Ltd., 1963.) 18.50 dollars; 132*s*. 6*d*.

IN the preface to *Cinemicrography in Cell Biology*, Dr. Rose states that, "Although not to be considered a general textbook of Cinemicrography, this volume brings together significant perspectives of an analytical approach. . .". However, while Dr. Rose has done a great service to every biologist who reads the book—for he has assembled a wide range of research papers linked by the common denominator, the use of cinematography in research—a great opportunity to produce a more comprehensive text, dealing fully with techniques, as well as results, has been lost.

The work is divided into three main sections, dealing respectively with 1, instrumentation; 2, tissues; 3, cells. The authors themselves are all respected workers in their own particular fields and their work discusses developments from many countries.

Part 1, read in conjunction with a good text on photomicrography, should enable any research worker to develop a time-lapse film laboratory, while avoiding some of the many pitfalls a beginner inevitably falls into. It is, however, interesting to note the number of times in the following papers that an author acknowledges the assistance of an established research film group which helped him with his instrumentation problems. There is little doubt that for effective use of film as a research tool, a sympathetic collaboration between film technologist and scientist is essential.

It is in parts 2 and 3 that an opportunity has been missed. Cinemicrography, in the biological field, deals essentially with the manipulation and photographic recording of living, growing tissues, observed during long periods of time: on the other hand, the technology of photomicrography deals only with manipulating the size and the optical/recording components of a system examining dead, or shortly to be dead, subjects. There is a wealth of literature on how to manipulate the photomicrographic systems in all their complex forms, but there is a serious dearth of comprehensive literature on the special methods required to maintain the growing cell or tissue, for observation and record, during long periods of time, while using film, and with the minimum of interference to the subject itself. The paper by Clay, Huff and Weathersby underlines this criticism when they say: "Such methods and techniques will be sufficiently discussed in other chapters of this volume, or in books devoted directly to tissue culture and photomicrography, to justify omitting description of them here". Unfortunately this material is not so easy to find—at all events collected between two covers—and a section devoted entirely to the effects of the filming process on various biological materials, on the methods of culture peculiar to the film technique, on the methods of constraint within the microscopic field, and on the methods and problems to be faced in analysing the photographic evidence would all have been most valuable.

However, in spite of possible omissions, *Cinemicrography in Cell Biology* will prove exceedingly valuable to any research worker; for it indicates the immense potential which film techniques in research can offer. It emphasizes the necessity for using film in parallel with the other methods of looking at any particular problem, and it underlines some of the instrumentation problems likely to be met when developing a cinematic technique. A wealth of references to papers discussing results could have been enhanced by a classified bibliography on methods, and the historical note found in the preface attributing the birth of microcinematography to an American scientific culture should be taken with a grain of salt.

On the cinemicrographic side this book is one of ideas, and further fields to explore, underlining ends rather than means. In fact, *Cinemicrography in Cell Biology* is a reference text which should be available in all research libraries.

E. LUCEY

AN ENCYCLOPAEDIA OF SPORE MORPHOLOGY

Morphologic Encyclopedia of Palynology

An International Collection of Definitions and Illustrations of Spores and Pollen. By G. O. W. Kremp. Pp. xiii+186+38 plates. (Tucson: The University of Arizona Press, 1965.) 15 dollars.

PALYNOLOGY—the science of pollen and spores—has developed, like many other things in the past twenty years, at a rate too great for comfort. A science which even a few years ago was unheard of even by many botanists has now two journals devoted to it, and palynological papers have intruded into more than two hundred journals in the allied fields of botany, geology, palaeontology and palaeobotany. With four to five papers on palynology appearing every week, and new species of fossil spores being discovered at a rate of a thousand a year, palynology is suffering some of the embarrassments of over-production. Many of the 'new' species of fossil spores reported more or less simultaneously from different parts of the world are evidently synonyms of one another. While consistent application of the International Code of Botanical Nomenclature will eventually resolve much of this, unfortunately no such code governs the plethora of terms used to describe the spores.

Morphologic Encyclopedia of Palynology sets out to elucidate the meaning of the 700 words coined expressly to describe the minute structure of spores—and the almost equal number of terms to which special palynological meaning has come to be attached. Prof. Kremp's book is in effect an exhaustive literature survey of this elaborate terminology: 1,280 terms are defined, their definitions quoted from original sources, and more than 800 are illustrated. In one sense, the book is an encyclopaedic dictionary, but no ultimate and definitive meaning of terms is given, nor is one usage advocated as being preferable to another. Prof. Kremp has been at pains not to impose his ideas on the reader: "it is not the object of this encyclopaedia to weed out 'incorrect definitions' and to tell other people what the author thinks 'correct definitions' should be". The "young worker in palynology . . . having before him the similar and related terms and definitions may make his own choices and judgements". A more assertive, Johnsonian interpretation of the prerogatives of a lexicographer might have helped to rid palynology of some of its superfluous terms.

This is the only serious negative criticism of the book; Prof. Kremp has done what he sets out to do with great care and painstaking thoroughness. He has produced an essential reference source for workers not only in palynology but in the neighbouring fields into which this vigorous science is overflowing.

W. G. CHALONER

THE NEW CENTER FOR THE LIFE SCIENCES AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

By Prof. IRWIN W. SIZER

Head, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

On December 2 and 3, 1965, an international symposium on the "Life Sciences" was held at the Massachusetts Institute of Technology on the occasion of the dedication of the Uncas A. and Helen F. Whitaker Building. This, together with the contiguous Dorrance Building (and certain ancillary laboratories), houses the new Center for the Life Sciences which comprises the Departments of Biology and Nutrition and Food Science. The Center provides an extensive teaching programme for undergraduate, graduate and post-doctoral students. The latter include a number of medical doctors who are being trained for research in basic medical sciences. In addition, the Center is devoted to research in specialized areas of the life sciences such as molecular biology, biophysics, biochemistry, microbiology, physiology, and developmental biology in the Department of Biology, and human and animal nutrition, food science and technology in the Department of Nutrition and Food Science. In both Departments, special emphasis is given to the application of the physical sciences and engineering to education and research in the life sciences.

The new 6-million dollar Whitaker Building (Fig. 1) has eight stories above ground and two below, providing 134,000 gross square feet of additional space for the Departments of Biology and of Nutrition and Food Science. It has a poured-in-place reinforced concrete frame, with metal and glass exterior curtain walls, and is air-conditioned throughout. It was financed in part by the National Institutes of Health, by the Second Century Fund of the Massachusetts Institute of Technology, and by a generous grant from Uncas and Helen Whitaker. Mr. Whitaker is a graduate of the Massachusetts Institute of Technology and is now a member of its Corporation. He is also chairman of the Board and chief executive officer of AMP, Inc. (formerly Aircraft-Marine Products). For many years he and Mrs. Whitaker have been interested in the encouragement of research and teaching in medicine and the life sciences in the United States.

The international symposium on the "Life Sciences", under the general chairmanship of Dr. Jerome B. Wiesner, dean of the School of Science, was opened by Dr. Julius A. Stratton, president of the Massachusetts Institute of Technology. In the first session, "Molecular Structure and the Functional Organization of Cellular Constituents", of which Prof. Salvador E. Luria, Sedgwick professor of biology at the Massachusetts Institute of Technology, was in the chair, major advances in translating molecular structure of nucleic acids into functional specificities of proteins and the relation of macromolecules to cellular structures were reviewed. Speakers in this programme included: Dr. F. H. C. Crick, Medical Research Council Laboratory for Molecular Biology (Cambridge); Prof. Daniel E. Koshland, jun., professor of biochemistry, University of California (Berkeley); Prof. Tracy M. Sonne-

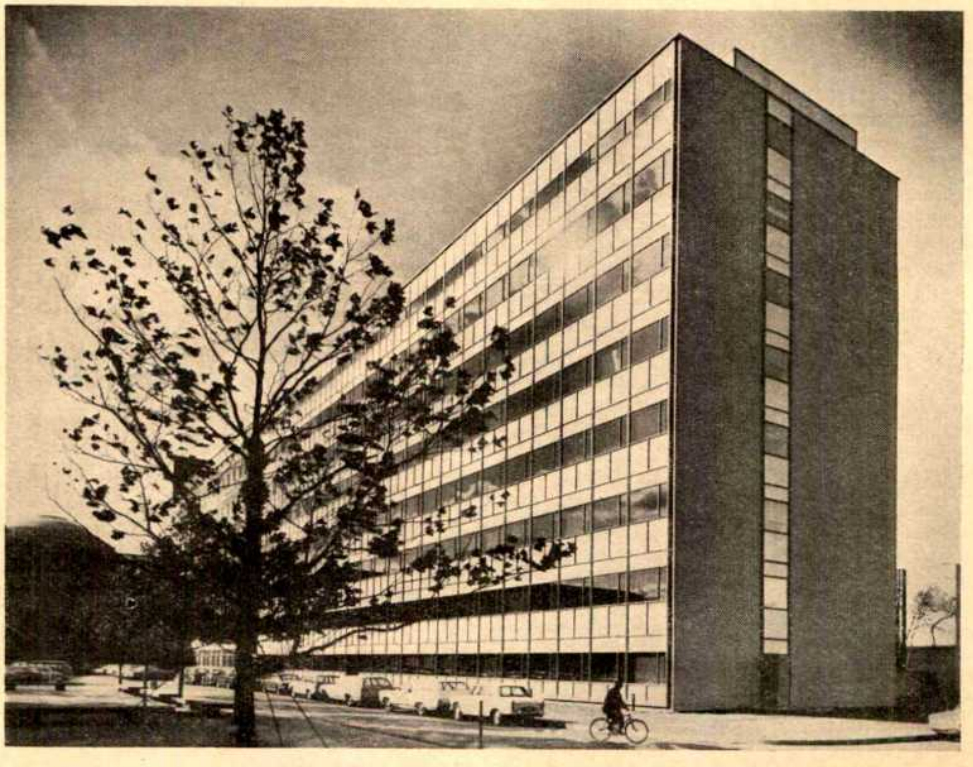


Fig. 1. Center for the Life Sciences, Massachusetts Institute of Technology; the Uncas A. and Helen F. Whitaker Building is in the foreground

born, distinguished service professor of zoology, University of Indiana.

The second session, arranged by Prof. Patrick D. Wall, professor of physiology at the Massachusetts Institute of Technology, was devoted to "Adaptation and Functional Co-ordination" and included a consideration of sensory mechanisms, the central analysis of sensory patterns and the production and control of behaviour patterns which adapt the animal to its environment. Participants in this session were: Prof. J. Z. Young, professor of anatomy, University College (London); Prof. Vincent G. Dethier, professor of zoology, University of Pennsylvania; Prof. Theodore H. Bullock, professor of zoology, University of California (Los Angeles).

In the third session, "The Future of Man and the Life Sciences", chaired by Prof. Samuel A. Goldblith, professor of food science at the Massachusetts Institute of Technology, the relevance of recent advances in basic and applied biology to the problems of over-population and man's food supply were discussed. Speakers in this session were: Prof. Ritchie Calder, Montague Burton professor of international relations, University of Edinburgh; Dr. Clement L. Markert, chairman of the Department of Biology, Yale University; Prof. Roger Revelle, Richard Saltonstall professor of population policy and director of the Center for Population Studies, Harvard University.

The theme of the third session was continued in an evening address on "Molecular Medicine" by Dr. A. Baird Hastings, head of the Laboratory of Metabolic Research, Scripps Clinic and Research Foundation, and in the dedication programme on "The Life Sciences and Society" by Dr. George W. Beadle, president of the University of Chicago.

The educational programme in the Center for the Life Sciences is devoted primarily to training 125 undergraduate majors and 175 graduate students. In addition, there are about 130 postdoctoral Fellows, many of them medical investigators who are receiving special training in research. Courses leading to a Ph.D. degree are offered in biophysics, biochemistry, microbiology, physiology, biology, nutrition, food science, biochemical engineering, and bioengineering. These teaching programmes are administered by a faculty of fifty professors in the life sciences.

Closely associated with the Center for Life Sciences is the new Clinical Research Center with its modern hospital facilities available to faculty who wish to carry out research using patients or student volunteers. Also closely allied with life sciences are the Communications Center and the Neurosciences Center, both of which have extensive programmes related to the nervous system. Research in the life sciences is also being carried out in most of the departments at the Massachusetts Institute of Technology, and indeed 25 per cent of all the research projects in the Institute are devoted to the life sciences. The scope of these projects is extremely broad, but in general they involve the application of science and technology to biological problems. Such equipment as nuclear reactors, giant computers, electron microscopes, cyclotrons and high-voltage X-ray generators, find many uses in research in the life sciences. The use of such facilities requires close collaboration between scientists and engineers throughout the Massachusetts Institute of Technology.

Biology has been taught at the Massachusetts Institute of Technology from the time of its foundation (1861) and was given emphasis in 1871 when a new course in natural history was established "whose ulterior objective is the special pursuit of geology, mineralogy, botany, zoology, or to prepare for medicine, pharmacy, or rural economy". In the years from 1883 until 1921, under the leadership of Prof. William Sedgwick, the Institute won renown as a centre for the life sciences relating to microbiology and public health. Sedgwick was the first to demonstrate that infectious disease could be caused by pollution of water supplies and as a result of this research became known as the "Father of American epidemiology". Work on water supplies soon led to bacteriological investigations of foods which, under Dean Samuel C. Prescott, led to the development of food sterilization and the canning industry in the United States. Research on food and nutrition at the Institute became so important that a graduate Department of Nutrition and Food Science was separated from the Department of Biology in 1942, making it possible for the latter Department to concentrate its efforts on the utilization of the physical sciences in basic research and teaching in the biological sciences. About half the undergraduates who major in the life sciences at the Massachusetts Institute of Technology are preparing for medical school and half for graduate school. The establishment of the new Center for the Life Sciences enables the two Departments to co-operate closely in teaching and research.

The Department of Nutrition and Food Science, under the leadership of Prof. Nevin S. Scrimshaw, will emphasize in its teaching and research programme such topics as clinical and biochemical nutrition, veterinary pathology, oral science, toxicology, and food preservation and processing as well as food chemistry. The Department of Biology, with Prof. Irwin W. Sizer as its head, will offer educational and research programmes which will concentrate on molecular biology, biophysics, cell physiology, neurology, biochemistry, microbiology, developmental biology, and bioengineering. The programmes of the two Departments plus the contribution of other departments and centres should make it possible for the Massachusetts Institute of Technology to play a leading part in the development of the life sciences in the world during the next decade.

NOTATION FOR GENETIC FACTORS OF HUMAN IMMUNOGLOBULINS*

IN a previous memorandum stating recommendations for a uniform notation for human immunoglobulins[1] it was considered advisable that analogous recommendations be made in the future for genes, genotypes and allotypes. In that memorandum, classes of human immunoglobulins which are common to all normal individuals of a given species were considered. These classes of immunoglobulins can be distinguished from each other by a number of means including their antigenic specificity (isotypic specificity).

Another kind of classification, which is at present based on serological typing, distinguishes variants which occur among proteins of the foregoing classes. The corresponding determinants, which are present in some groups of individuals within the species, define antigenic specificities which in animals are commonly termed 'allotypic specificities' and in man 'factors'. Many of these antigenic specificities determine genetic polymorphisms in the different races of man and other animals.

The genetic factors of human immunoglobulins are at present identified by serological tests which measure the inhibition by the test material of the agglutination of erythrocytes pre-coated with incomplete antibody. The

* This memorandum was drafted by us following discussions held during the meeting of the World Health Organization Scientific Group on Genes, Genotypes and Allotypes of Immunoglobulins, held during May 31–June 5, 1965, in Geneva.

agglutinating antibodies occur in selected sera from patients with rheumatoid arthritis or from normal subjects. The incomplete coating antibodies are usually IgG immunoglobulin molecules and the agglutinating reagents are usually IgM molecules. The whole serum, an isolated immunoglobulin, or an immunoglobulin sub-unit may be used in such an inhibition test.

The term 'factor' defines a particular property of certain immunoglobulins which enables them to be identified by a given set of reagents, but it should be noted that the characterization of factors by these means does not preclude the possibility of a cross-reaction.

Family studies have shown that the factors involved are inherited according to Mendelian principles. Two series of factors controlled by independent genetic systems are known; one (the Gm system) is associated with the heavy polypeptide chain of some IgG molecules, and the other (the Inv system) is associated with light polypeptide chains of immunoglobulin molecules.

The nomenclature at present in use is based on serological tests, with genetical analysis providing linkage group information. In higher organisms, the term locus (and the corresponding terminology for genes and genotypes) may be ambiguously used for indicating either a complex chromosomal region or a structural gene, and owing to the nature of serological analysis a one-to-one correspondence between one factor and one gene or one region of a gene cannot be inferred at the present time. For these reasons, the term 'system' is used here for a unit of closely linked genetic information which cannot be separated into sub-units at the level of resolution allowed by formal human genetics.

A new notation should provide a symbol which suggests the relation of the system to the structure it influences, which does not go beyond established fact and which is sufficiently flexible to meet future needs. It should relate to prior terminology for the same system if at all possible, and provide for different systems a series of symbols which differ from each other by at least two letters when practicable. The following recommendations have been designed to meet these requirements so far as possible.

Notation for the factors of the Gm and Inv systems. At the present time, the accumulated information is insufficient to permit precise correlation of the genetic loci for the human immunoglobulins with the structures they influence. Consequently, it seems advisable at this time to retain the present symbols Gm and Inv to describe the two systems currently recognized. For these systems, particularly at the Gm locus, an ever-increasing complexity has been developing in recent years. To avoid these complexities and the implication of possibly false relationships, we recommend a non-committal, flexible numerical notation, similar to that suggested for the *Salmonella* antigens, Rh blood groups, and histocompatibility antigens in the mouse.

The assignment of numbers to the already known Gm and Inv factors has been done using the principles outlined below and considering also the order of their discovery and the known relationships of the Gm factors to the sub-groups of the polypeptide chain of IgG.

Table 1. Notation for the Factors at the Gm Locus*

Original	New
a	1
x	2
b^w and b^2	3
f	4
b and b^1	5
c	6
r	7
e	8
b^α	9
b^β	10
b^γ	11
b^3	12
b^4	13
b^5	14

* A number has not been assigned to Gm (D) because it has been found to be an artefact and not a Gm factor.

Table 2. Notation for the Factors at the Inv Locus

Original	New
l (lower-case L)	1
a	2
b	3

Either of two methods is suitable for recording the phenotype of an individual.

(*a*) Record the result obtained with each set of reagents: for example, Gm(1,−2,3,4,5); Inv.(1,−2,3). Punctuation includes commas between the reactions with each set of reagents. This would mean that the sample was tested with reagents which correspond to the factors Gm(1), Gm(2), Gm(3), Gm(4), and Gm(5) and Inv(1), Inv(2), and Inv(3); and that it was positive with the test systems for Gm(1), Gm(3), Gm(4), and Gm(5), and negative for Gm(2); and positive for Inv(1) and (3), and negative for Inv(2).

(*b*) Record only the positive results: for example, Gm(1,3,4,5); Inv.(1,3). In this case, the factors tested cannot be determined from the recorded phenotype and reference must be made to the list of reagents used for this information.

It is recognized that studies with several sets of reagents of Gm factors 7–14 inclusive have not yet been extensively published. Nor is the closeness of the relationship between Gm(3) and Gm(4) precisely known at the present time. These factors have nevertheless been assigned separate numbers here. Further decisions in the form of deletions of some numbers are foreseen.

The phenotypes of isolated discrete (for example, myeloma) proteins may be recorded on the same basis as whole serum and by adding the symbol for the immunoglobulin sub-type if known. For example: phenotypes γ_2b : Gm(1,2); or γWe : Gm(1,2); κ : Inv(1); etc.

Notation for genes. Symbols for genes should be underlined when typed and italicized when printed. The factors determined by the gene should be indicated by superscript digits.

It is understood that the assignment of a genotype to a phenotype can be done only after genetic analysis, that is, the observation of family segregation. Assignment of the 'most probable' genotype in the absence of family data should be clearly indicated.

Table 3. Examples

Phenotype*	Population	Most probable genotype
Gm(1,5)	Caucasoid	Gm^1/Gm^5
Gm(1,5)	Negroid	$Gm^{1,5}/Gm^{1,5}$
Gm(1,2)	Caucasoid or Mongoloid	$Gm^{1,2}/Gm^{1,2}$ or $Gm^{1,2}/Gm^1$

* All samples were tested for Gm(1), Gm(2) and Gm(5).

It should be emphasized that findings in other immunological systems indicate that serological specificities may appear in the phenotype which may not be represented in the genotype (that is, specificities resulting from interaction of different genes). Although such specificities have not yet been found in the human immunoglobulins, the proposed system of nomenclature may be used to record them.

The use of genetic symbols containing several numbers is a convenient notation to describe the inheritance of complex patterns of serological reactions (phenogroups). The symbols do not imply that regions corresponding to the individual numbers do or do not exist in the relevant genes. For example, a gene $Gm^{1,2}$ does not necessarily have part of its structure determining factor 1 and a different part determining factor 2.

Notation for new systems. It is possible that new factors in addition to those at present known will be discovered and shown to be inherited independently of any of the known systems. If this occurs it seems advisable to relate the system symbol to the immunoglobulins and to the appropriate polypeptide chain and to re-name the Gm and Inv systems at present established so that their

symbols reflect the structural units they control. In this event, it is suggested that the symbol 'I' be used as the initial letter in the description of all the loci for the immunoglobulins.

Notation for new factors. There is no wish to restrict the freedom of investigators to publish data in any way they desire. On the other hand, the usefulness of the proposed system of notation will be seriously jeopardized if numerical symbols are added to the present list in an unco-ordinated way. For these reasons, the following recommendations are made:

(*a*) If the serological and family data are insufficient to meet the criteria for recognition of new factors set out below, either because of insufficient quantity of the reagent or because the frequency of the factor in the population precludes definitive family studies, it is recommended that the author give the new factor a provisional name using a two-letter abbreviation derived from town name, name of patient, etc. This should be preceded by the non-specific generic term I, rather than a specific system symbol, for example, I(pr) and I(−pr) for the phenotypes.

(*b*) If the factor which has not yet been assigned a number is known to be part of the Gm or Inv systems, it can be indicated in the parenthesis after the numbered factors, for example: Gm(1, −2,3,4,5,pr) or Gm(1,3,4,5,pr).

(*c*) If the author believes that the criteria are fulfilled and that the linkage relations have been established, the use of a number instead of the provisional name is warranted. To avoid duplicate use of the same number a request for a number should be sent to the World Health Organization International Reference Centre for Genetic Factors of Human Immunoglobulins, addressed to Dr. C. Ropartz, Directeur, Centre départemental de Transfusion sanguine et de Génétique humaine, 609, Chemin de la Bretèque, 76–Bois-Guillaume, France.

(*d*) If the factor is shown to belong to one of the already known linkage groups (systems), it would be assigned the next available number of that group, for example, Inv(4).

(*e*) If the factor is shown to belong to a different system, it would be given the number 1. If it is located on a particular immunoglobulin class, the system symbol could be chosen according to the suggestions made here. In choosing the system symbol for a factor not localized on one particular class of immunoglobulins, transposition of the provisional letter into the system symbol might be useful. The phenotype I(pr) would in this instance be changed to IPr(1).

None of these suggestions should be taken to imply any formal control over the future use of numbers to describe factors inherited in the immunoglobulins. The sole intention is to avoid duplication of numbers or their use for poorly defined factors, and to assist in the rapid inclusion of reported factors into the proposed system of notation. Circulation of data relevant to their identification to other investigators would be helpful in this respect.

Criteria for recognition of new factors. To establish that a test system detects a 'new' factor, it is recommended that the following conditions be fulfilled:

(*a*) The reaction system employed should show complete reproducibility. With the present haemagglutination-inhibition technique, the difference between 'inhibiting' and 'non-inhibiting' normal sera from the same population should be three tubes or higher in a twofold dilution test system.

(*b*) Since antisera may contain mixtures of antibodies of different specificities, steps should be taken to show that the observed 'new' reaction patterns are not due to an obvious mixture of antibodies of previously known specificities. (Absorption experiments using cells coated with selected antibodies, inhibition tests with purified myeloma proteins, and tests for consistency using cells coated with antibodies from different persons may give information concerning these matters.)

(*c*) It is essential to specify all reagents used in any study because, (1) two reagents (antiglobulin and a cell coat) are necessary to define a factor; (2) the same coated cells in some cases may be used for defining more than one factor; and (3) the antiglobulin from one person may be used with different cell coats to define more than one factor (see Table 4).

Table 4. ANTIGLOBULIN FROM ONE PERSON USED WITH DIFFERENT CELL COATS TO DEFINE MORE THAN ONE FACTOR

Factor	Anti-globulin	Dilution	Cell coat	Dilution
Gm(5)	Dr.*	1/8	Anti-D V.S.*	1/5
Gm(13)	Th.*	1/8	Anti-D V.S.*	1/5
Gm(6)	Bomb.*	1/32	Anti-D Warren*	1/10
Gm(5)	Bomb.*	1/32	Anti-D Berg*	1/10

* Identification of reagent.

(*d*) Enough family data should be obtained to establish the pattern of inheritance of the new factor.

(*e*) Tests with isolated immunoglobulins from positive and negative sera should be performed to show that the new factor is associated with immunoglobulins. Tests with agammaglobulinaemic sera might also be useful.

(*f*) The reactions observed with the new set of reagents should be different in at least one race from those of any previously described set of reagents. It is suggested that a useful standard for deciding that the new factor differs from known factors is a χ^2 test of the comparisons of the reaction with different reagent sets, giving values with a $P \leqslant 0{\cdot}02$. However, one clearly established and inherited difference, confirmed in several laboratories, may be acceptable as evidence for a new factor.

Reference panel of sera. In order to maintain uniformity in reagent sets used to detect established differences between the immunoglobulins of different individuals, the specificities of the reagent sets should be defined by their reactions with a reference panel of normal human sera. It is hoped that such a panel will be made available through the World Health Organization International Reference Centre for Genetic Factors of Human Immunoglobulins.

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[1] *Bull. Wld. Hlth. Org.*, **30**, 447 (1964).

EXISTENCE OF A SIGNIFICANT LATITUDINAL VARIATION IN DENSITY FROM 200 TO 800 KILOMETRES

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USING satellite orbital decay data, the effect of longitude on density, known as the diurnal (time-of-day) effect, has been well established. With these data, until recently, it has not been possible to prove definitely the existence of any significant latitudinal variation in density. Different investigators have reported slight latitudinal density variations, but the various findings are not in agreement[1–3]. Now, the existence of an appreciable latitudinal density gradient has been demonstrated by May[4], who analysed data from the *Discoverer* series of satellites, the altitudes of which ranged between 190 and 260 km and perigees from 80° S to 80° N. He concluded that the air density at a fixed height is a function of latitude and is about 30 per cent smaller at the poles than at the equator. May's conclusion is corroborated by the following independent analysis performed by me that is based on a different method of deriving the behaviour of density with latitude.

The upper-atmosphere density ρ, in g cm^{-3}, can be represented by:

$$\rho = K'' S'^{N} \qquad (1)$$

where S' is a daily indicator of the extreme ultra-violet radiation of the Sun, expressed in units of the 10·7-cm flux S (10^{-22} W m^{-2} (c/s)$^{-1}$). These radio astronomy units will be understood to always apply to S' in this article. K'' and N are constants for a given altitude h and local time t. They are presented in tables[5] for selected altitudes from 200 to 800 km and every hour, where the local time t = 12 h (local noon) corresponds to a zero angular difference between the right ascension of the local meridian and the right ascension of the Sun; t = 24 h (local midnight) corresponds to a difference of 180°.

Using equation (1) together with tables, the density and its variations can be readily calculated as a function of S'. The index S', in turn, is derived from S and drag data for satellite 1958 α, by correcting for the amount by which S fails to predict exactly the satellite drag when it is substituted in equation (1) (ref. 5). Thus, S' represents the total extreme ultra-violet radiation from the Sun, estimated from satellite orbital decay data for 1958 α by assuming that its drag variations result only from changes in the extreme ultra-violet radiation that affect atmospheric density.

Fig. 1 exhibits a curve of S', based on monthly averages from 1958 to 1962. The curve for S is displayed for comparison. The S' curve is noticeably different from the S curve. Two marked features appearing in Fig. 1 are (*a* the relative minimum values of S' for either June or July during the 5 years from 1958 to 1962, and (*b*) the semi-annual variation exhibited in the S' curve (but not the S curve) in 1961 and 1962, with maxima around the equinoxes and minima around the solstices. The most likely explanation for the semi-annual variation in S' in 1961 and 1962 is that the S' values reflect a latitude variation in density. Thus, S', taken as a measure of the daily output of extreme ultra-violet radiation from the Sun, is calculated by assuming that satellite drag (density) changes are due only to variations in the extreme ultra-violet radiation. While S' is independent of changes in longitude of the perigee of 1958 α, the method of calculating S' did not take into account changes in the latitude of perigee. Since the amount of extreme ultra-violet radiation intercepted by the Earth's upper atmosphere should vary with latitude, except in the summer, when the daily insolation is nearly independent of latitude, therefore S' should vary with latitude also.

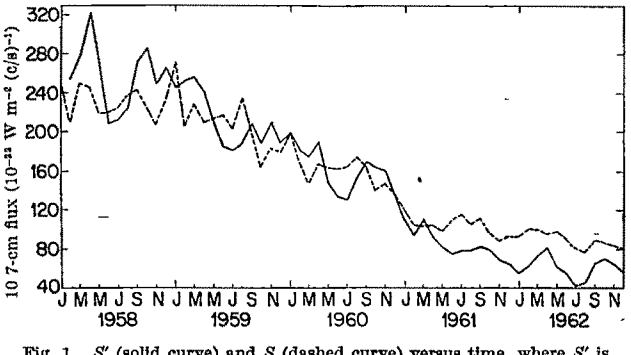


Fig. 1. S' (solid curve) and S (dashed curve) versus time, where S' is derived from satellite 1958 α by comparing calculated rates of orbital decay with observed values and S is the 10·7-cm solar flux

The diurnal effect is one in which the density varies with local time or the longitude difference between the given location and the sub-solar point. This indicates that any analysis of the density variation with latitude should take into consideration the latitude variation of the sub-solar point. The density at any point at a given altitude above a given geographical location on Earth should vary with the difference between the latitude of this location and the latitude of the sub-solar point, which changes in the course of the seasons. If, instead of having a fixed point above the Earth, satellite perigee is used, which also has latitude variations, then the density should vary with the difference between the latitude of perigee and the latitude of the sub-solar point. Since density is represented as a function of S' (equation 1), the nature of the density variation with latitude can be determined by analysing how S' varies with the combined movement of satellite 1958 α and the Sun north and south of the equator.

Curve *a*, Fig. 2, shows how the perigee for 1958 α varies with latitude in 1962. Positive latitude denotes perigee north of the equator, while negative latitude is for the perigee south of the equator. The same convention is followed for the latitude of the sub-solar point (declination of the Sun). Curve *b*, Fig. 2, presents the variation of the declination of the Sun during the year. Curve *c*, Fig. 2, the result of subtracting curve *b* from curve *a*, illustrates how the angular distance of the perigee north or south of the sub-solar point, θ', varies in 1962. The perigee reaches the maximum distance south of the sub-solar point ($\theta' = -56°$) when it is farthest south of the equator near the end of June. Likewise, the perigee reaches the maximum distance north of the sub-solar point ($\theta = +56°$) when it is farthest north of the equator in both December and January. Curve *c*, Fig. 2, is typical for other years from 1958 to 1961; the perigee reaches the maximum distance south of the sub-solar point near the summer solstice in the northern hemisphere (June or July) and the maximum distance north near the winter solstice (December or January).

Fig. 3 gives curves of the absolute value of θ' (curve *a*) and S' (curve *b*) for 1961 and 1962, where the S' data points are plotted on a scale increasing downwards. The S' data points are monthly averages corrected for the

solar cycle trend by normalizing them to January 1962. A comparison between the two smooth curves a and b, drawn to the data point averages of θ' and S', shows that S' inclines to be a maximum at the sub-solar point, decreasing both north and south of it, thus indicating a definite relationship. This preliminary analysis supports the assumption that S' is affected by latitude variations in the perigee of satellite 1958 α. In the following paragraphs, S' values are analysed in detail by a method devised to uncover the effect of latitude. Since seasonal effects are also indicated to be important, the method used treats the S' data by season for both the northern and southern hemisphere.

The S' values for 1961 and 1962 were calculated using satellite orbital decay data of Jacchia and Slowey[6], and Izsak[7,8]. Since the decay values (P) were available usually only every fourth day, the calculated S' values were plotted on an S' versus time (days) graph, a smooth curve was drawn through the data points, and daily S' values were derived by interpolation. These daily values of S' were corrected for two effects: one is the long-term (solar cycle) variation in the output of extreme ultra-violet radiation by the Sun; the other is the short-term variation in extreme ultra-violet radiation due to the rotation of the Sun. For the former, Fig. 1 discloses that the long-term trend of S' is down from 1958 to 1962. The daily S' values for 1961 and 1962 were corrected for this trend by normalizing them to January 1962 by means of a tendency curve fitted to 6-month average S' values. The following procedure was adopted to minimize the effect of the 27-day periodicity in S': the daily S' values for 1961 and 1962, normalized to January 1962, were grouped according to 10-degree intervals of θ', by season, where May, June and July, the three consecutive months corresponding to the maximum positive declination of the Sun, are taken as northern hemisphere summer (the other seasons follow according to the hemisphere). Then average S' values were computed. These are displayed in Table 1 together with the number of cases for each average. Averages were not included in Table 1 if, (a) the number of cases was less than 10; (b) an average for 1961 differed by more than 20 per cent from that for 1962 for the same season and θ' interval; (c) an average for a given season deviated by more than 10 per cent from the average above or below it in Table 1. These criteria ensured that the S' averages in Table 1 were not unduly affected by an unequal distribution of daily S' values, favouring either high or low values, within a 27-day period. More accurate trends with latitude and season could be established by the elimination of inconsistent or doubtful averages. Another source of error in the S' averages could arise from errors in the satellite orbital decay values. The tracking data used to derive the P values are sporadic and frequently inaccurate. Jacchia and Slowey[9] have described one type of error in P for 1958 α, consisting of drag oscillations of period of approximately 3·5 days and amplitudes that reach 40 per cent. By averaging over a season it should be possible to eliminate these shorter period errors.

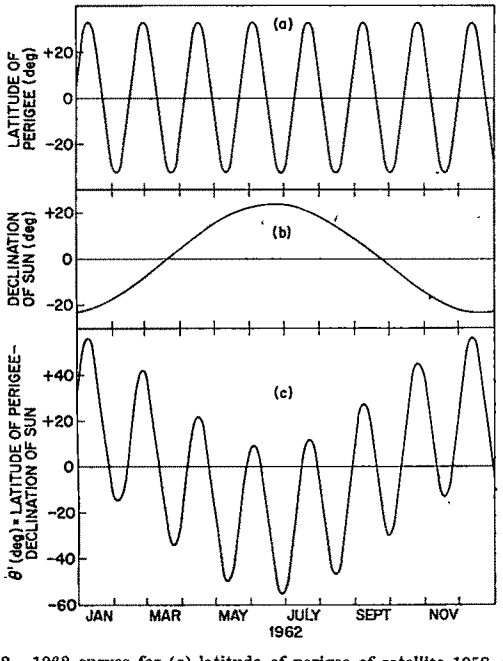


Fig. 2. 1962 curves for (a) latitude of perigee of satellite 1958 α; (b) declination of Sun (latitude of sub-solar point), (c) latitude of perigee of 1958 α minus declination of Sun (curve a–curve b)

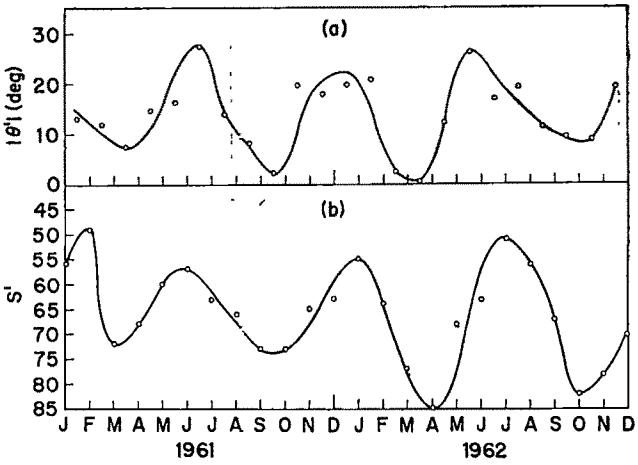


Fig. 3. 1961 and 1962 curves for (a) the absolute angular distance north or south of the sub-solar point, [θ'], (b) S'. Note that the S' scale increases downwards

The S' averages in Table 1 were plotted on an S' versus θ' graph, in Fig. 4, by placing the S' data points at the mid-points of the respective θ' intervals. The data points for the respective 3-month seasons were connected with solid straight-line segments. The resulting solid curves all indicate meaningful trends with latitude. Their average slope is in good agreement with May's Fig. 2 (ref. 4), which is a plot of density against latitude for *Discoverer* satellites, except for the northern hemisphere autumn curve. This anomaly will be discussed later. It should be pointed out that May's plot extends over the period from August 1959 to December 1960, while the data points in Fig. 2 of this article are for 1961 and 1962. The average value of the S' data points in Fig. 4 on the solid curve

Table 1. AVERAGE S' VERSUS θ' AND SEASON FOR 1961 AND 1962

Latitude perigee–latitude Sun (deg)	Nov. Ave. S'	Dec Jan. No. cases	Feb. Ave. S'	Mar. Apr No. cases	May Ave. S'	June July No. cases	Aug. Ave. S'	Sep. Oct. No. cases
+46 to +56	61·2	34						
+36 to +45	62 1	23					81 0	17
+26 to +35	65·6	21	65 6	17			81·8	16
+16 to +25	66·0	20					79·8	23
+6 to +15	67·8	23	69·0	19	60·0	37	74·0	18
+5 to −5	65·9	31	69·1	20	62·7	28	67·9	22
−6 to −15			64·6	23	63·4	21	66·0	20
−16 to −25			63·6	29			65·2	24
−26 to −35					60·5	17		
−36 to −45			62·3	10	56·7	25	62·7	13
−46 to −56								

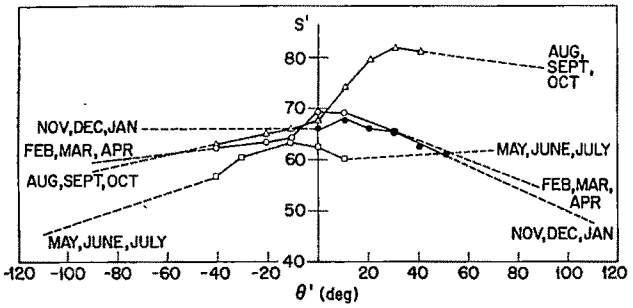


Fig. 4. S' versus θ', the angular distance north or south of the sub-solar point, for the four seasons in 1961–1962. The solid curves connect S' data points; the dashed curves are extrapolations to the poles based on astronomical consequences of upper-atmosphere heating by solar extreme ultra-violet radiation. ●, Nov., Dec., Jan.; ○, Feb., Mar., Apr.; □, May, June, July; △, Aug., Sept., Oct.

for May, June and July (corresponding to geographical latitudes 20° S. to 30° N.) is 60·6. This value is 6·0 per cent less than the average of 64·5 for the points on the November, December and January curve for the same latitude band. There should be 6·5 per cent less extreme ultra-violet radiation intercepted by the Earth's upper atmosphere in July than January because the Earth is farther from the Sun in July. A calculation, based on taking an average Earth–Sun distance for 3-month intervals, indicates that the average S' for May, June and July should be 5·3 per cent less than the average for November, December and January due only to this distance variation. This value of 5·3 per cent compares well enough with the 6·0 per cent value already given to suggest that the difference between the two solid curves can be explained on the basis of the varying Earth–Sun distance alone.

The average latitude of the sub-solar point, $\bar{\theta}_s$, is close enough to the equator ($\theta_s \simeq 0$) during the spring and autumn so that the average geometric latitude, $\bar{\theta}$ (where $\theta = \theta' + \theta_s$), is approximately equal to the average θ' or $\bar{\theta} \simeq \bar{\theta}'$. Also, $\bar{\theta} \simeq \bar{\theta}' - 20°$ for the winter in the respective hemisphere, and $\bar{\theta} \simeq \bar{\theta}' + 20°$ for the summer. Therefore, the north pole is at $\bar{\theta}' \simeq 110°$ during the northern hemisphere winter. In Fig. 4, the solid curve for this season is extrapolated to the north pole by a dashed straight line, continuing the trend manifested by the last two data points. The southern hemisphere winter (May, June and July) curve is linearly extrapolated by drawing a dashed line between the S' value for the south pole (taken to be 5·3 per cent less than the north pole winter value) and the last data point. To account for the fact that during the summer the daily exposure to the extreme ultra-violet radiation of the Sun (represented by S') at the pole is nearly the same as at the equator, the southern hemisphere summer curve is extrapolated to the south pole by drawing a dashed straight line between the last data point and an S' value at the south pole equal to the value at the equator. The same procedure was followed in extrapolating the May, June and July curve to the north pole. The remaining curves for spring and autumn were linearly extrapolated to the poles by drawing dashed lines to continue the trends denoted by the last two data points at the ends of the solid curves.

The S' data points in Fig. 4 substantiate the picture of upper-atmosphere seasons as derived from astronomical considerations, except for the northern hemisphere autumn season. The rise to high S' values from the equator to mid-latitudes, with maximum at 30°–40° N., exhibited by the August, September and October curve is quite unusual. This unexpected feature is fully supported by the S' data, both in 1961 and 1962. Apparently, there is additional heating, not caused by solar extreme ultra-violet radiation, that affects the northern hemisphere at mid-latitudes near the autumn equinox. Heating associated with geomagnetic activity is one possibility. Jacchia[10] has demonstrated that there is a conspicuous drag effect on satellites associated with major magnetic storms. Dessler[11] has proposed one mechanism for such heating involving hydromagnetic waves, but it is difficult to find a physical explanation for asymmetric heating with regard to both the equinoxes and the two hemispheres. The curves of Fig. 4 will not be representative of gradients in the auroral zones during major magnetic storms. Jacchia and Slowey[12] have indicated from the analysis of the atmospheric drag affecting the *Injun* 3 satellite that the heating accompanying geomagnetic perturbations in the auroral zones is 4 or 5 times greater than the heating experienced during these perturbations in low latitudes. However, the model embodied in Fig. 4, based entirely on variations of the extreme ultra-violet radiation, best explains the S' variations in Fig. 1.

The following qualitative description of the upper-atmosphere global density distribution is a consequence of Fig. 4 and equation (1): The density at a given altitude decreases from the equator to the poles, except for the summer, where there is little or no gradient, and the autumn in the northern hemisphere, where the density increases from the equator to 30°–40° N. The season with the lowest density is winter. The winter in the southern hemisphere is less dense than the same season in the northern hemisphere because the Earth is farther from the Sun during May, June and July. Therefore, since the minimum density in each hemisphere occurs over the respective pole during the winter, the absolute minimum density over the Earth is at the south pole during this season. Latitude 30°–40° N. has the maximum density (autumn season). The autumn and spring density gradients are intermediate with respect to those of summer and winter, except for the autumn season in the northern hemisphere the gradient of which is anomalous and unexplained. It will be shown that when the diurnal (longitude) effect on the density is taken into account, the foregoing picture is modified by the introduction of a marked latitude gradient above 400 km in the polar regions for all seasons.

Table 2 presents S' values by season and geometric latitude θ from 90° S. to 90° N., as selected from the curves of Fig. 4 by using the appropriate conversions between $\bar{\theta}'$ and $\bar{\theta}$. Displayed also are the ratios $S'(\theta)/S'(0)$, representing the value of S' at latitude θ relative to its value at the equator ($\theta = 0$), both for the same local time. Since $N = 1$ in equation (1) at 200 km, these ratios express the relative density variation with latitude for a given season at this altitude. For example, in winter the densities at both poles are 27 per cent lower than at the equator for the same local time. Later on, it will be clear that Table 2 is most accurate for the early morning hours, 5–7 h local time. Fig. 5, drawn from the $S'(\theta)/S'(0)$ values in Table 2, gives the relative S' (density at 200 km) variation with latitude in the form of curves for each of the four seasons. The hemispheric parts of the curves correspond-

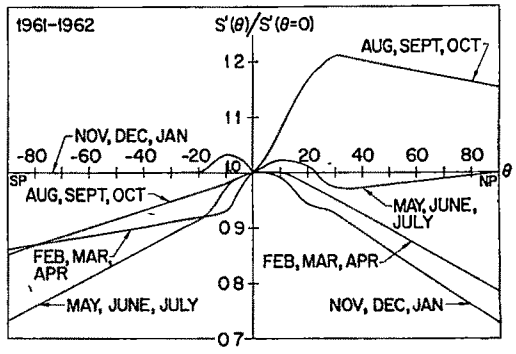


Fig. 5. $S'(\theta)/S'(\theta = 0)$ versus θ (latitude) for the four seasons in 1961–1962 from pole to pole, where $S'(\theta)$ is the value of S' at latitude θ, $S'(\theta = 0)$ is the S' value at the equator, NP is the north pole, and SP is the south pole. These curves represent the relative S' (or density at 200 km) variation with respect to the equator

ing to winter, summer and spring are roughly symmetric with respect to the equator.

The relative density variation with latitude can be calculated for a higher altitude up to 800 km by using the S' values from Table 2 together with the K'' and N values (equation 1) corresponding to the selected altitude and local times in the following equation:

$$\rho(\theta)/\rho(0) = K''(\theta)S'(\theta)^{N(\theta)}/K''(0)S'(0)^{N(0)} \qquad (2)$$

where $\rho(\theta)$ and $S'(\theta)$ are the density and S' at latitude θ, respectively, and $\rho(0)$ and $S'(0)$ are the density and S' at the equator, respectively. The local times at θ and the equator are accounted for through the K'' and N values corresponding to these times, namely, $K''(\theta)$, $N(\theta)$ and $K''(0)$, $N(0)$. Normally the same local time will be used for θ and the equator, but for calculations involving the polar regions, different times may have to be used. There should be only minor diurnal fluctuations in density at the poles during the spring or autumn seasons. During the summer there should be practically no diurnal variation, since the altitude of the Sun, which is never far above the horizon, and the extreme ultra-violet radiation do not change significantly with time of day. During the winter, of course, there is no Sun. Therefore, a reasonable estimate of the time for relative density calculations involving the pole is $t = 5$ h for the winter, $t = 6$ h (sunrise) for spring and autumn, and $t = 7$ h for summer.

Table 3 illustrates how the hemispheric latitudinal gradient can change with altitude for the various seasons. The ratios of the polar to equatorial density, $\rho(N)/\rho(0)$ and $\rho(S)/\rho(0)$, are given at 200, 400, 600, and 800 km and the four seasons, where $\rho(N)$ and $\rho(S)$ are the densities at the north and south pole, respectively, and $\rho(0)$ is the density at the equator. The local time t is taken to be 6 h for convenience; hence, the ratios are most representative for the spring and autumn seasons. When the ratios are less than one at 200 km, they decrease with altitude, and when they are greater than one, the ratios increase with altitude, due to N growing with altitude. In both cases, the magnitude of the horizontal density gradient between the equator and the pole increases with altitude.

From Table 3, the density at 800 km at the winter pole is 41 per cent lower than the density at the equator at $t = 6$ h. The density at the north pole is 28 per cent higher than at the equator during August, September and October. Variations for other seasons and altitudes range between these values.

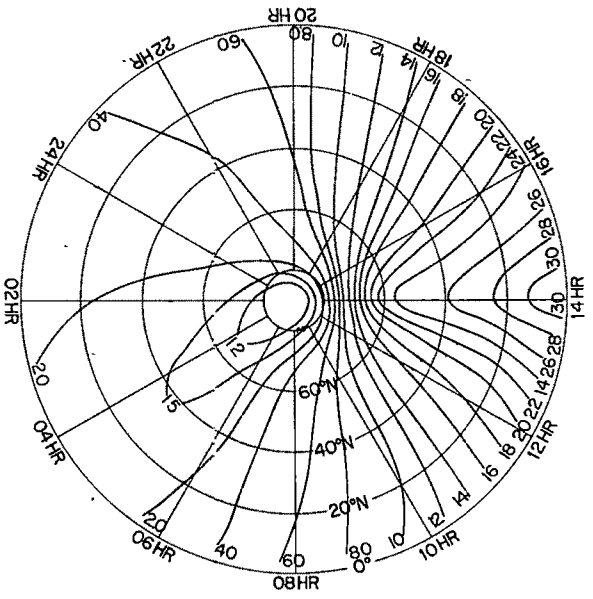


Fig. 6. 800-km relative density contours (north pole = 1·0) for northern hemisphere winter (Nov., Dec., Jan.)

Figs. 6, 7, 8, and 9 present contours (isopycnics) showing the relative density variation at 800 km (north pole = 1·0) in the northern hemisphere for winter, spring, summer and autumn, respectively. The latitude effect is taken into account by using the S' values in Table 2; the longitude (diurnal) effect is calculated from the tables given in ref. 5. In the figures, the longitudes are labelled in local time. These time labels apply up to about 70° N. The local time selected for the north polar region (above 70° N.) is 5 h for winter, 6 h for spring and autumn, and 7 h for summer, irrespective of longitude. In Fig. 6, winter, the latitude variation in density is much less than the longitude variation over most of the hemisphere, except for the region between 60° N. and the pole on the day side of the Earth. The maximum gradient occurs along the 14 h meridian. This same feature occurs on the maps for the other three seasons, although the gradient is not so great. The maximum density in the northern hemisphere in the winter occurs on the equator, where it is 30 times greater that at the north pole. In Fig. 7, spring, the minimum

Table 2. S' AND $S'(0)/S'(0)$ VERSUS θ AND SEASON FOR 1961 AND 1962

Geometric latitude (deg)	Nov S'	Dec. Jan $S'(\theta)/S'(0)$	Feb S'	Mar. Apr. $S'(\theta)/S'(0)$	May S'	June July $S'(\theta)/S'(0)$	Aug. S'	Sept. Oct. $S'(\theta)/S'(0)$
+90	48 0	0·73	54·5	0·79	62·0	1·00	78·0	1·16
+80	50 0	0·76	56·3	0 81	61 6	0·99	78·6	1·16
+70	52·2	0·79	58·1	0·84	61·2	0·99	79 2	1·17
+60	54·3	0·82	60·0	0·87	61·0	0 98	79·8	1·18
+50	56·6	0·86	62 0	0·90	60·7	0·98	80·6	1·19
+40	58·8	0·89	63 9	0 92	60·3	0·97	81·0	1·20
+30	61·2	0·93	65·8	0·95	60·0	0·97	81·7	1·21
+20	62·1	0 94	67·5	0·98	62 7	1 01	79·0	1·17
+10	65 6	0 99	69 1	1 00	63 4	1·02	73·3	1·08
0	66 0	1 00	69·2	1·00	62·0	1·00	67·5	1 00
−10	67·8	1·03	64 5	0·93	60·5	0·98	66·0	0·98
−20	65·9	1·00	63·5	0·92	56·7	0·91	65·1	0·96
−30	66·0	1·00	63·0	0·91	55·0	0·89	64·0	0·95
−40	66·0	1·00	62·1	0·90	53·5	0·86	63·0	0 93
−50	66 0	1·00	61·8	0 89	52·0	0 84	62·0	0 92
−60	66·0	1·00	61·2	0 88	50·2	0 81	61·0	0 90
−70	66·0	1·00	60 8	0 88	48·7	0·78	59·9	0 89
−80	66 0	1 00	60 1	0 87	47·0	0·76	58·9	0·87
−90	66 0	1 00	59·6	0·86	45·5	0·73	57·8	0·86

Table 3. RATIO OF POLAR TO EQUATORIAL DENSITY VERSUS ALTITUDE AND SEASON FOR 1961 AND 1962

Altitude (km)	Nov. Dec. Jan $\varrho(N)/\varrho(0)$	$\varrho(S)/\varrho(0)$	Feb. Mar. Apr. $\varrho(N)/\varrho(0)$	$\varrho(S)/\varrho(0)$	May June July $\varrho(N)/\varrho(0)$	$\varrho(S)/\varrho(0)$	Aug. Sept. Oct. $\varrho(N)/\varrho(0)$	$\varrho(S)/\varrho(0)$
200	0·73	1 00	0 79	0 86	1·00	0·73	1·16	0·86
400	0·65	1 00	0·73	0 82	1 00	0·65	1·22	0 82
600	0·61	1 00	0·70	0 79	1·00	0·61	1 26	0·79
800	0·59	1·00	0 68	0 78	1 00	0·59	1·28	0·78

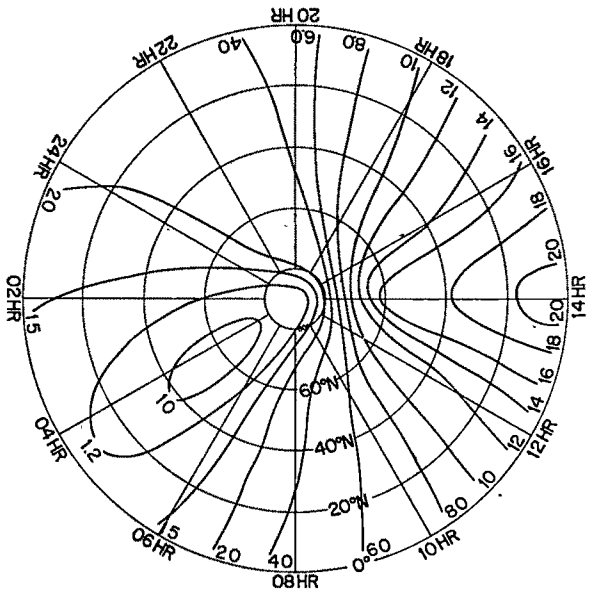


Fig. 7. 800-km relative density contours (north pole = 1·0) for northern hemisphere spring (Feb , Mar., Apr.)

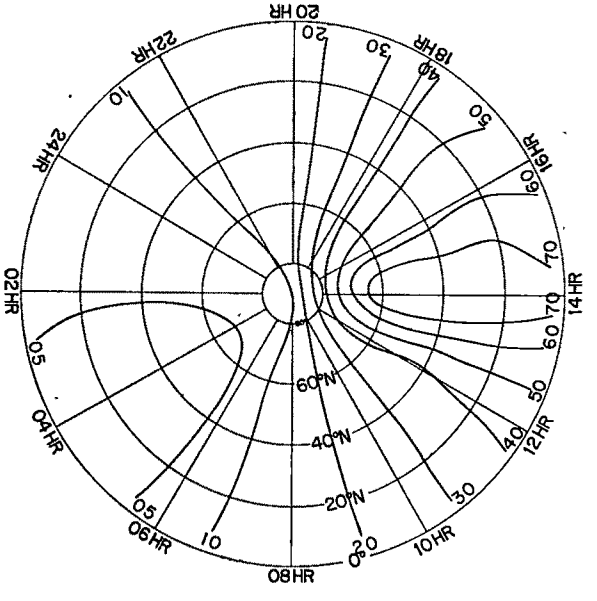


Fig. 8. 800-km relative density contours (north pole = 1 0) for northern hemisphere summer (May, June, July)

density has shifted south, from the pole to 60° N., while the maximum is on the equator at the average latitude of the sub-solar point. In Fig. 8, summer, the minimum has moved farther south to 30° N. latitude, while the maximum has moved north to about 20° N. In Fig. 9, autumn, the minimum is at about 60° N., but the maximum has not retreated to the equator with the Sun as expected; it has moved farther north to around 30°–40° N. This feature is a result of the anomalous heating indicated in the northern hemisphere autumn curve of Fig. 5.

It is obvious that the atmospheric density bulge caused by the Sun is not symmetric with latitude, except possibly during spring in the northern hemisphere. Contour charts drawn for other altitudes from 400 to 800 km will have their main features the same as those at 800 km. The contour configurations and locations of the maximum and minimum densities would not change with altitude, although the horizontal density gradients would decrease as the altitude diminishes. Below 400 km, the diurnal effect decreases in comparison with the latitude effect as the altitude diminishes, so that its influence on the contour pattern becomes much less. At 200 km, the contours (not shown; see ref. 13) are oriented more east–west than north–south, as at 800 km, so that the latitude density gradient is several times greater than the longitude density gradient over most regions. In winter and spring the lowest density is at or near the pole. The contours appear almost symmetric with respect to the pole, except for a slight bulge toward the pole along the 14-h meridian and a slight dip away from the pole along the 5-h meridian. In summer, the minimum density has shifted south to 50° N., while two maxima in density are indicated at 20° N. and 50° N. along the 14-h meridian. The gradients in summer are much weaker than spring and winter. In autumn the maximum density is at about 40° N., with a zone of relatively high density extending along this latitude on the day side of the Earth. At lower latitudes the contours are almost symmetric with the equator. King and Kohl[14] have used a density gradient from the 200 km contours[13] to deduce a mid-latitude meridional wind that is not greatly different from winds determined by means of sodium trail experiments.

The general configuration of the contours in Figs. 6–9, incorporating both the latitude and diurnal effects, will be quite similar for the southern hemisphere during the respective season and for the same altitude, except for autumn. For this, the northern hemisphere spring map can be taken to represent approximately the southern relative density pattern at the same altitude. The contour patterns at 400 km will be intermediate between those at 200 km and 800 km with respect to contour orientations and magnitudes of the gradients. It is not expected that the relative density variation with latitude at a given altitude and season, that is a consequence of relative variations of extreme ultra-violet radiation with latitude, would change greatly with changes in solar extreme ultra-violet radiation activity. The variations manifested in Table 2 and Fig. 5 should hold, at least as a first approximation, over the solar cycle, except possibly for the northern hemisphere autumn season. The contour configurations and gradients displayed in Figs. 6–9 should appear about the same at the maximum of the solar cycle. Calculations made with equation (2) indicate that the latitudinal gradients would intensify only slightly at sunspot maximum.

Therefore, a density variation with latitude and season can explain the semi-annual effect exhibited by S' in 1961 and 1962. The semi-annual effect is caused by the combined movement of satellite 1958 α and the Sun north

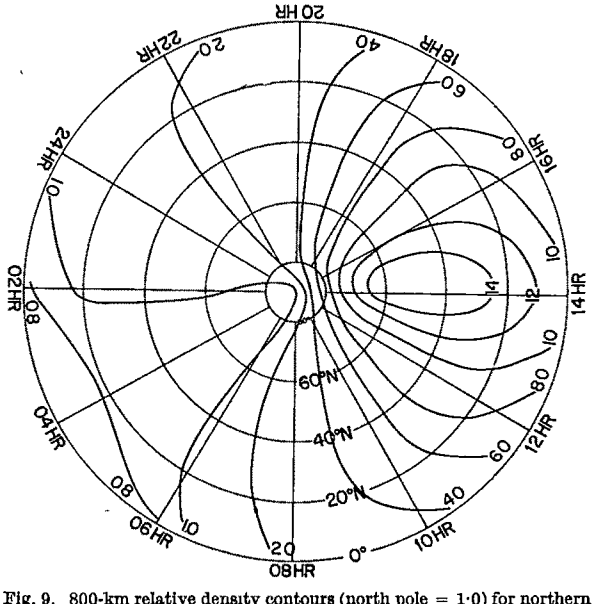


Fig. 9. 800-km relative density contours (north pole = 1·0) for northern hemisphere autumn (Aug., Sept., Oct).

and south of the equator. The marked minima in S' in either June or July from 1958 to 1962 result from a latitude variation in density and the perigee of 1958 α being at a maximum distance south of the sub-solar point in these months. A minimum should also be expected in S' in December or January. Such a minimum is indicated in 1961 and 1962; however, it is not apparent in 1958–1960. This minimum should not be so great as the June or July minimum because the Earth intercepts less extreme ultra-violet radiation during these latter months due to the greater Earth–Sun distance. The magnitude of the June or July minima cannot be completely explained on the basis of the seasonal curves in Fig. 4, but it is expected that curves for the individual months containing the solstices would indicate much greater latitudinal density gradients. The analysis of the data was unavoidably based on 3-month seasons because of the limited amount of S' data. The latitudinal density gradients for the individual months of a given season will differ from the 3-month curves, especially near the solstices. If a marked latitude density gradient does exist in the winter hemisphere near the solstices, as indicated by this analysis, then it should be reflected also in the P values for satellites other than 1958 α as their perigee latitudes change north and south of the equator. Other satellites do show the existence of a marked latitudinal density gradient, especially near the time of the solstices[15]. Thus, there seems to be little doubt that perigee latitude and the latitude of the sub-solar point are factors that must be taken into account in any analysis of the P variations, particularly near the solstices.

This work was supported by the Lockheed Independent Research Programme.

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OBITUARIES

Dr. F. H. Pollard

Frederick Henry Pollard was born on June 6, 1907, in Swindon. After leaving school, he entered the University of Bristol in 1923, and took a bachelor's degree in 1927, reading chemistry as special subject. After taking a Diploma in Education in 1928, also at Bristol, he was appointed head of the Science Department at Thornbury Grammar School, Gloucestershire, from 1929 to 1937. In 1937 he moved to Hanley High School, where he proceeded to teach for a further 5 years. While at Thornbury Grammar School, Pollard was persuaded by Prof. W. E. Garner to work part-time for his Ph.D. The research work he carried out was on the infra-red spectra of carbon monoxide flames and he was awarded a Ph.D. in 1935. This interest, which he developed, he maintained until his untimely death.

In 1941, Pollard accepted the post of lecturer in inorganic chemistry at Bristol and was appointed senior lecturer in 1951, reader in 1954, and finally awarded the degree of D.Sc. in 1959. During the Second World War he worked with Dr. W. J. Dunning and others on the explosive *RDX*. Among the reactions he investigated were those between the oxides of nitrogen and low-molecular weight organic compounds; for example, formaldehyde and acetic acid. This interest, together with his responsibilities to the Department of Chemistry at Bristol, led him into the field of inorganic chemistry research. However, Pollard soon became convinced that more powerful methods of analysis would greatly increase the scope of inorganic chemistry. He began his research career relatively late in life, but, as is internationally recognized, he published an impressive collection of nearly one hundred scientific papers. The papers he published can be grouped under five headings: (*a*) inorganic chromatography; (*b*) gas chromatography studies; (*c*) thermal decomposition of organic nitrates; (*d*) carbides, silicides, nitrides and their reactions; and (*e*) co-ordination chemistry of new analytical reagents. Pollard began his work on inorganic chromatography in order to investigate how far paper and ion-exchange chromatography could be applied to the analysis of inorganic compounds with two ultimate aims: the development of a complete scheme of qualitative analysis; and the application of quantitative methods to the investigation of inorganic reaction mechanisms. At the time of his death, he was engaged on the establishment of a course in advanced analytical chemistry leading to the degree of M.Sc.

Pollard energetically shouldered the major burden of organizing the teaching of inorganic chemistry at Bristol, and was responsible for building up a vigorous school of teaching and research. It can be said that the foundation of inorganic chemistry at Bristol was his chosen life's work.

He took an active part in many University matters and had a knowledge and experience of schools and especially sixth-form work which was of great value in the matters of entrance requirements and student selection. Pollard was a member of the committee responsible for policy decisions on all building aspects of the new School of Chemistry, with the special duty of co-ordinating the scheme. He also found time to take an active part in the local and central activities of the Chemical Society, and the Society for Analytical Chemistry, serving on the councils of both bodies. In addition to this, he never severed his links with the teaching profession, in that he organized and lectured at conferences for teachers. In committee, Pollard's contributions, although often outspoken, were given with sincerity and deep feeling, in order that the correct choice might be made. He loved playing 'devil's advocate', a quality which often caused him trouble, being so easily misunderstood.

In spite of all these calls on his time, Pollard always took a very keen interest in the welfare of students, and anyone was welcome to ask his advice on problems of a personal nature. His quip to all of us was, "My door is always open". Characteristic of him was the effort, time and thought he gave to the development of student interchanges, especially between the Department of Chemistry, Bristol, and the Anorganische Institut, Technischen Hochschule, Brunswick, an association which developed from the lectures he gave in Germany in 1953 under the auspices of the Rockefeller Interchange Scheme.

His sudden death at the age of fifty-eight left his career sadly incomplete. During his life he never spared himself to serve his own generation, together with those generations in his charge which made up the University, to the best of his ability. Those of us who were his students are

deeply grateful. Although he listened with sympathy he could be a task-master—to all of us he was our 'father figure'. In the School of Chemistry at Bristol he will long be missed.

G. Nickless

Dr. Allen B. Du Mont

Dr. Allen B. Du Mont, the television pioneer of Cedar Grove, New Jersey, died on November 14, 1965, at the age of sixty-four. He was best known for his work in perfecting the cathode-ray tube.

Dr. Du Mont was born in Brooklyn, New York, on January 29, 1901, and he became a ship's wireless operator at an early age. He received his degree in electrical engineering from the Rensselaer Polytechnic Institute, Troy, New York, in 1924, and afterwards received honorary degrees from the Rensselaer Polytechnic Institute, the Brooklyn Polytechnic Institute, Fairleigh Dickinson University, New York University, and Montclair State College.

At the Westinghouse factory in Bloomfield, New Jersey, Dr. Du Mont worked on the development of high-speed manufacturing and test equipment for radio tubes. Later, as chief engineer of the De Forest Radio Company, of Passaic, New Jersey, he worked closely with Lee De Forest. In 1928 they established the first synchronized sight and sound television station *W2XCD*, using the Nipkow disk mechanical scanner at both the camera and receiver.

Recognizing the limitations of a mechanical television system, he then started his own company in 1931, later known as Allen B. Du Mont Laboratories, Inc. Dr. Du Mont afterwards expanded the company to produce a variety of cathode-ray tubes, the first commercially sold television receivers, television broadcast transmitters and studio equipment and an extensive range of scientific apparatus. He started the Du Mont Network with Station *WABD* before the Second World War and later linked it with the companion Station *WTTG* in Washington, D.C. From 1934 until the merger in 1960 with Fairchild Camera and Instrument Corporation, Dr. Du Mont served as director, president or chairman of the board of the Allen Du Mont Laboratories, Inc.

During the 'thirties, Dr. Du Mont worked on the development of radar systems and cathode-ray displays for both the Signal Corps and the Navy. His company contributed greatly in these fields during the Second World War and also to the Loran and Manhattan Projects, and later to nuclear propulsion instrumentation.

Dr. Du Mont served on the National Television System Committee, contributing to the formulation of broadcast standards for both monochrome and colour television. He was United States delegate to the International Radio Consultative Committee for consideration of international colour television standards. He collaborated extensively with the Federal Communications Commission in matters pertaining to the allocation of very high and ultra-high frequency television channels.

In 1943 he became the first president of the Television Broadcasters Association, which later combined with the National Association of Broadcasters for both radio and television.

Through the Allen B. Du Mont Foundation he took part in the establishment of Educational Television in co-operation with the Montclair State College in New Jersey and served on the New Jersey Commission for Educational Television. The Foundation also participated in and financed research in medical electronics.

Among his many awards, Dr. Du Mont received the Marconi Award of Achievement, the Horatio Alger Award, and he was made Chevalier de la Légion d'Honneur. In 1927 he was awarded the Westinghouse Award for Most Outstanding Accomplishment, in 1943 the American Television Society Award for Advancement of Commercial Television, and in 1944 the Television Broadcasters Association Award for the development of the cathode-ray tube.

Dr. Du Mont was one of the Public Governors of the American Stock Exchange and was vice-chairman of the Board of Trustees of Rensselaer Polytechnic Institute.

As an enthusiast for power boats, Dr. Du Mont won many awards for navigational accuracy in predicted log racing. With his boats, *Hurricane II* and *Hurricane III*, he did much to discover and interpret the multi-path and propagation effects of both television and radar signals.

Thomas T. Goldsmith, jun.

NEWS and VIEWS

Steacie Prize : Prof. N. Bartlett and Prof. J. C. Polanyi

Prof. Neil Bartlett, of the Department of Chemistry, University of British Columbia, and Prof. J. C. Polanyi, of the Department of Chemistry, University of Toronto, will share the 1965 Steacie Prize. The Prize consists of the income from the E. W. R. Steacie Memorial Fund to which colleagues and friends of the late president of the National Research Council contributed. It is awarded annually for outstanding work done in the natural sciences by younger people. Prof. Bartlett is cited for the first preparation of a chemical compound of a noble gas (*Proc. Chem. Soc.*, 218; 1962). The compound, $XePtF_6$, was prepared by the reaction of xenon with platinum hexafluoride. This startling experiment evolved logically from work on the fluorination of platinum and platinum compounds that Prof. Bartlett had been doing with D. H. Lohmann. The result immediately stimulated a flurry of experiments in other countries and soon a number of compounds of xenon and krypton had been prepared. The ramifications will not soon be exhausted. Prof. Polanyi was the first to observe infra-red chemiluminescence (Cashion, J. K., and Polanyi, J. C., *J. Chem. Phys.*, 29, 455; 1958), the occurrence of which he had predicted theoretically two years previously. The experimental techniques that he evolved have now been refined, and a wide range of atomic and molecular excitations caused by chemical reactions are being studied in Toronto and elsewhere. Five years ago, he published a proposal for a chemical laser (*J. Chem. Phys.*, 34, 347; 1961) and it has recently been shown to be practical. Both Prof. Bartlett and Prof. Polanyi are graduates of British universities. The former received a Ph.D. in 1958 from the University of Durham and the latter a Ph.D. in 1952 and a D.Sc. in 1964 from the University of Manchester.

Principal of the University of Stirling: Prof. T. L. Cottrell

Prof. T. L. Cottrell, professor of chemistry in the University of Edinburgh, will take up full-time duties as principal of the new University of Stirling in August. Prof. Cottrell graduated with honours in chemistry at the University of Edinburgh in 1943, and joined the Research Department of the Explosives Division of Imperial Chemical Industries, Ltd., at Ardeer. After working in the Physical Chemistry Laboratory at Oxford, he returned to Imperial Chemical Industries (Nobel Division) and became head of the Physical Chemistry Research Section in 1950. He was awarded the Meldola Medal of the Royal Institute of Chemistry in 1952. At the time of his appointment to the chair of chemistry in

Edinburgh in 1959, he was personal assistant to Sir Ewart Smith, deputy chairman of Imperial Chemical Industries, Ltd. Prof. Cottrell's main research interest in the University of Edinburgh lies in the field of energy transfer, with particular reference to the theory of chemical reaction rates. The research school which he has built up and directed continues to investigate transfer efficiency in molecule–molecule and electron–molecule collisions: the energy transfer from low-energy electrons to rotational or vibrational modes of molecules has received special attention. In addition to research papers in the scientific journals, Prof. Cottrell's published work includes *The Strength of Chemical Bonds* (Butterworths), *Molecular Energy Transfer in Gases* (with J. C. McCoubrey) (Butterworths), *Chemistry* (Oxford University Press) and his recently published *Dynamic Aspects of Molecular Energy States* (Oliver and Boyd). At the same time, he has been actively engaged in the teaching, planning and supervision of academic programmes, with special attention to undergraduate tutorial arrangements. One major change has been the establishment in Edinburgh of an honours B.Sc. degree in chemical physics, providing chemists with a more rigorous physical and mathematical approach to the subject. Formerly a member of the University of Essex Academic Planning Board, Prof. Cottrell is now a member of the corresponding Academic Advisory Committee, and thus brings to his new post a wide experience of teaching, research, planning and administration.

Materials Science at the U.S. National Bureau of Standards: Dr. H. E. Sorrows

The U.S. National Bureau of Standards has recently announced the appointment of Dr. Howard E. Sorrows as assistant to the Director of the Institute for Materials Research. Until his appointment, Dr. Sorrows was manager of the Space and Environmental Science Programs of Texas Instruments, Inc., a position that combined scientific management with profit responsibility. From 1959 until 1963, he initiated and managed a department for technical intelligence, long-range planning, and new product development. Dr. Sorrows has previously worked at the Office of Naval Research, the Navy Bureau of Ordnance, and at the White Oak Naval Ordnance Laboratory. He began his professional career at the National Bureau of Standards. Dr. Sorrows holds degrees from Baylor University and George Washington University and a Ph.D. from Catholic University. He has been active in a number of scientific societies, including the Institute of Electrical and Electronic Engineers, the American Institute of Aeronautics and Astronautics, Sigma Pi Sigma, Sigma Xi, the Dallas-Fort Worth Council of Scientific Societies and the Metropolitan Philosophical Society. He was chairman of the Dallas Section of the Institute of Electrical and Electronic Engineers during 1964.

United Kingdom Expenditure on Space Research

In a written answer in the House of Commons on January 25, the Secretary of State for Education and Science, Mr. A. Crosland, stated that the estimated expenditure by the Science Research Council on scientific space research in 1965–66 was £3·55 million. This included provision for the United Kingdom contribution to the European Space Research Organization of £1·75 million, most of which should be regarded as expenditure outside the United Kingdom. There was also a little expenditure outside the United Kingdom for purchase of equipment which had to be imported. In a further written answer on January 26, Mr. Crosland added that the Science Research Council organized the scientific space programme, which existed to make it possible for individual scientists, or groups of scientists, in universities or other institutions, to carry out researches in space. Hitherto, the main opportunities had been in the series of Anglo–American satellites. In future, the main opportunities would be in the European Space Research Organization and the National Aeronautics and Space Administration satellites. Also in a written answer, on January 25, the Minister of Technology, Mr. F. Cousins, stated that under its contract with the European Space Research Organization, the Atomic Energy Authority had completed its design study of the payload for the astronomical satellite project in which it makes proposals for satellite-borne instruments to enable ultra-violet and X-ray studies to be made of stars and nebulae. The Authority's design would be in competition with two other contract studies requested by the Organization, which was expected to take several months to assess them. In a further written answer, on January 27, the Minister of Aviation, Mr. F. Mulley, stated that the total estimated expenditure in 1965–66 by the United Kingdom on the space programme amounted to about £22 million, or 0·07 per cent of the gross national product, or a *per capita* expenditure of 5*s*. 3*d*. compared with 1*s*. in Belgium, 9*s*. 6*d*. in France, 6*s*. in the Federal Republic of Germany, and 2*s*. 3*d*. in The Netherlands. About 3,800 persons in the United Kingdom were employed on the British space programme and £15·32 million was provided in the 1965–66 programme for expenditure by the Ministry of Aviation on space research and development. This did not include expenditure to meet specific requirements of the Ministry of Defence and the Science Research Council, but does include a net payment of £2·21 million for the European Launcher Development Organization transactions.

Forestry Commission: Plans for Expansion

In reply to a question in the House of Lords on February 3, Lord Mitchison, Joint Parliamentary Secretary to the Minister of Land and Natural Resources, said that the Government had completed its review of planting to be carried out by the Forestry Commission. The programme for Great Britain provided for the Commission to plant 450,000 acres during the years 1964–73 and it had been decided that planting in England and Wales should continue within this programme. In Scotland it had been decided, in view of circumstances set out in the White Paper on the Scottish Economy, 1965 to 1970 (Cmnd. 2864), to increase the programme from the present level of 30,000 acres a year to 36,000 acres a year from 1969. In a written answer in the House of Commons on February 2, the Secretary of State for Scotland, Mr. W. Ross, stated that the Forestry Commission intended, as soon as circumstances permitted, to increase the number of professional, scientific and supporting staff in Edinburgh from seventeen to about forty. The location of the new building required was already being considered.

United Kingdom Atomic Energy Authority: Non-nuclear Research

In a written answer in the House of Commons on February 3, the Minister of Technology, Mr. F. Cousins, stated that scientific effort of the Atomic Energy Authority on work not directly connected with nuclear-energy was equivalent to that of about eighty professionally qualified or graduate scientists and engineers.

United States–Poland Exchange Programme

The U.S. National Academy of Sciences and the Polish Academy of Sciences have announced plans for a programme of exchange visits by scientists of the two countries. The agreement, which covers a 3-year period, became effective from January 1 and provides for exchanges in all the disciplines in which the two Academies are mutually competent: natural sciences, including mathematics; engineering sciences; and behavioural sciences. The new programme is flexible in its provisions

for both short-term visits by individual scientists to lecture, to conduct seminars, to survey research, or to exchange professional views and, for longer stays of up to 1 year, to carry out laboratory research or investigations in the field. Visits in each direction, totalling approximately 40 man-months per annum, are provided under the programme, with no necessary matching of either the number of exchanges or the scientific fields in which they occur. In addition to its provisions for the exchange of individual scientists, the agreement provides for co-operation between the two Academies in the field of scientific publications and documentation, including the exchange of information on research and documentation and programmes, and mutual assistance and advice on the exchange of books and scientific literature. This agreement is similar to the agreement which came into effect on January 1 between the U.S. National Academy of Sciences and the Council of Academies of Yugoslavia. Similar arrangements are also anticipated between the U.S. National Academy of Sciences and the academies of Romania, Hungary and Czechoslovakia.

Polish Academy of Sciences: Four-year Plan

The basic provisions of the 1966–70 Plan of the Polish Academy of Sciences Agencies are outlined by W. Nowacki in the Academy's *Review* for April–June, 1965 (10, No. 2; 38). The twenty-one institutes and forty-nine autonomous centres and agencies of the Academy now employ 5,600 persons, including 2,210 scientific workers, of whom 460 are independent; by 1970 it is anticipated that the present shortages in some disciplines will have been eliminated. In the social sciences, the research and development plan will continue the policy initiated a few years ago, but special attention will be given to the further development of the economic sciences. The establishment of an Institute for the Economic Sciences is proposed, as also is the development of research in pedagogies and psychology. In the biological sciences, the importance of biochemical and biophysical research is emphasized, and the development of microbiology and genetics is proposed. In medical and agricultural science and forestry, collaboration with the biological sciences will be strengthened, and research on the nervous system, on experimental and transplantation surgery, the physiology of ageing and of labour, and on human genetics, medical anthropology and human ecology is specially mentioned. In physics, special attention will be given to the development of the physics of solids, quantum electronics and the physics of low temperatures. In physical chemistry, the mechanism and laws of physico-chemical change, the methodology of separating multi-component mixtures, the physico-chemical foundations of metallic corrosion and the erosion of structural materials will be studied. In organic chemistry, the mechanism of reactions, the kinetics and catalysis of organic reactions, the utilization of indigenous raw materials and physiologically active substances are among the subjects proposed for research. Work on the physics of the rare and radioactive elements and the chemistry of plasma is also proposed. Research is also to be concentrated on the application of large automatic systems, new applications for analogue computers, the modelling of neuronal systems and on the methodology of planning.

The Cumberland Basin, Bristol

All large cities in Britain are to-day faced with an ever-growing traffic problem, and in this Bristol is no exception. In recent years, especially near the city centre and the city docks, traffic conditions at certain times became quite chaotic, particularly when the old swing bridge was opened at intervals during the day to permit the passage of shipping in and out of the docks. This swing bridge was the focal point for an enormous flow of traffic from the North, the Midlands, South Wales and South-West England; when ships were moving in and out of dock, road traffic built up into queues stretching for miles along either side of the Cumberland Basin. The problem has been considerably eased, however, by completion of what is known as the Cumberland Basin Bridges Scheme, a brilliantly conceived and executed design of bridges, viaducts and a new swing bridge, an account of which is contained in an illustrated article entitled "The Cumberland Basin Bridges and Viaducts", recently published (*Concrete Quarterly*. No. 66; July–September 1965. London: Cement and Concrete Association. 5*s*.).

The new lay-out is essentially an interchange of elevated roads on which north–south traffic crosses the river on the Avon Bridge, a three-span pre-stressed concrete structure. On the north bank of the river is constructed a spiral slip road and a branched ramp which take traffic to and from the old swing bridge. Just north of this interchange is the new swing bridge carrying the road across the entrance lock. Then, just north of this, the carriageways divide into four separate lanes; north-bound traffic can go straight on into Bristol City, or it can turn westward down a curving ramp towards Avonmouth via the Avon Gorge; two more curved ramps carry south-bound traffic up on to the new swing bridge. It is here that the swing bridge control tower is situated, whence changeable signs are operated to divert traffic streams down ramps towards the old swing bridge (east end of Cumberland Basin) when the new swing bridge is open for shipping. This most informative article gives technical details of the designs of the viaducts, the new Avon Bridge, and the new swing bridge. Apart from a panoramic photograph of the entire project, taken from the heights of Clifton, which clearly shows how traffic can flow in all directions without interruption, there are also photographs of a scale model of this scheme and its orientation, and of the Avon Bridge. A material factor in the overall design is that it has vastly improved the approaches to the city and has transformed much that was ugly into something much more pleasing which harmonizes the dockland with the well-known picture westwards, ". . . the towering cliffs of the Avon Gorge and Brunel's dramatic suspension bridge".

Improved Analysis of Earthquakes

According to a recent paper entitled "The Application of Phased Arrays to the Analysis of Seismic Body Waves", by J. W. Burtill and F. E. Whiteway (*Phil. Trans. Roy. Soc.*, No. 1091, 258, 421–493. London: H.M.S.O., 1965. 44*s*.; 6.60 dollars), the analysis of seismic body waves (that is, waves which have travelled through the earth rather than over its surface) has been facilitated by the use of phased arrays. This method has enabled more data concerning natural earthquakes and explosions to be obtained than has been so using the more conventional three-component set of seismographs. The array may first be used (often by signal delay and addition) to cut out random noise and microseisms, and then as a velocity and azimuth filter to eliminate unwanted waves, so that signal recognition is vastly improved. By these methods and by the use of correlation techniques, the velocities of various pulses across the array and their arrival time at one particular seismometer can be determined accurately. This information may then be used for the determination of the Earth's crustal structure by well-known methods. At ranges less than 25° seismograms are very complex. However, for ranges from 30° to 90° the effect of the propagation path has been shown to be small, and the results from explosions are in agreement with a simple symmetrical, impulsive source function. Nearly all natural earthquakes investigated, however, had asymmetrical source functions. At Yellowknife, waves having a length of at least 20 km have been observed and arrays of comparable dimensions are therefore necessary. Experiments have been, and are being, performed with line,

circular and cluster arrangements of seismometers and with various methods of weighting the signals (signal/noise power ratio, Tchebyscheff polynomials, etc.) in order to improve pulse recognition and measurement. Work on data analysers (chiefly for velocity and azimuth) is in progress. It might be added that seismological arrays are now in operation at Eskdalemuir, Scotland (United Kingdom Atomic Energy Authority), in Canada, in India, in Australia and in the United States.

Action of Ammonium Salts on Concrete

APART from ammonium sulphate, data on the action of ammonium salts on concrete are limited and, in some cases, conflicting. The problem has been investigated by Dr. F. M. Lea (Building Research Station), whose paper on "The Action of Ammonium Salts on Concrete" has recently been published (*Magazine of Concrete Research*, 22, 17; September 1965. Cement and Concrete Association, London, S.W.1). Dr. Lea studied the action of 5 per cent solutions of ammonium salts on a Portland, a Portland–pozzolana, and a high-alumina cement concrete. He found that ammonium chloride and nitrate solutions had a strong leaching potential, acting as dilute acids consequent on loss of ammonia by reaction with lime. Strengths of concrete cubes with all three cements fell by at least half in 4 years, without outward signs of deterioration. Examples are quoted of comparative compressive strengths (in lb./in.^{2}) of 4-in. cubes of 1 : 2 : 4 concrete; in the case of Portland cement, storage in water for 1 year gave the figure 6,310; in 5 per cent ammonium nitrate 1-year storage reduced this to 4,340; after 4 years the figure was 2,310; a 60 per cent Portland cement and 40 per cent spent oil-shale pozzolana mix gave corresponding figures of 4,960, 3,500 and 2,440; cubes of high-alumina cement showed a similar trend with corresponding figures 9,340, 7,180 and 4,400. Chemical analyses of some specimens tested show large reductions in the lime content of the cements.

In a further significant series of tests, to determine compressive strengths (in lb./in.^{2}) of 50 cm^2 cubes of 1 : 2 : 4 concrete, the following results were obtained: for Portland cement stored in water for 1 year, 7,320; the same stored for 3·5 years, 8,420. In 5 per cent ammonium nitrate, the corresponding figures were 4,525 and 3,050. In 5 per cent ammonium chloride, the figures for 1-year and 3·5-year storage were 4,140 and 2,110; and in 5 per cent ammonium sulphate, stored for 1 year, the figure was 825. High-alumina cement revealed similar reductions under the same conditions: in water the figures were 9,830 and 9,730 (storage for 1 and 3·5 years, respectively); with 5 per cent ammonium nitrate the reduction was from 4,685 to 1,520; with 5 per cent ammonium chloride the reduction was from 7,810 to 5,720; but with 5 per cent ammonium sulphate, only 10,110 to 9,115. (All the foregoing results are means of tests on four cubes.) Regarding the reaction of ammonium sulphate on Portland cement, the formation of calcium sulpho-aluminate producing expansion is dominant, and Dr. Lea attributes this to its solubility being lower than that of the nitro- or chloro-aluminates.

Control of Tuberculosis

International Work in Tuberculosis, 1949–1964, is the title of a reprint from the *WHO Chronicle* which reviews the tuberculosis programme of the World Health Organization (Pp. 32. Geneva: World Health Organization; London: H.M.S.O. 1965. 2 Sw. francs; 3*s*. 6*d*.; 0.60 dollars). In 1948, the World Health Assembly decided to make tuberculosis control one of the priority programmes of the World Health Organization. Initially, there were two main aspects of the work of the World Health Organization: the demonstration of modern methods of tuberculosis control to developing countries and collaboration with the United Nations Children's Fund in mass BCG vaccination programmes. In 1959, the work took a new direction. Research into the epidemiology of tuberculosis assisted by the World Health Organization had shown that some of the conventional ideas about the disease were erroneous, and clinical research assisted by the World Health Organization had shown that domiciliary treatment of tuberculosis by drugs was as effective as, and cheaper than, institutional treatment. The experience of the World Health Organization with classical methods of control had shown that training and demonstration projects would not, by themselves, promote new programmes and that mass BCG programmes also had limitations. A new approach to the control of tuberculosis was therefore evolved, namely, the concept of the national tuberculosis programme adapted to the needs and resources of each country and planned by a national tuberculosis institute. The first of these institutes was opened in Bangalore in 1959, and the success of the programme developed there exceeded expectations. This concept is now being used to deal with the widely differing problems met with in countries now receiving help from the World Health Organization for tuberculosis control. Research has been, since 1949, an integral part of the tuberculosis control programme of the World Health Organization, and thirty-four national institutions in twenty-four countries are now taking part in this work. The activities range from the establishment of a standard tuberculin test to the development of cheap and effective standard chemotherapy. This publication contains seven sections on various aspects of control programmes and five sections on research and diagnostic procedures, epidemiology, immunization, chemotherapy and chemo-prophylaxis, and systems analysis.

Effect of Experimental Haemorrhage on Haemoglobin Plasma Iron and Total Iron-binding Capacity in Dairy Calves

WHEN K. C. BREMNER, of the Division of Animal Health, and J. A. Ronald, of the Wheat Research Unit, C.S.I.R.O., withdrew blood samples of 10, 20 and 30 ml./kg body-weight from healthy dairy calves, the plasma total iron-binding capacity increased (*Austral. J. Exp. Biol. and Med. Sci.*, 43, Part 5; October 1965). Plasma iron concentrations also increased significantly after bleeding at the two higher levels, but were unaffected by a blood loss of 10 ml./kg. Calves bled daily for 2 weeks at rates of 2, 4 or 6 ml./kg/day also had significantly increased plasma total iron-binding capacity. Blood losses of 2 or 4 ml./kg/day resulted in an elevation of plasma iron concentration. However, phlebotomies of 6 ml./kg/day eventually caused a marked depression of plasma iron levels, but when the bleedings stopped, plasma iron concentrations increased to abnormally high values. It is suggested that the increased plasma iron levels were due to an increased absorption of dietary iron. Labile iron reserves in the experimental calves were estimated to lie within the range 1·0–1·4 g, and were associated with liver concentrations of non-haem iron of about 30 μg/g wet liver. In calves, levels of non-haem iron in liver tissue of less than 20 μg/g appear to be indicative of iron deficiency.

Exchange Scheme between Britain and India for Younger Scientists

DURING the past 2 years there has been in operation an exchange scheme designed to establish or maintain contact between British universities or research institutes and their counterparts in India through visits by younger scientists. The scheme, originally proposed by the (then) scientific adviser to the Indian High Commission in London, provides for six visits of between 6 weeks and 4 months duration by younger British scientists to India,

arrangements for attachments to suitable institutions being made by the Indian University Grants Commission. A similar number of visits by Indian scientists to Britain are also provided for, attachments being arranged through the British Council. Nine British scientists have visited India under the scheme since its inauguration and it is hoped to arrange a further six visits during the period April 1, 1966–March 31, 1967. Applications from British scientists, preferably between the ages of 27 and 35, are now invited. Further information and forms of application can be obtained from the Director, Science Department, the British Council, 59 New Oxford Street, London, W.C.1. The closing date for applications is March 15, 1966.

The Paul Instrument Fund Awards

THE Paul Instrument Fund Committee has made grants as follows: £3,250 to Prof. A. F. Gibson, professor of physics in the University of Essex, for the development of devices to extend the application of infra-red lasers; £700 to Dr. E. T. Hall, director of the Research Laboratory for Archaeology and History of Art, Oxford, for the construction of a geophysical magnetic simulator; £5,000 to Mr. A. R. Luxmoore, of the Engineering Department, University College, Swansea, for the construction of a 'Moiré-scope'—a special optical instrument sensitive only to the deformations of a body, and not affected by any rigid body movements (closely comparable with the conventional polariscopes used in photo-elastic measurements of stress). The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much-improved types of physical instruments and apparatus for investigations in pure or applied physical science".

Council for National Academic Awards : Degree Courses

Statement No. 3 from the Council for National Academic Awards deals with the approval of courses in science, technology, arts and social studies, leading to the award of the honours or ordinary degrees of B.Sc. or B.A. (Council for National Academic Awards. *Statement No. 3.* November 1965. Pp. 15 London: Council for National Academic Awards, 1965). For this purpose the Council has established two Boards of Studies: the Committee for Science and Technology, and the Committee for Arts and Social Studies. The *Statement* deals with the admission of students to courses, the duration and content of courses including training in sandwich courses, with teaching and with examination arrangements. Students who successfully complete honours courses will be granted degrees in four classes: first-class honours; second-class honours (first and second divisions); and third-class honours.

University of Newcastle upon Tyne : Vice-Chancellor's Report

THE Vice-Chancellor's report to the Court and Council of the University of Newcastle upon Tyne for the period August 1, 1964–July 31, 1965, is accompanied by departmental reports and lists of publications, arranged under departments (Pp. 152. Newcastle upon Tyne: The University, 1965. n.p.). It records 3,996 undergraduate and 587 postgraduate students; 1,554 students entered in October 1965, compared with 8,702 applications, making a total of 628 postgraduate and 4,247 undergraduate students. Deep concern is expressed over the erosion of the value of recurrent grants in the present quinquennium. A grant from the Department of Scientific and Industrial Research enabled the University to establish the first centre in Britain for retrieving information on medical science. A university grant enabled members of the University to participate in the 1965 Fezzan Expedition, while the Exploration Society sent an expedition to Afghanistan and undergraduate expeditions to Iceland and to Greece.

Announcements

DR. J. E. RICHEY, formerly district geologist, H.M. Geological Survey, Scotland, has been awarded the Neill Prize for the period 1963–65 by the Council of the Royal Society of Edinburgh, in recognition of his investigations on the structure of the tertiary volcanoes of Scotland and Northern Ireland.

MR. W. T. COOPER has been appointed national chairman of the Institute of Fuel, Australian Membership, in succession to Prof. N. Y. Kirov.

THE Deutsche Akademie der Naturforscher, Leopoldina has announced the appointment of the following new members: Prof. E. Hasche (Bad Berka); Prof. E. Lesky (Vienna); Prof. H. von Mayersbach (Nijmegen); Prof. H. Merxmuller (Munich); Prof. K. W. Merz (Freiburg).

A COURSE on "Information on a Shoe-string" will be held by ASLIB on March 17. Further information can be obtained from the Education Officer, ASLIB, 3 Belgrave Square, London, S.W.1.

A CONFERENCE on "Stresses in Service", under the auspices of the Joint British Committee for Stress Analysis, will be held at the Institution of Civil Engineers during March 23–25. Further information can be obtained from the Secretary of the Conference, Institution of Civil Engineers, Great George Street, London, S.W.1.

A ONE-DAY meeting of the Biological Group of ASLIB to discuss the problem of training for university students, research workers and librarians in the biological field will be held at the Northampton College of Advanced Technology on March 25. Further information can be obtained from Miss P. I. Edwards, Department of Botany, British Museum (Natural History), Cromwell Road, London, S.W.7.

A SYMPOSIUM on "Potatoes for the Food Processing Industries", sponsored jointly by the Fruit and Vegetable Preservation Research Association and the Department of Food Science and Technology of the Borough Polytechnic, will be held at the Borough Polytechnic on March 17. Further information can be obtained from Dr. D. B. Smith, Department of Food Science and Technology, Borough Polytechnic, London, S.E.1.

A COURSE of six evening lectures on "The Mechanical and Metallurgical Aspects of Metal Forming" will be held at the Bradford Institute of Technology on Wednesdays during February 23–March 30. The programme will include: introductory metallurgical principles; crystal structures and imperfections; application of dislocation theory; introduction to the theory of plasticity; mechanics of metal deformation. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

A CONFERENCE on "Electronic Processes in Low Mobility Solids", sponsored jointly by the Departments of Physics and Glass Technology of the University of Sheffield, will be held in the University during April 18–20. The purpose of the meeting is to discuss transport phenomena, optical, dielectric and other properties associated with electron (or polaron) hopping and tunnelling in solids in which normal band theory may not be applicable. Further information can be obtained from Dr. A. E. Owen, Department of Glass Technology, The University, Northumberland Road, Sheffield 10.

FIELD AND GLASSHOUSE EXPERIMENTS

A SYMPOSIUM on the "Layout, Management and Recording of Experiments in Field and Glasshouse" was held in London on November 24, 1965, by the Horticultural Education Association, in association with the Association of Applied Biologists and the British Ecological Society. In opening the symposium, Prof. L. Broadbent, president of the Horticultural Education Association, said that members had requested an opportunity to learn something about the principles underlying experimental work without becoming too involved in statistics.

The first paper was by Dr. S. C. Pearce (East Malling Research Station), who discussed "Determining the Objects of the Experiment" so that data could be analysed and results interpreted. Before starting an experiment its objective should be clearly defined and a different hypothesis, suggested by examination of the data, should not be tested although the arithmetic involved might be virtually the same. Variability within even apparently uniform material existed and, indeed, was the basis of the 'analysis of variance'. In good designs, which were based on randomization and replication, variability from external causes, such as soil and environment, could be calculated and removed from the total variation and the likelihood of incorrect results in an experiment could be determined. Although the 'coefficient of variability' was often useful in comparing one experiment with another in a series of experiments, it could be most misleading when used in isolation, as its numerical value depended to some extent on the inherent variability of the material used in the experiment.

D. Cooke (University of Sussex), in considering "The Design of the Experiment", said that a critical examination ought to be made after data had been analysed. This should reveal whether the design selected had been 'efficient', that is, whether the experiment had been worth doing at all and what inferences could be made. He suggested that perhaps it was a pity that there were no critics in science comparable to art critics. He explained why the appropriate unit to use with many experiments involving glasshouse crops is a whole glasshouse and not part of one. It should be recorded in this connexion that nine similar glasshouses have been built at the Glasshouse Crops Research Institute in order that multi-factorial experimental designs may be used.

He also suggested that the effect of 'scaling up' in horticulture should be investigated. Experience had shown that marked effects in experiments were often very much reduced when applied on a large scale, but in the horticultural industry, unlike chemical engineering, no scale models were available.

In discussing the 'three R's' of experimentation—restriction, randomization and replication—Cooke recommended that the major sources of variation often warranted a careful examination. If the residual variation per plot, after allowing for variation due to treatment and block, was large, a different design for future experiments should then be considered to see whether a greater amount of variation could be accounted for and so removed in the analysis of variance.

D. Price Jones (Imperial Chemical Industries, Ltd.), opening the discussion on the foregoing two contributions, commented that pilot trials, general surveys and small simple experiments which could be repeated over a wide range of soils still played an important part. Judging by the many and varied questions which followed, there is a real desire for guidance from statisticians on such technical matters as the number of plots to use, how to score when qualitative effects are being assessed, how precise measurements should be, and on many other day-to-day matters which have to be decided by experimentalists, even if a statistician is available for overall planning.

"Multivariate Analysis", the subject of J. N. R. Jeffers (Forest Research Station), was introduced by the chairman, Prof. A. H. Bunting (University of Reading), as a logical basis for understanding biological situations. Now that data are processed by electronic computers, lengthy arithmetical calculations may quickly be done, but the real advantage, as Jeffers said, is not in the speed of working but in being able to use techniques hitherto not even contemplated because of their complexity. In the past, when a number of different variates have been measured in an experiment (for example, dry weights, lengths, number of leaves), analyses have been carried out for individual variates, or two of them have first been combined in some way (for example, as a proportion or percentage). In multivariate analysis, however, even when variates are measured in different units (grams, centimetres, etc.), provided they are independent (that is, there is not a constant relation between two of them), a mathematical relationship which includes all the variates may be found to describe a biological situation and comparisons may then be made between different situations. Such a mathematical equation does not immediately show the relative importance which may be attributed to the different variates, but Jeffers outlined how this problem could be solved by a calculation known as principal component analysis. In this analysis data are transformed at the outset to counteract the effect of different units being used and to ensure that one factor is not overweighted because of the arbitrary selection of the original dimensions (for example, measuring in millimetres rather than in centimetres). Two of the transformations in use involve finding a variance-covariance matrix between every set of variates and the computation of a correlation matrix. Jeffers concluded by referring to canonical analysis, another technique which may be used when comparisons are made of biological material and data for a number of variates exist. He forecast that fewer experiments would probably be done in the future, but that they would be larger and better planned and would require the advice of a statistician at all stages, particularly in the interpretation of the results.

It was stimulating to hear of the possibilities of obtaining so much information from one experiment, provided a statistician was at hand to clarify the complex situation which would arise. The ecologist, on the other hand, starts with a complex interacting system containing plant and animal species, different soils and so on, and attempts to describe it mathematically. Dr. M. B. Dale (University of Hull) discussed numerical techniques in ecological research and showed how investigations fall into two main groups, those in which the frequency with which a particular factor is present is recorded and those in which only their presence or absence is noted. In both cases, much depends on the initial decision as to which items to investigate. Samples are taken from appropriate sites, either at random or in some systematic way, rather like taking records at stated intervals of time. The data may be analysed by an 'ordination' technique when the samples are put in sequence, so that the two which differ most are

at the ends. A number of such sequences are possible according to the factors chosen as being most important. A more usual method of handling the data is by 'classification' when like samples are grouped together. In both cases a personal decision as to the relative importance of the different factors involved determines the results; it should be remembered that what seems unimportant at the time of sampling in ecological work may be of great interest in later years. Although both principal component analysis and canonical correlation analysis are being tried, some other analysis, more easily interpreted, is required. Dale is hopeful that, by introducing some form of 'weighting', he will be able to produce a satisfactory classification method.

In the final general discussion, Jeffers said that suitable statistical techniques were becoming so advanced that the service of one professional statistician would be required to about six research workers, if full value was to be obtained from an experiment. With the present shortage of statisticians that ideal seems remote, but members of the symposium welcomed Prof. Bunting's announcement that, beginning in October 1966, there would probably be a one-year M.Sc. course in biometry at the University of Reading. Nevertheless, research workers will still have to formulate a hypothesis, be able to discuss the layout of the experiment with a statistician, supervise the day-to-day management and obtain appropriate data. Finally, even if the analysis is obtained for him by the statistician, with the use of an electronic computer, he must be able to discuss the interpretation of the results and translate them into biological terms. A working knowledge of the 'language' of statistics and of the underlying principles would seem to be increasingly necessary for all those responsible for field and glasshouse experiments and for those interested in the application of their results. It is hoped that another symposium will be arranged to continue the education of the non-statistician who uses statistical tools.

Members of the three organizations responsible for the symposium are greatly indebted to Mr. J. H. Walker, of East Malling Research Station, for arranging this symposium and to the various speakers for presenting their specialized subjects to an audience of horticulturists and biologists, many of whom had had no formal training in statistical methods.

M. E. Marston

EUROPEAN JOURNAL OF CANCER

THERE is, and has been since 1936 when the International Union Against Cancer was founded, extensive international collaboration and exchange in the field of cancer research. This co-operation has been stimulated in recent years by the idea of an international institution of cancer research located in Europe and also by the efforts of the World Health Organization. At the European regional level such groups as the European Committee for Cancer Chemotherapy and the European Committee for Human Tumour Investigation are also playing their part in encouraging inter-European collaboration between clinicians and scientists engaged in the study of cancerous diseases. It is logical that these European groups and their associated workers should now have a journal which will provide a forum for the exchange of ideas and information. This journal is the *European Journal of Cancer* which made its first appearance in June of last year*.

The editorial advisory board of the new journal consists of workers of repute in the cancer field drawn from nine European countries, and the policy of the board will be to publish original work in the field of experimental and clinical cancer research, papers giving the results of planned clinical studies and also general reviews on subjects of particular relevance to these researches and studies.

The first issue contains eight papers from laboratories and centres in Belgium, France, Holland and Italy, so justifying the adjective European in the journal's title. There are two papers from the Radiological Institute of the Netherlands. One by L. M. van Putten presents the results of studies on the effects of different storage techniques on mouse and monkey cell suspensions, and the other is by H. S. Reinhold, in which he describes a combined mechanical and enzymatic method for obtaining a single cell suspension from solid tumour tissue. The method allows a concentrated suspension of a large number of cells to be prepared in a short time. The paper from Milan by G. G. Guidotti and others is on the inhibiting effects of aliphatic aldehydes on protein synthesis in Yoshida ascites hepatoma cells. The paper from France consists of a review by G. Mathé on immunological reactions in their relationship to the development of cancer and the possibility of immunotherapy in its treatment. The four remaining papers come from Belgium. R. van Nie and P. J. Thung contribute a paper on the hormonal influence of pregnancy on mouse mammary tumours and its implication for tumour behaviour and progression. J. Hildebrand and C. Coers report six cases of polyneuritis with severe damage to peripheral nerves in patients receiving intravenous injections of 3–5 mg vincristine per week for varying periods. Vincristine is an alkaloid extracted from the periwinkle flower which, although very toxic, has shown promise as an effective agent in Hodgkin's disease and certain leukaemias, and to a much lesser extent in other tumours. This study confirms previous reports of polyneuropathy caused by vincristine treatment. Another paper dealing with a chemotherapeutic agent is that by Y. Kenis and others, again from Brussels, which reports the results of treating patients suffering from Hodgkin's disease, various lymphomata and leukaemias, with a methylhydrazine derivative. The fourth paper, from a centre in Belgium, consists of a general review of the literature reporting cases of metastatic liver cancer associated with renal dysplasia. This paper is contributed by G. Buyse and others from the hospital at St. Gilles and Brussels University.

The November issue contains the papers given at the International Symposium on the biological characterization of human tumours which was held in May of last year. That these papers should have appeared in print so soon after their formal presentation shows a promptness in symposium publication which is as rare as it is commendable.

The standard of typography and lay-out, the quality of the paper, and the illustrations are of the high standard which we have come to expect from the Pergamon Press. A particularly pleasing aspect is the way in which the bibliographical references are given in full with the titles of the papers quoted. This greatly increases the value of the list of references and is a house rule which could well be copied by other journal publishers. The annual subscription price to individuals is a very reasonable £5 5*s*. The annual subscription price to libraries is £14, which follows this publisher's usual practice of being at a higher differential rate: a practice which I deplore.

D. A. Brunning

* *European Journal of Cancer*, **1**, No 1 (June 1965). Pp 1–74. Published quarterly. Subscription rates: (*A*) 280*s*. (40 dollars) per annum for libraries, government establishments and research institutions, (*B*) 105*s*. (15 dollars) per annum for individuals who write directly to the publisher and certify that the journal is for their personal use. (Oxford Pergamon Press, Ltd., 1965.)

THE BAAS BECKING BIOGEOLOGICAL RESEARCH LABORATORY, CANBERRA, AUSTRALIA

THE Baas Becking Biogeological Research Laboratory, which will shortly move into its permanent home at the new Bureau of Mineral Resources Building in Canberra, was established in July 1965, under the joint sponsorship of the Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.), the Bureau of Mineral Resources (B.M.R.) and the Australian Mining Industry Research Association (A.M.I.R.A.). The broad objective of the research work is to investigate the biological and chemical processes associated with the formation of mineral deposits and with other geological phenomena.

The Laboratory is named after the late Dr. L. G. M. Baas Becking, who was one of the first to recognize the possibility that biological, in particular microbial, activity may play an important part in the genesis of sulphide ores.

Laurens G. M. Baas Becking was a research scholar of wide interests, equally renowned in the United States, Java, Australasia and his native Netherlands. His investigations of microbial involvement in the syngenetic deposition of minerals arose from an interest in the milieu of life and its environmental limits. This interest first brought him to Australia in 1936 to investigate, in collaboration with Dr. J. Reuter, life in salt lakes, as part of his research into the effect of organisms on changes in soil, lakes, estuaries and on mineralization.

For a long period, the mode of deposition of certain types of large base-metal ore deposits was open to conjecture; two theories were advanced, sedimentary deposition and hydrothermal emplacement. By the early part of the twentieth century, the hydrothermal theory was widely accepted. Most ore deposits, and virtually all sulphide ore deposits, were believed to have been formed by a process of hydrothermal introduction of the ore minerals at high temperature, into fractures and by metasomatic replacement, and it was assumed that the textures of the ore minerals were the result of this emplacement. It followed that these textures could therefore be used to determine the depositional history of the deposits.

In the 'fifties the view gained ground that this was not the only way in which base metal ore deposits were formed. Some of the ore deposits emplaced in sedimentary and metamorphic rocks were in fact laid down with these rocks. This process was termed syngenesis. The mechanisms of syngenetic deposition are still mostly unknown, but the possibility has been recognized that biological factors might be involved.

In Australia, during the late 'fifties and early 'sixties, some investigations into the general problem of syngenetic sulphide ore formation were initiated. Laboratory research was pursued into the low-temperature formation of sulphide minerals, crystallization of amorphous base metal sulphides at high temperature under defined conditions and the biological formation of mineral sulphides. The biological studies were undertaken by Dr. Baas Becking, who succeeded in demonstrating the formation of a number of metal–sulphide minerals by sulphate-reducing bacteria in artificial sea water at room temperature.

In 1960, a number of mining companies, operating through the Australian Mineral Industries Research Association, joined with the B.M.R. and the C.S.I.R.O. in financing a small research unit to enable Dr. Baas Becking to pursue his studies. Ill health unfortunately prevented Dr. Baas Becking from doing this, but his work was generally confirmed and appreciably extended by Dr. K. L. Temple during the period 1961–63. In general, the work reached the stage where it could be visualized that under certain conditions the biogenic formation of sulphides might be an important factor in the formation of some economic ore deposits. As a result of this work, it was decided to embark on a more ambitious research programme and the three supporting organizations formed the Baas Becking Biogeological Research Laboratory.

Initial emphasis will be placed on investigations to establish the relationship of biological factors to the natural physico-chemical environment with particular reference to the possible role of these factors in the formation and transformation of sulphide minerals. Investigations will include the response of micro-organisms to heavy metals; biochemistry and physiology of oxidative and reductive sulphur transformations; role of organisms in the concentration of mineral elements; physico-chemistry of low-temperature mineral synthesis; mobility of sulphides under the influence of temperature and pressure and the interaction of mineral types; and biological leaching of low-grade sulphide minerals.

It is planned to extend research at a later stage to include other important minerals of possible biogenic origin such as carbonates and phosphates. Dr. P. A. Trudinger and Mr. W. M. B. Roberts will be in charge of research operations, responsible to a committee of management under the chairmanship of Mr. Haddon F. King.

J. E. FALK

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LOCAL HEALTH SERVICES

EXPENDITURE on health by local authorities has been rising faster than expenditure on the National Health Service as a whole. The National Health Service accounted for more than 10 per cent of the total expenditure in 1964, compared with little more than 7 per cent in 1949. Expenditure on health services by local authorities more than trebled between those years, to reach a total of £107 million for the United Kingdom in 1963–64. This information is given in an Office of Health Economics report * which emphasizes the changing pattern of work now being carried out by local health authorities.

The largest proportional increases in expenditure are due to the provision of more 'home helps' (from £2·3 million to £12·3 million) and to extending the services for the mentally ill in the community (from £1·2 million to £9·3 million). The number of 'home helps' employed rose from about 19,000 in 1949 to more than 60,000 in 1964. Four-fifths of all their work is looking after elderly people at home; the report points out that, for every elderly person visited by a 'home help', there are two others who need help with their housework and are not getting it. Practically no community care was provided for the mentally ill before the start of the National Health Service. By 1964, 156,000 people outside hospital were benefiting from local mental health services.

* Office of Health Economics. *The Local Health Services*. Pp. 40. (London: Office of Health Economics, 1965.) 2*s*. 6*d*.

The largest actual increase in expenditure has been in the ambulance services. This has risen from £6·5 million in 1949 to £18·8 million in 1964. The total number of patients carried has risen even more, from 7 million in 1949 to almost 20 million in 1964. Four-fifths of ambulance work consists of carrying patients to and from out-patient departments, and a much larger proportion of such patients are now travelling by ambulance. At the start of the Health Service only one out of every ten journeys between the home of an out-patient and the hospital was made by ambulance; now, one in every four journeys is by ambulance.

The report emphasizes how difficult it seems to be to persuade people to accept immunization against infectious diseases. Only 66 per cent of infants born in 1961 were immunized against poliomyelitis. It comments that "the dangers of infectious diseases have diminished to an extent undreamed of 30 years ago; but there can be one disadvantage of a successful vaccination campaign. This is the comfortable but false assumption by the general public that it has been a once-for-all battle which has eliminated the threat forever".

The report directs attention to the large differences between local health expenditures in different parts of Britain. They range from the expenditure by Northampton County Council of £1 3*s*. per head of population to that of £3 1*s*. 2*d*. by the Cardigan County Council. In terms of personnel, this means a two-fold variation in numbers of health visitors, and a three-fold variation in numbers of 'home helps', nurses and midwives. The report comments that "in this case, geographical factors are undoubtedly important; but, in general, it is not easy to say whether variations between the level of provision in different areas are due to differences in need, differences in financial resources, or differences in the appreciation of the value of the services". These differences in levels of expenditure between different parts of Britain are expected to continue during the next ten years.

The report calls for "a substantial operational research programme" to "evaluate in depth the services actually provided by typical high spending and low spending areas". The results, it says, could be coupled to those of community studies; these have already suggested that there are many needs unmet, especially in the case of the elderly at home.

Commenting on plans for the future, the report points out that "the trend towards community care and the consequent necessity for close co-operation between the various sections of the tripartite Health Service have provided an opportunity for the local health authorities to play a more prominent part". However, the plans for the next ten years prepared by the local health authorities "imply a slackening rate of expansion in their local health services which becomes very marked after the end of this decade".

The report suggests that the falling off in expansion of the services planned by the authorities must be taken primarily as a reflexion of the difficulty of forecasting for a situation in which there are still so many unknowns. The changing responsibilities of the general practitioner, the increasing skill in early diagnosis and the development of day hospitals are but a few of the external factors which must affect the function of the local health authority. What is important is to maintain flexibility, and, above all, to avoid recrimination if the present rate of expansion of the local health services in fact continues far into the 1970s. Those who framed the National Health Service made a "miscalculation of sublime proportions" in assuming that its costs would not rise as the Service developed, and much criticism of actual expenditure resulted. The ten-year plans of the local health authorities inevitably appear to contain a similar fallacy, and it would be unfortunate if these were eventually to result in similar misunderstanding.

"In this connection", says the report, "it is encouraging to note that the new National Plan takes a more expansive view of the local health services in the future. While the local authorities forecast an increase in current expenditure of only 4 per cent in 1968–69, with an annual average of 1·5 per cent in the following five years, the National Plan refers to the annual rate of increase having risen to 6·5 per cent in 1969–70. Whereas local authorities forecast an increase in net revenue expenditure of 30 per cent in the decade from 1964–65, the National Plan forecasts an increase of 34 per cent within the first five years."

THERMODYNAMICS OF NEW GENERALIZED TRANSPORT LAWS FOR LIQUIDS, GASES AND ELECTRONS IN MATTER

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It is well known that thermodynamics constitutes the foundation of numerous conventional principles and theories in the physical, life and engineering sciences. Some important parts of thermodynamics are the criteria of thermal equilibrium. It has been almost invariably assumed that for any thermodynamic system in thermal equilibrium, the chemical potential is constant everywhere in the system for each constituent. Such a criterion, as will be shown in this article, can only be justified if the particle concentration in the system is uniform everywhere for each constituent. The last statement is substantiated by at least one case treated in the classical work of J. W. Gibbs, to be referred to later in this article.

For the particular case of electrons in solids, it is well known that these particles obey the Fermi–Dirac[1,2] statistics, and that at constant temperature the Fermi-level is equal to the chemical potential. Thus, it has been conventionally concluded that in thermal equilibrium the Fermi-level for electrons must also be constant throughout any solid. This assumption alone constitutes a foundation for much of the transport theory of electrons in solids as it is known to-day.

It has been pointed out that the validity of assuming that the Fermi-level is constant in thermal equilibrium would not be justifiable for electrons in non-degenerate *p-n* junctions[3]. Similar objections have been raised for electrons in any heterogeneous solid[4]. If the arguments in refs. 3 and 4 are valid, one would expect that the conventional criteria of thermal equilibrium as a whole might involve some basic misconceptions. If so, it should then be possible to show these misconceptions by new direct arguments and conventional thermodynamics. Attempting to point out and treat this serious matter will be the main objective of this article.

To investigate the criteria of thermal equilibrium, new phenomenological laws will be formulated. These will describe the transport of conduction electrons in any form of matter, and more generally the transport of particles in fluids enclosed in stationary containers, under

the action of any finite number of processes of different nature, in and near static equilibrium. The new transport laws lead to the fact that in thermal equilibrium generally the differentials of the chemical potential, the Helmholtz free energy, and the Gibbs function must all become exact differentials rather than necessarily vanish, as conventionally assumed.

The new phenomenological criteria of thermal equilibrium will be verified quantum statistically for boltzon, fermion and boson particles. It will be shown that these criteria, unlike the conventional ones, make it possible to formulate what is to be called the equation of internal state for liquids. The latter equation is based on the virial theorem. It will be further demonstrated that Fick's law for ideal classical isothermal liquids is a consequence of the new transport laws and the equation of internal state for liquids.

The transport laws to be formulated are meant to describe steady-state conditions associated with time-independent processes. Under such conditions conceivably the concentration of particles is not time-dependent. Thus, there is no accumulation of particles anywhere and for charged particles there are no displacement[5] currents at any point. These assumptions clearly hold in static equilibrium and for many other interesting situations in dynamic equilibrium.

(1) Transport Correspondence Principle

Since the discovery of the Seebeck effect[6] in 1826, the Peltier effect[7] in 1834, and other transport phenomena in solids, the electron transport theory in its conventional form has developed along lines almost completely different from those of what is at present known as network or circuit theory. The non-network transport theory seems to be essentially based on thermodynamics and some supposedly accepted criteria of thermal equilibrium.

Circuit theory, on the other hand, has been largely based on seemingly different laws. These include Ohm's law and Kirchhoff's voltage and current laws. It is rather difficult to explain why the foundations of circuit theory and the rest of the transport theory have been formulated differently. Perhaps the conditions of circuit theory appeared to be considerably more special than those of the transport theory in general. Thus it so happened that the fundamental laws, which simultaneously link nearly all electron transport phenomena in matter, including those of circuit theory, and more generally systems of mobile particles, seem to have passed unnoticed. This article attempts to describe this missing link and some of its fundamental new implications. We begin in this section with postulating what is to be called 'the transport correspondence principle'. This principle may be stated as follows: to every time-independent law in network theory there corresponds another to hold in general for the electron transport in any form of matter, in and near static equilibrium, provided that the network law and its conditions are entirely describable in terms of non-restrictive physical quantities. As will be explained in section (4), a similar statement may be made for any system of mobile particles, if the system is enclosed in a stationary container.

The term 'non-restrictive' used here is meant to describe physical quantities, which are not characteristic of only one process of a specific nature. Rather they must signify a universal physical quantity. Thus, we may describe the electron force, energy, and volume concentration as non-restrictive physical quantities. On the other hand, the electric or magnetic-field intensities, according to the definition here, are restrictive physical quantities. Each one is characteristic, and indicative of the existence of a specific physical situation of a specific nature.

The following sections will describe a number of interesting general consequences of this new fundamental principle.

(2) The Electron Force–Current Generalized Law in Passive Media

This section is concerned with formulating the first electron generalized transport law. To explain this law, consider Fig. 1, which represents a loss-less battery with an e.m.f. V_B connected to a resistor R. The current I_R through R may be described in terms of the voltage V_R across R by Ohm's law as follows:

$$I_R = V_R/R \tag{1}$$

Notice here that all the three quantities in equation (1) belong to one element; namely, the resistor R.

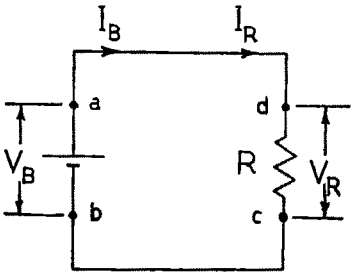


Fig. 1. A loss-less battery connected to a resistive element R

Let us next write an expression for the battery current I_B. This will be:

$$I_B = V_B/R \tag{2}$$

Notice here that while I_B and V_B both belong to the battery, R does not belong to that circuit element. Rather it belongs to an element outside the battery. Thus we may state the following: The current through any resistor is determined by Ohm's law in terms of all the variables of that resistor. However, the current through an e.m.f. source cannot be determined by all the variables of the source. Rather complete information for the whole closed loop is needed to determine the current in the e.m.f. source.

Let us next transform equation (1) and the statement just made into a non-restrictive form so that the transport correspondence principle of section (1) may be applied. To do so, consider a point P within the resistor. Let the electric field intensity at P be $\mathbf{E}$. The electron current density $\mathbf{J}_n$ at P may be expressed as:

$$\mathbf{J}_n = \mu' n(q\mathbf{E}) \tag{3}$$

Here μ' and n are respectively the electron mobility and volume concentration at point P, and q is the magnitude of the electron charge. It should be noticed here that equation (3) can be derived from equation (2) and the definition of mobility.

It is apparent that $(-q\mathbf{E})$ in equation (3) represents the electrostatic force $\mathbf{f}_e$ acting on the electron at P. Let us now imagine that $\mathbf{f}_e$ at P has been replaced somehow by another equal force $\mathbf{f}$ of any nature. It is conceivable that the electron current density $\mathbf{J}_n$ would remain unchanged, provided that $(\mu' n)$ remained constant. Therefore we may write:

$$\mathbf{J}_n = -\mu' n\mathbf{f} \tag{4}$$

and say that $\mathbf{f}$ is the generalized force per electron at P. The nature of this force here, of course, is totally unimportant.

It must be remembered here that equation (4) is a non-restrictive transformation of Ohm's law described by equations (1) and (3). Equation (1) is only valid for a resistor but not an e.m.f. source. Similarly, equation (3) is only valid in solid regions not containing e.m.f. sources. Such regions will be referred to as 'passive regions'.

In order to apply the transport correspondence principle to this last-mentioned condition, again we must state it in a non-restrictive way. Regions involving only e.m.f. sources, hereinafter called 'active regions', have merely spatially varying electron energy not caused by electric

currents. This will mean that the electron energy in such regions has gradients everywhere at zero currents. These energy gradients will be defined as 'motive' or 'active' forces.

Examples of active regions in solids may be the transition region in a p-n junction or generally the heterogeneous regions in solids, isothermal or otherwise. The term heterogeneous here is used in the same sense as is defined in ref. 4. In all such regions, as is well known, the electron energy varies spatially everywhere even in thermal equilibrium, that is, when there is no electron current anywhere.

Examples of passive regions in solids include all homogeneous isothermal regions such as uniformly doped p- or n-regions in p-n junctions. In such regions the electron energy is constant so long as the electron current is zero everywhere, that is to say, in thermal equilibrium.

On the basis of the preceding discussion, one may state the first generalized transport law for electrons in any form of matter as follows. Near and in static equilibrium, the steady-state electron current density $\mathbf{J}_n$ at a point P is related to the time-independent force per electron $\mathbf{f}$ at that point by: $\mathbf{J}_n = -\mu' n \mathbf{f}$, provided that P lies only in a passive medium. Specifically this relation is meaningless if P is in an active medium. A similar statement of this law may be made for any system of mobile particles, provided that the system is enclosed in a stationary container.

(3) Loop Conservation of Energy for Conduction Electrons in Matter

The object in this section is to discuss what is to be called the 'second generalized transport law' for electrons in any form of matter. Using the same line of reasoning as that of the previous section, one may formulate this law, which can be stated as follows. Under steady-state conditions, the line integral of the active force per electron around any closed loop in any form of matter must be equal to the line integral of the passive force per electron around the same loop. This is so whether or not there are currents at various points of the medium. When the actual electron flow along the loop is taken to be a reference for direction, active and passive, forces along the reference direction must be assigned the same sign. For example, both forces must be considered positive. As will be explained in the next section, a similar statement may be made for any system of mobile particles, provided that the system is enclosed in a stationary container.

It should be indicated here that the aspect of conservation of energy of this law is, of course, quite familiar. What is new, however, is the statement of the law in terms of the force per particle in active and passive media.

(4) Generalized Criteria for Static Equilibrium of Electrons in Matter and of Mobile Particles Generally

The object in this section is to arrive at the necessary and sufficient condition for the static equilibrium of any one constituent of a system of particles, compressible or otherwise, if the system is enclosed in a stationary container and is subject to any finite number of time-independent processes of different nature. Before discussing this general problem, a derivation will be presented for a system of a specific form, namely, electrons in various forms of matter.

For electrons in solids, the conditions to be sought have been stated in ref. 4 on the basis of a seemingly paradoxical argument. It is now possible on the basis of the first and second generalized transport laws to derive it on more rigorous grounds. To do so, consider any medium containing active and passive regions. Let the conduction electrons in the medium be in static equilibrium everywhere. Thus, according to the first generalized transport law, the force per electron is zero, at every point lying in a passive region. Imposing this relation on the second generalized transport law, expressed in the previous section, we obtain:

$$\oint \mathbf{f}.\mathrm{d}\mathbf{s} = 0 \qquad (5)$$

Here $\mathbf{f}$ represents the generalized force per electron in the active regions of the considered medium and the integration is carried around every closed loop in the system. In view of Stokes's theorem[8], equation (5) may be expressed in the microscopic form as:

$$\nabla \times \mathbf{f} = 0 \qquad (6)$$

The generalized conditions for the static equilibrium of electrons in various forms of matter expressed by equations (5) and (6) may be further described in a third form. From the point of view of energy, the quantity $\mathbf{f}.\mathrm{d}\mathbf{s}$ merely represents the infinitesimal work done, $\mathrm{d}W$, per electron when it is transported a distance $\mathrm{d}s$. If the integral of $\mathrm{d}W$ must vanish around every closed loop, it follows that everywhere:

$$\mathrm{d}W = \text{an exact differential} \qquad (7)$$

Such a property would constitute a third generalized criterion for the static equilibrium of electrons in any form of matter.

So far the main concern has been to treat the transport of electrons in matter near and in static equilibrium. Such sub-atomic particles indeed constitute one form of a system of mobile particles. A question of considerable interest may be raised as to whether it would be possible to apply all the generalized transport laws formulated for electrons for all other mobile particles near and in static equilibrium. If a positive answer exists, one must be able to show that conduction electrons in matter are at least as general hydrodynamically and otherwise as any other type of particles such as fluids.

Let us first investigate the question of compressibility for electrons in matter. That electron conduction can involve electron compressibility even without a recombination process may be directly shown by considering, for example, the conduction in any medium free of such a process. Under steady-state conditions, it is well known that the continuity equation[9] for electrons leads to:

$$\nabla \mathbf{J}_n = 0 \qquad (8)$$

It is also well known that the density of the electron current is related to the carrier flow velocity $\mathbf{v}$ at any point by:

$$\mathbf{J}_n = -qn\mathbf{v} \qquad (9)$$

From equations (8) and (9), it follows that:

$$\nabla.(n\mathbf{v}) = 0 \qquad (10)$$

The implication of the preceding equation is that $\nabla.\mathbf{v}$ will vanish only if n is constant. Hence, whenever conduction by electrons occurs in any medium and $\nabla n \neq 0$, then $\nabla.\mathbf{v} \neq 0$. Examples, for which $\nabla n \neq 0$, are the interfaces between any two different metals or between a solid and a liquid. It is well known hydrodynamically[10] that a fluid is considered to be 'incompressible' if $\nabla.\mathbf{v} = 0$, and conversely. Thus, conduction by electrons in any medium where $\nabla n \neq 0$ indeed involves compressibility even in the absence of the process of recombination. This particular statement will have some especially important consequences to be discussed in section (6) concerning the question of validity of the conventional thermodynamic criteria of thermal equilibrium.

From the preceding discussion, it seems reasonable to assume that the two generalized transport laws and condition for static equilibrium do apply generally to systems of mobile particles which are enclosed in stationary containers. In further support of this statement, it should be indicated that, when the first generalized law of transport is applied to particles moving in a one-phase fluid, it reduces essentially to Stokes's law[11]. For such particles, of course, the fluid represents a passive region.

The applicability of the new generalized condition for static equilibrium to various kinds of systems of particles

subject to various types of processes may be further illustrated by some interesting examples. Consider a closed bottle containing water. Let this bottle be shaken before it is placed on a stationary table in an isothermal neighbourhood. After a sufficiently long time in the absence of any gravitational fields other than that of the Earth, there will be no flow in the water at any point. Static equilibrium will be established in this case despite the fact that every particle of the liquid of mass m is acted on by a gravitational force $\mathbf{f}_g = m\mathbf{g}$. Here $\mathbf{g}$ is the gravitational acceleration. Notice here that $\nabla \times \mathbf{f}_g = 0$, and equation (6) is satisfied.

Let us next consider a conducting solid near an antenna but electrically isolated from it. Clearly if the antenna is radiating electromagnetic waves, currents will be induced in the solid. Let now the antenna be fed from a d.c. source, so that only an electrostatic field will exist around the antenna where the solid is. Conceivably conduction electrons in this case will be in static equilibrium everywhere in the solid. According to Maxwell's equations[12], however, when the electric field $\mathbf{E}$ is not time-varying, then curl $\mathbf{E} = 0$ everywhere around the antenna including at all points of the solid. Thus curl $\mathbf{f}_e = 0$. Here $\mathbf{f}_e$ is the electrostatic force per electron at any point on or within the solid. Thus equation (6) is again satisfied.

Another interesting example lies within the domain of circuit theory. It is well known that the most general condition for a vanishing current everywhere in a multi-mesh network is that the e.m.f. around every closed loop is zero. This condition is equivalent to saying that the work done per electron around any closed loop is zero. Static equilibrium of electrons in this case will be established, regardless of whether the passive circuit elements were linear or non-linear. Notice here that equation (5) is satisfied.

From equations (5), (6) and (7), and the preceding examples, it is apparent that the generalized conditions necessary and sufficient for static equilibrium may be stated as follows. If a system of mobile particles, enclosed in a stationary container, is involved in any finite number of time-independent processes of any nature, it will be in static equilibrium everywhere within the container, if for each constituent due to each and every process: (1) the work done per particle around any and every closed loop is zero; (2) everywhere within the container the infinitesimal work done per particle dW when transported a distance ds is an exact differential; or (3) there exists an acting force $\mathbf{f}$ per particle the derivatives of which exist and which is irrotational everywhere within the container.

Thus far, the generalized conditions of static equilibrium for systems of mobile particles have been discussed for a number of processes which are not thermodynamic in nature. In the remaining sections, it will be demonstrated that these general criteria of static equilibrium indeed will equally apply to purely thermodynamic processes.

(5) Fundamental Thermodynamic Equations, the Chemical Potential, and the Thermodynamic Transport Energy Function

This section will be primarily concerned with summarizing the four fundamental thermodynamic equations associated with a thermodynamic system. From one of these equations, a rigorous new definition will be arrived at for the important quantity known as the chemical potential. The concept of thermodynamic fields will also be introduced.

Consider a one-constituent thermodynamic system. Let part of that fluid consist of N particles and occupy a volume V. Let U be the internal energy of the N particles, T its absolute temperature, P its pressure, and S its entropy. Let V be sufficiently small so that the pressure and temperature of the fluid are uniform throughout V. In an infinitesimal quasi-static[13] change involving a reversible process for which N is constant[14]:

$$dU = T\,dS - P\,dV \qquad (11)$$

Here $(T\,dS)$ represents the increment of heat energy given to N and $(P\,dV)$ is the amount of work done by the particles while expanding.

The preceding equation is a consequence of the first and second law of thermodynamics. Suppose, however, that during the occurrence of the process a small number dN of new particles entered quasi-statically the volume V. The first law of thermodynamics then will require that:

$$dU = T\,dS - P\,dV + \mu\,dN \qquad (12)$$

Here the quantity $(\mu\,dN)$ represents the amount of work done on the original particles by the dN particles, which entered the volume V. It has been assumed here that each of the dN particles has the same μ. This particular point will be further discussed shortly. The quantity μ is generally known as the 'chemical potential'. This fundamental quantity was probably first introduced in the classical work of J. W. Gibbs[15].

It is important to arrive at a rigorous definition for μ, which can be used consistently to describe generally the transport phenomena under isothermal conditions and even otherwise. Any basic definition for μ must clearly be expected to stem from the preceding fundamental thermodynamic equation. Since according to that equation $(\mu\,dN)$ represents the work done on the N particles by the dN particles introduced into the volume V, it must be that μ is the average work done by each particle introduced into V, due to a purely thermodynamic process.

The definition just stated for μ lacks completeness and, in fact, general validity. The reason for this is that the definition assumes simply that μ can always exist mathematically throughout the entire thermodynamic system, inside and outside the volume V. Unfortunately, this is not the case. As will be discussed in section (7), the quantity $d\mu$ will not necessarily be an exact differential except under isothermal conditions. Thus, under non-isothermal conditions, the integral of $d\mu$ between any two points must in general depend on the path linking these points. If we still wish to define μ uniquely under non-isothermal conditions, we must then specify a path and an arbitrary value for μ at some arbitrary point on the chosen path.

An analogous situation to this arises in electromagnetic wave theory. For example, in a medium in which an electromagnetic wave is propagated, it is not possible to specify the electric potential V at every point of the medium. This is so since, according to Maxwell's equations[12], the electric field intensity is rotational at every point. Thus, the quantity dV is not an exact differential and the integral of dV between any two points must depend on the path. If, however, a path is specified, such as a certain thin open loop of wire, and a reference point chosen on the path for measuring V, then it is possible to define uniquely the electric potential at every point of the wire.

To summarize the preceding discussion, we may define the chemical potential μ at any point within a sufficiently small volume V enclosing N particles to be the average work done, under the action of a purely thermodynamic process, on the N particles by each particle introduced into V after having been transported quasi-statically from a reference point along a prescribed path. It is meant by a sufficiently small volume here that the pressure and temperature are uniform throughout the volume V.

In problems connected with the transport of mobile particles it would be convenient to think in terms of the average work done on, rather than by, a transported particle. Thus, it would be desirable to introduce a new quantity ν which may be defined generally at any point A within a sufficiently small volume V enclosing N particles to be the average work done, due to a purely thermodynamic process, on each particle transported from an arbitrary

reference point along a prescribed path to point A. Let this function be called the thermodynamic transport energy function, or the transport function.

Comparison of the definition of μ and of ν clearly indicates that:

$$\nu = -\mu + C \tag{13}$$

Here C is an arbitrary constant, which will be zero if the reference points for both ν and μ coincide.

For the particles described earlier, one may write the important thermodynamic relation[16]:

$$U = ST - PV + \mu N \tag{14}$$

It is now possible to derive a fundamental expression for the differential of the chemical potential $d\mu$. This can be done by equating dU, as described by equation (12), to that determined from equation (14), and solving for $d\mu$. Carrying out these steps, we obtain:

$$d\mu = -(S/N)\,dT + (V/N)\,dP \tag{15}$$

The quantities (S/N) and (N/V) may be defined as the entropy per particle, s, and the particle concentration, n, respectively. Thus, equation (15) may be rewritten as:

$$d\mu = -s\,dT + (1/n)\,dP \tag{16}$$

or as:

$$\nabla\mu = -s\,\nabla T + (1/n)\,\nabla P \tag{17}$$

From equations (13) and (17), it follows that:

$$\nabla\nu = s\,\nabla T - (1/n)\,\nabla P \tag{18}$$

Since the dimension of ν is that of energy, clearly the dimension of $\nabla\nu$ is that of a force. Thus we may define $\nabla\nu$ as the thermodynamic generalized (acting) force per particle, and denote this quantity by $\mathbf{f}_t$. The term generalized refers to the fact that the two components of $\mathbf{f}_t$, although thermodynamic in nature, are of fundamentally different characteristics.

The reader will recognize the last term in equation (18) to be what has been called in refs. (3) and (4) the 'diffusion force per particle' and designated by f_d. From the original derivation of $\mathbf{f}_d$, in ref. (3), it is apparent that it is a mechanical force, caused by a pressure gradient. The middle term in equation (18), however, would exist only when there is a temperature gradient. Since its dimension is that of a force, it may be defined as the thermal effective force per particle, and will be designated by $\mathbf{f}_T$.

In view of the preceding definitions, and equation (18), the thermodynamic generalized force per particle may be expressed as:

$$\mathbf{f}_t = \mathbf{f}_T + \mathbf{f}_d \tag{19}$$

where:

$$\mathbf{f}_T = s\nabla T,\ \mathbf{f}_d = -(1/n)\,\nabla P \tag{20}$$

Equations (16) and (17) are well-known relations[17], even though they are not commonly used. It so happens, however, that these equations have some particularly interesting implications concerning the new criteria of thermal equilibrium to be formulated in section (7).

In addition to equation (12) there are three other fundamental thermodynamic equations. In the differential forms, these are:

$$d\mathcal{F} = -S\,dT - P\,dV + \mu\,dN \tag{21}$$

$$dG = -S\,dT + V\,dP + \mu\,dN \tag{22}$$

$$dH = T\,dS + V\,dP + \mu\,dN \tag{23}$$

Here $\mathcal{F}$ is the Helmholtz free-energy function, G is the Gibbs function, and H is the heat function or enthalpy.

(6) Critique of Conventional Criteria of Thermal Equilibrium

It is well known that in thermal equilibrium, for any thermodynamic system, the temperature must be constant everywhere. In addition to this condition, there are others commonly assumed. The object in this section is to show that, except for the condition of constant temperature, for some thermodynamic systems, the other conventional criteria of thermal equilibrium do not necessarily hold.

(*A*) *The pressure and chemical potential.* It is generally assumed that under thermal equilibrium conditions, the chemical potential for almost any thermodynamic system is constant everywhere. This assumption is considered to hold, for example, for electrons in any solid, regardless of the spatial distribution of the electron concentration. The criterion of thermal equilibrium just described follows at once from equation (16) only if the pressure is constant throughout the thermodynamic system.

Let us first discuss whether the conventional assumption concerning pressure is really true for all systems. One may be able to cite, of course, some examples for which $dP = 0$ in thermal equilibrium. On the other hand, one can find also many other interesting systems for which this assumption does not hold. Examples of these are: (1) an isothermal atmosphere around an isolated planet, the gravitational field of which is sufficiently strong to hold the gas; (2) the electrons in two different isothermal solid crystals in physical contact.

In the first example, the pressure can be shown[18] to decrease exponentially as a function of height in thermal equilibrium. In the second example, since the vapour pressure P for electrons may be defined[19,20] quantum-mechanically on the basis of the virial theorem, P will indeed be a function of the concentration n. The latter is not the same in the two different solids under consideration in thermal equilibrium. Therefore, across the interface between the two solids $dn \neq 0$ and $dP \neq 0$ in thermal equilibrium.

The conclusion that $d\mu \neq 0$ whenever $dP \neq 0$ in the two preceding examples is an inevitable consequence of equation (16). It might be of historical interest to state that the example of an atmosphere in thermal equilibrium happens to have been treated by Gibbs[21], who also has concluded that for that case $d\mu \neq 0$ in thermal equilibrium. Clearly it may now be stated that under thermal equilibrium conditions, $d\mu$ will never vanish in any system wherever $dP \neq 0$; that is, wherever $dn \neq 0$.

The conventional argument supposedly leading to a constant μ in thermal equilibrium is not usually based on the aspect of pressure as just explained. Rather it is based on an equation similar to (21), which may be written as follows:

$$d\mathcal{F} = -S\,dT - P\,dV + \sum_k \mu_k\,dN_k \tag{24}$$

The last term is a summation of more than one product, since it is needed here supposedly to test whether or not the particles would have the same chemical potential at different points.

The conventional argument supposedly proving that for electrons in solids $d\mu = 0$ in thermal equilibrium proceeds as follows. First it is assumed that $d\mathcal{F}$ is zero. This particular assumption will be shown in the next section to be unjustifiable. For the time being, however, let us accept the conventional assumption that $d\mathcal{F} = 0$ in thermal equilibrium. It is then reasoned conventionally that if dN particles were transferred from point A to B at constant volume, then:

$$d\mathcal{F} = (\mu_a - \mu_b)\,dN = 0 \tag{25}$$

From equation (25) it would follow that $\mu_a = \mu_b$.

The weakness in the preceding argument is that if dN particles were transferred from point A of high particle concentration n_a to point B of low particle concentration n_b, this could not possibly be done at constant volume. To explain this point let us calculate specifically the volumes occupied by dN particles at point A and at point B. At the former point, the volume $dV_a = (dN)/n_a$, and at point B, the volume $dV_b = (dN)/n_b$. Thus $dV_b = (n_a/n_b)\,dV_a$. If points A and B are on either side of, for example, an interface between a metal and a p-type semiconductor, respectively, the ratio of (n_a/n_b) could be 10^{16} or higher. Thus, the assumption that dN particles could be transported at constant volume in any system is obviously invalid. Clearly the volume will remain unchanged only if $n_a = n_b$, that is, in any region where $\nabla n = 0$. This particular conclusion was arrived

at in section (4) in connexion with the hydrodynamic interpretation of equation (10).

(*B*) *The function G.* It is generally stated that across the boundary line between a liquid and its vapour, in thermal equilibrium $dG = 0$. The conventional reasoning supposedly leading to this result uses equation (22), which is here rewritten for convenience, $dG = -S\,dT + V\,dP + \sum_k \mu_k\,dN$.

To show supposedly that $dG = 0$ in thermal equilibrium across the boundary line between two phases, it is conventionally stated that if dN particles were transferred from phase i to j, then this would be done at constant pressure. Thus $dG = (\mu_i - \mu_j)\,dN$. Since it is conventionally believed that $\mu_i = \mu_j$, it is then concluded that $dG = 0$.

There are two objections in the preceding argument. The first concerns the validity of assuming that $\mu_i = \mu_j$. This matter has been already discussed in the preceding subsection. The second objection is less obvious. It is concerning the assumption that across the boundary, $dP = 0$, which can be shown to contradict the virial theorem. This particular point will be further discussed in section (8).

(7) New Criteria of Thermal Equilibrium

In the previous section, a number of arguments were presented challenging the validity of the conventional criteria of thermal equilibrium. The object in this section is to formulate new criteria of thermal equilibrium. It will be shown that under such conditions, wherever the concentration is non-uniform, each one of the quantities $d\mu$, $d\mathcal{F}$, dG and dH must become an exact non-vanishing differential, and the thermodynamic generalized force per particle must become irrotational.

(*A*) *The chemical potential, and the thermodynamic generalized field.* In section (5) it was demonstrated that under the action of a purely thermodynamic process, the work done per particle transported a distance ds is merely dv and that is equal to $(-d\mu)$. According to equation (7), the condition necessary and sufficient for the static equilibrium of any system of mobile particles enclosed in a stationary container is that the work done dW per particle transported a distance ds must be an exact differential. Therefore, it may be concluded at once that at constant T:

$$d\mu = -dv = \text{exact differential} \qquad (26)$$

Thus from equations (17), (18), (19) and (20) it follows that, in thermal equilibrium, $\mathbf{f}_t = \mathbf{f}_d$ and each must become irrotational.

Equation (26) is clearly a consequence of the new transport laws, which are phenomenological. Before discussing any of its consequences, equation (26) must be verified quantum-statistically.

Let us first consider an ideal classical gas, which has concentration gradients in thermal equilibrium. An example of this will be electrons in a non-degenerate p-n junction or gas in an isothermal atmosphere of a planet. The equation of state for either of the two cases is well known[22] to be:

$$P = nkT \qquad (27)$$

The quantity $d\mu$, at constant T, may be found from equations (16) and (27) to be:

$$d\mu = (kT/n)\,dn = d(kT \ln n) \qquad (28)$$

Equation (28) is clearly an exact non-vanishing differential wherever $dn \neq 0$. If $dT \neq 0$, then $d\mu$ would not be an exact differential necessarily.

The non-vanishing exactness of $d\mu$ in thermal equilibrium should be possible to verify quantum-statistically in any general situation wherever $dn \neq 0$. Consider, for example, conduction electrons in any solid crystal, degenerate or otherwise, in which the electrons have a concentration gradient in thermal equilibrium. Examples of this situation are p-n junctions, heterojunctions, or any solid–solid interfaces. Let the volume energy density of quantum states[23] $g(\varepsilon)$ be parabolic. It is well known that electrons in solids generally obey the Fermi–Dirac statistics. For such particles, it has been shown in ref. 4 that in thermal equilibrium $\mathbf{f}_d$ must become irrotational. In view of equations (16) and (20), it can be shown that $d\mu$ must become an exact non-vanishing differential wherever $dn \neq 0$. It can be further shown that $\mathbf{f}_d$ may be rotational and $d\mu$ inexact if $dT \neq 0$.

(*B*) *Helmholtz free energy* $\mathcal{F}$. It will be demonstrated here that in thermal equilibrium, and only then, $d\mathcal{F}$ becomes an exact non-vanishing differential wherever $dn \neq 0$. To do so, consider equation (21), which in general will be:

$$\begin{aligned} d\mathcal{F} &= -P\,dV + \mu\,dN - S\,dT \\ &= -d(PV) + V\,dP + d(\mu N) - N\,d\mu - S\,dT \end{aligned} \qquad (29)$$

From equations (15) and (29), at constant T, it follows that:

$$d\mathcal{F} = -d(PV) + d(\mu N) \qquad (30)$$

Thus, $d\mathcal{F}$ is an exact non-vanishing differential at constant T, since $d\mu$ is so.

(*C*) *Gibbs function G.* It is also possible to show that in thermal equilibrium, dG becomes an exact non-vanishing differential wherever $dn \neq 0$. To do so, consider equation (22), which is:

$$dG = V\,dP + \mu\,dN - S\,dT \qquad (31)$$

or:

$$dG = V\,dP + d(\mu N) - N\,d\mu - S\,dT \qquad (32)$$

From equations (15) and (32), it follows that:

$$dG = d(\mu N) \qquad (33)$$

This completes the proof.

(*D*) *The heat function, or enthalpy H.* It is again possible to demonstrate that in thermal equilibrium, dH becomes an exact non-vanishing differential wherever $dn \neq 0$. To do so, consider equation (23), which is:

$$dH = T\,dS + V\,dP + \mu\,dN \qquad (34)$$

In view of equations (31) and (34), we may write:

$$dH = d(ST) + dG \qquad (35)$$

Since, in thermal equilibrium, dG is an exact non-vanishing differential wherever $dn \neq 0$, so also is dH.

(*E*) *Reconstruction of the principle of detailed balancing.* It has been pointed out in refs. 3 and 4 that the common interpretation of the conventional principle of the detailed balancing implies a fundamental conflict with the existence of the Peltier effect. The cause of the conflict may here be explained to be simply a violation of the first generalized transport law. According to that law, the rate of steady-state flow cannot be expressed at any point in an active region only in terms of the physical constants there. Thus, for example, one cannot say that in the transition region of a p-n junction, in thermal equilibrium, the diffusion and drift current densities are equal and opposite at every point. What one can say for this particular case and for any region containing mobile particles, without violating the new transport laws, may be described by the new statement of the principle of detailed balancing, as follows. At any point in any system in static equilibrium, for every particle, of a specific constituent, which is crossing a given area per unit time in one direction, under the action of each process of a specific nature, there is another particle of the same constituent crossing in the opposite direction.

Thus, according to this new law, under static equilibrium conditions, each process must become conservative and hence independently gives rise to a vanishing flow for each constituent at each point, within the stationary container enclosing the fluid. This is in sharp contrast with the conventional statement of the principle, which assumes that the balance in thermal equilibrium is not caused by the individual processes; rather it is caused by all the processes combined.

(8) The Equation of Internal State for Liquids, and the Coexistence of Liquid–Vapour Phases

The conventional concept of pressure in liquids is probably one of the oldest in physics. The concept, for example, presents itself in connexion with the law of buoyancy discovered by the Greek scientist Archimedes (287–212 B.C.). In the eighteenth century, this concept has been used by Daniel Bernoulli (1700–1782) to formulate some well-known laws of hydrodynamics.

The conventional concept of pressure for liquids, henceforth called 'external pressure', is in sharp contrast with what is to be named the 'internal pressure'. The latter will be defined for liquids according to the virial theorem. Internal pressure, as the name implies, describes a physical quantity which cannot be measured directly. This is much like the absolute internal energy of a thermodynamic system or the so-called 'internal potentials' in a p-n junction or an interface between any two different metals. For the latter two examples it is well known that 'internal potentials' cannot be directly measured. Despite the inaccessibility of the internal pressure of a liquid, the transport of its particles would be expected to depend on it.

The external pressure in a liquid, if considered to be a thermodynamic quantity, as conventionally done, presents at least two serious conflicts. The first conflict lies within the domain of the kinetic theory. According to the latter, the pressure P should be defined according to the virial theorem. This particular assumption is used, for example, for gases and for electrons in solids, but not for liquids. The reason for this is that if P is calculated, for a liquid, it can be shown that, depending on the temperature, P could be orders of magnitude higher in the liquid than it would be in its vapour, in thermal equilibrium. Obviously this result represents a fundamental contradiction to the conventional criteria of thermal equilibrium. Despite this conflict, applying the kinetic theory to liquids is not discarded altogether. For example, the velocity distribution at room temperature is generally assumed to obey the Maxwell–Boltzmann statistics.

The other conflict may be explained on the basis of the material presented in section (5). In that section, it has been shown that $(-\mathrm{d}\mu)$ represents the work done per particle transported a distance $\mathrm{d}s$. If $\mathrm{d}P = 0$ in thermal equilibrium across the boundary of a liquid and its vapour, as conventionally assumed, then according to equation (16) at constant T, $\mathrm{d}\mu = 0$. This will be so anywhere within the boundary, and otherwise. If this conclusion were valid, it should be possible to transport particles from the liquid phase to the vapour phase at constant T without expending energy. This will mean that the latent heat of vaporization would be zero if the process occurred quasi-statically. This conclusion clearly is seriously contradicted by well-known observations.

The two conflicts just described may now be resolved through the new criteria of thermal equilibrium, and the fundamental new transport laws they are based on. The existence of the latent heat of vaporization is evidence that energy must be expended to transport particles quasi-statically from points in the liquid to points in the vapour at constant T. Thus by the definition of chemical potential introduced in section (5), $\mathrm{d}\mu \neq 0$ at points across the boundary between the two phases, at constant T. From equation (16), it follows inevitably that $\mathrm{d}P \neq 0$ across the boundary at constant T. This result was mentioned earlier to be an inevitable consequence of the virial theorem.

To explain this result further, one can imagine that the concentration n of the particles will vary across the boundary all the way from its value within the vapour n_v to that within the liquid n_l. It is conceivable that somewhere within the boundary, there exists a point Q at which n may be found to be (αn_v), where α is a number, let us say, less than 10. If the vapour pressure is sufficiently small, the phase at point Q will be gaseous, and the pressure at Q and at all points across the boundary between Q, and the uniform vapour, can then be calculated from equation (27). Therefore at Q, $P_Q = knT = k\alpha n_v T$. Thus, across the boundary at least within the gaseous portion of the transition region, it can be said that $\mathrm{d}P = kn_v T\mathrm{d}\alpha = 0$, in thermal equilibrium.

That static equilibrium can still occur at constant T, even when $\mathrm{d}P \neq 0$, is a matter already discussed phenomenologically in sections (4) and (7). In the latter section, it was concluded that in thermal equilibrium, and only then, $\mathrm{d}\mu$ must become necessarily an exact differential. This particular result was confirmed in section (7) to be the case for ideal boltzon and fermion particles. Since particles in most liquids, if not classical, would obey the Bose–Einstein statistics[24,25], it remains to be shown that, for such particles, $\mathrm{d}\mu$ is an exact non-vanishing differential in thermal equilibrium wherever $\mathrm{d}n \neq 0$.

To do so consider the Bose–Einstein function:

$$B(\varepsilon,\varepsilon_b) = \{\exp[(\varepsilon-\varepsilon_b)/kT] - 1\}^{-1} \qquad (36)$$

where ε_b represents the amount of energy measured from the Bose-level to the reference from which the energy ε is measured. It should be noticed that here ε_b is always a negative quantity. In this case the particle concentration and average kinetic energy per particle will be given respectively by:

$$n = \int_0^\infty g(\varepsilon)\, B(\varepsilon,\varepsilon_b)\, \mathrm{d}\varepsilon \qquad (37)$$

$$<\varepsilon> = (1/n)\int_0^\infty \varepsilon\, g(\varepsilon)\, B(\varepsilon,\varepsilon_b)\, \mathrm{d}\varepsilon \qquad (38)$$

It is now possible to formulate quantum-statistically the internal pressure in a liquid, the particles of which are bosons, on the basis of the virial theorem. This will be:

$$P = (2/3)n<\varepsilon> - P_0(n) = (2/3)\int_0^\infty \varepsilon\, g(\varepsilon)\, B(\varepsilon,\varepsilon_b)\, \mathrm{d}\varepsilon - P_0(n) \qquad (39)$$

Here $P_0(n)$ is the component of pressure contributed by the inter-particle forces. At constant T, this quantity may be expressed, at least in principle, by a Maclaurin's series in n.

If $g(\varepsilon)$ is assumed to be parabolic, then in view of (16), (36), (37) and (39), at constant T, it can be shown that:

$$\mathrm{d}\mu = \mathrm{d}\varepsilon_b - \mathrm{d}\varepsilon_0 \qquad (40)$$

Here $\mathrm{d}\varepsilon_0$ is an exact differential equal to $(1/n)\mathrm{d}P_0(n)$. From the definition of ε_b, it follows at once that in thermal equilibrium, $\mathrm{d}\mu$ is an exact non-vanishing differential for Bose liquids wherever $\mathrm{d}n \neq 0$. Obviously this conclusion is valid also for gases. This result explains the co-existence of the liquid and vapour phases when their particles are bosons.

(9) Diffusion in Isothermal Ideal Classical Liquids

This section presents the theory of diffusion in isothermal liquids on the basis of the new concept of liquid internal pressure, and the first generalized transport law.

Consider any isothermal liquid in which there are particles of another ideal Maxwell liquid. Let the concentration of these particles be non-uniform but time-independent.

According to the first generalized transport law, for positively charged particles, the current density is related to the driving force per particle, in any passive region near static equilibrium, by equation (4). For neutral particles, it is more appropriate to use the rate of particle flow $\mathbf{\Gamma}$ per unit area, rather than $\mathbf{J}$. In view of the Einstein mobility-diffusion relationship[26], and equation (4), $\mathbf{\Gamma}$ may be expressed by:

$$\mathbf{\Gamma} = (\mu'/q)n\mathbf{f} = (D/kT)\mathbf{f} \qquad (41)$$

For the situation under consideration, the quantity f is merely the diffusion force per particle. According to equations (19) and (20), at constant T, this force is given by:

$$\mathbf{f} = \mathbf{f}_t = \mathbf{f}_d = -(1/n)\nabla P \qquad (42)$$

From equations (27), (41) and (42), it follows that:

$$\Gamma = -D\nabla n \qquad (43)$$

Equation (43) will be recognized as what is generally called Fick's law[27]. It is interesting to notice that across a liquid–vapour boundary, $\nabla n \neq 0$, and in fact, ∇n is exceedingly high in thermal equilibrium. Notice here that, according to the first generalized transport law, equation (43) would not apply in that region. Rather, at constant temperature, the particles must be in static equilibrium.

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FERROMAGNETISM AND COLOUR ZONING IN SOME MALAYAN CASSITERITE

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LARGE cassiterite crystals from quartz-orthoclase-fluorite vein lodes at Pelepah Kanan, north-east Johore, possess conspicuous colour zoning and a small but strongly ferromagnetic fraction. From decrepitation tests it can be established that the mineral was formed at temperatures between 350° C and 360° C. Individual zones, which may be black, brown or colourless, range from a few microns to more than 1 mm in width. By reflected and transmitted light microscopy it is evident that the colour and ferromagnetic properties are not due to any resolvable foreign inclusions, nor are the refractive index and specific gravity values appreciably different. Furthermore, although a few weak anomalous lines exist which cannot be indexed as cassiterite, X-ray diffraction revealed no discernible difference between dark and colourless zones. Yet the dark ferromagnetic zones both here and in several samples from Thailand and Australia are found to possess slightly large 'a' unit cell parameters.

Chemical and spectrographic analyses of dark and colourless, strongly ferromagnetic and non-magnetic cassiterite fractions showed only slight compositional differences, which scarcely seem consistent with the properties of these fractions (Table 1). These analyses were confirmed by electron probe microanalyses, which showed no consistent relationship between the total iron content and colour of individual zones. Thermogravimetric analyses and Mossbauer spectra of the dark magnetic fraction, however, clearly ruled out the existence of any SnO which conceivably could be present as a separate phase.

Separation of crushed zoned cassiterite crystals into fractions of differing magnetic susceptibility showed that the dark brown or black fraction is the most ferromagnetic, having a susceptibility of 63×10^{-6} c.g.s., whereas the colourless variety is essentially non-magnetic. Thermal demagnetization analyses using a horizontal-type translation magnetic balance revealed two distinct inflexions which could both be interpreted as Curie points (Fig. 1). The second of these, at 580° C, can be assigned to magnetite, as confirmed by X-ray diffraction after completion of run. Furthermore, observations using hot-stage microscopy reveal that the dark ferromagnetic cassiterite crystals on heating to above 750° C become more translucent in appearance due to coagulation of the fine dispersion of stannate and/or magnetite particles. It appears, therefore, that the ferromagnetism arises from a second phase within the unheated cassiterite where it forms an extremely fine cloud-like dispersion which on heating to 380° C coagulates and reverts to magnetite. For reasons

Table 1. CHEMICAL ANALYSES OF CASSITERITE FRACTIONS FROM PELEPAH KANAN, NORTH-EAST JOHORE

	Ferromagnetic cassiterite	Non-magnetic cassiterite
SnO_2	98·10	99 00
Total iron as Fe_2O_3	1 80	0 60
TiO_2	nil	nil
MnO	0 005	nil
Total	99 905	99 60

Analyst. W. H. Hersdman

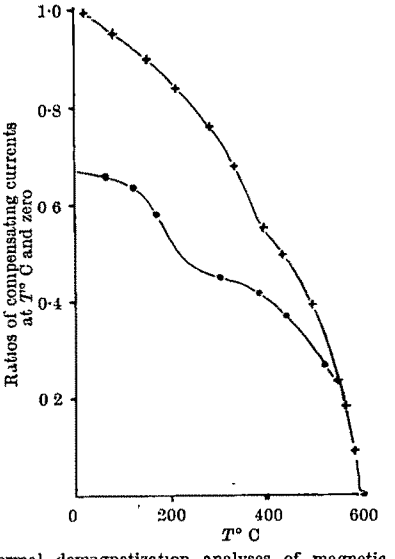


Fig. 1. Thermal demagnetization analyses of magnetic cassiterite from Pelepah Kanan. +, Heating, ●, cooling

to be discussed, this dispersed ferromagnetic phase is probably a ferrous stannate, and in support of this it is noteworthy that magnetite forms the chief decomposition product of the synthetic compound after calcining at 1,093° C (ref. 1). But by the same token considerable caution is required in examining cassiterite by electron diffraction techniques as the extreme temperatures to which specimens are necessarily subjected have been observed to have the same effect.

Owing to the difficulty of determining Fe^{2+} in cassiterite by direct chemical means, and in order to investigate further its magnetic character, Mössbauer spectra were obtained from dark ferromagnetic and light non-magnetic fractions using the 14·4 keV γ-ray of iron-57 and the 23·8 keV γ-ray of tin-119. Absence of observable magnetic splitting for both nuclei indicates that only small fractions of iron ($< \sim 10$ per cent) and tin ($< \sim 1$ per cent) atoms can be in a ferromagnetic or antiferromagnetic environment. This suggests that the ferromagnetism does not persist throughout a magnetic crystal but is localized in the minute domains already outlined.

The iron-57 Mössbauer spectra revealed quadrupole splittings and chemical shifts characteristic of Fe^{2+} and Fe^{3+} (Fig. 2). These spectra suggest that the magnetism and the coloration of the cassiterite are caused by a paramagnetic ferrous stannate which could be either $SnFeO_3$ or $SnFe_2O_4$.

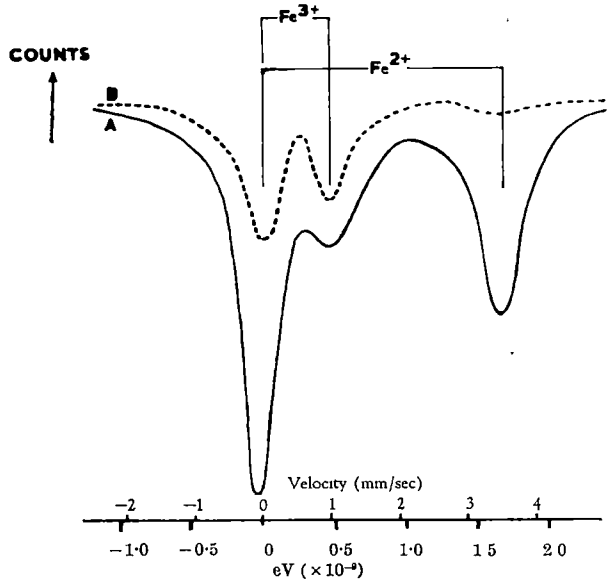


Fig. 2. Iron-57 Mossbauer spectra (relative to a 310 stainless steel source) for cassiterite (relative intensities for *A* and *B* not normalized). ——, Dark magnetic cassiterite; – – – –, colourless cassiterite

The crystal chemistry of Sn^{4+} and Ti^{4+} is not dissimilar, and it is suggested that the origin of ferromagnetism in cassiterite could be comparable to the behaviour of the solid solutions TiO_2–Fe_3O_4 or TiO_2–Fe_2O_3 (ref. 2). First in the TiO_2–Fe_3O_4 series, spinel-type titanomagnetites having the general formula $Ti_mFe_{3-m}O_4$ are derived from magnetite Fe_3O_4 by the substitution of Ti^{4+} for Fe^{3+} ions together with a change of ionization of an Fe^{3+} ion to Fe^{2+}. As m increases, the molecular saturation moment and Curie point (4 μ_B and 578° C for magnetite) fall regularly as the unbalancing of magnetic moments decreases, and for $m = 1$ ($TiFe_2O_4$, ulvöspinel), the phase becomes paramagnetic. Although this system provides a possible explanation for the origin of the magnetism in cassiterite, the absence of any detectable magnetite or tin analogue of titanomagnetite in the fresh magnetic cassiterite, together with the relatively low susceptibilities of titanomagnetite analogues in the region of $TiFe_2O_4$ compared with the overall susceptibilities of the magnetic cassiterite fractions, has led us to prefer the second alternative. In this, the rhombohedral series $Fe_{2-m}Ti_mO_3$ has end members haematite Fe_2O_3 and ilmenite $FeTiO_3$, which are both antiferromagnetic; but near the composition $Fe_{4/3}Ti_{2/3}O_3$, it is possible to obtain considerable unbalancing of magnetic moments between neighbouring cation layers, so that the compound is strongly ferromagnetic with a molecular saturation moment of about 2 μ_B and a Curie point of 150° C. Nagata[3], moreover, has shown that if the alternating cation layers had compositions $(\overrightarrow{Ti^{4+}_{1/6}Fe^{2+}_{1/6}Fe^{3+}_{2/3}})$ and $(\overleftarrow{Fe^{2+}_{1/2}Ti^{4+}_{1/2}})$, this would give the required molecular moment (arrows represent directions of magnetic moments). Now for cassiterite, the apparent correlation between the ferromagnetic character and the ferrous ion content would favour a tin analogue of $Fe_{4/3}Ti_3O_{2/3}$, since this would more likely be found interspersed in a matrix of the ferrous compound than would a counterpart of titanomagnetite. This conclusion is of course based on the (probable) assumption that $SnFeO_3$ is paramagnetic.

We thank Dr. A. D. Wadsley of the C.S.I.R.O. Division of Mineral Chemistry, and Prof. J. M. Cowley of the Department of Physics, University of Melbourne, for their advice. Special thanks are also due to Dr. L. G. Parry of the Department of Physics, University of New South Wales, for obtaining the thermal demagnetization analyses, and to Dr. J. Sanders of the C.S.I.R.O. Division of Tribophysics for taking several electron diffraction patterns. For conducting one of us over the deposit and beneficiation plant we also thank the mine manager and staff of the Pelepah Kanan Tin Mine.

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EXCIMER X-RAY INVESTIGATION

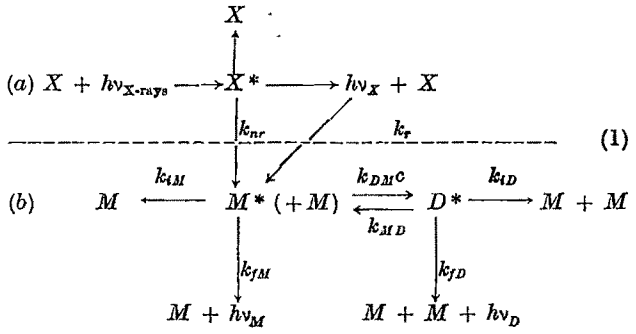
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THE monomer-excimer emission spectra of pyrene in solution[1,2] and of 2-ethyl and 1 : 2-dimethyl naphthalene in the liquid phase[3], excited by ultra-violet light, were reported previously. Birks *et al.*[4] have also used phosphorus-32 β-radiation to excite a 10^{-3} M solution of pyrene in toluene and reported identity of photofluorescence and scintillation spectra. In this article we investigate the excimer formation in solutions and in pure liquids under X-ray excitation and we determine the binding energy of excimers formed in organic liquids.

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Solutions. Oxygen-free solutions of pyrene in *cyclo*hexane were excited at room temperature by 230-keV X-rays. The relative quantum intensities, I_λ, were measured by a Bausch and Lomb 500-mm grating monochromator for various solute concentrations, c, and are plotted in Fig. 1. The spectra are normalized at a fixed wave-length $\lambda = 410$ mm, as were the photofluorescence spectra[2]. The shape and position of the monomer-excimer bands are identical to those obtained under ultra-violet excitation. The ratio of the excimer quantum yield, I_D, to that, I_M, of the monomer, I_D/I_M, varies linearly with c up to at least 10^{-2} M.

If X and M are the solvent and solute molecules, respectively, and D^* is the excimer, then we may consider the following two-step process:



In step (*a*) we consider only the species X^*, a solvent molecule in its lowest excited singlet state, from which electronic excitation energy is transferred to M radiatively and non-radiatively with rate constants k_r and k_{nr}, respectively. In step (*b*), which is the case of normal fluorescence (that is, only monomers are initially excited), k_{fM}, k_{tM}, k_{fD}, k_{tD}, $k_{DM}c$ and k_{MD} are the rate parameters for M fluorescence, M internal quenching, D^* fluorescence, D^* internal quenching, D^* formation, and D^* dissociation, respectively. Thus, if it is assumed that X is initially excited and that M is afterwards excited by energy transfer from X, the formation and emission of D^*, and the emission of M^*, which follow M^* formation, should be independent of the mode of excitation of X. Considering the kinetics of step (*b*), we have ($c \leqslant 10^{-2}$ M):

$$\frac{I_D}{I_M} = \frac{k_{fD}\, k_{DM} c}{k_{fM}(k_{fD} + k_{tD} + k_{MD})} = Kc \qquad (2)$$

For pyrene oxygen-free solutions in *cyclo*hexane, K is found equal to 640 mole l.$^{-1}$ and is independent of X-ray energy E_X (120 keV–240 keV) and/or X-ray intensity I_X (5 m.amp–20 m.amp). This value for K is $\sim$4 times smaller than that obtained under ultra-violet excitation (= 2,500 mole l.$^{-1}$) of the same system[4]. For pyrene oxygen saturated solutions in *cyclo*hexane, K is again smaller (500 mole l.$^{-1}$) for X-ray excitation than its value under ultra-violet excitation[2] (1,110 mole l.$^{-1}$) by a factor of $\sim$2·2.

The rate parameters k_{DM}, k_{MD}, and k_{tD} in equation (2) were found[6] to depend on the temperature, T, the solvent X, and the solute M, while k_{fM} and k_{fD} were found to be a function of the refractive index, n, of the solvent (increase with n) and independent of T. T, X and M were the same for ultra-violet and X-ray excitation.

Ion recombination of the solute which results in a higher population of triplets than singlets (thus affecting K) is not likely to be important in these experiments, where the energy absorbed directly by M is negligible.

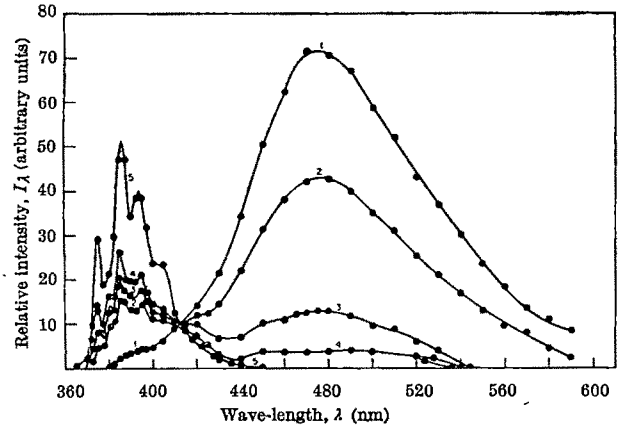


Fig. 1. Emission spectra of pyrene in *cyclo*hexane (oxygen-free solutions). Curves (1) 10^{-3} M; (2) 5×10^{-3} M; (3) 10^{-4} M; (4) 5×10^{-4} M; (5) 10^{-4} M

Oxygen-free solutions of 1 : 2-dimethyl naphthalene in *n*-heptane at various c were also excited by X-rays and a value of 0·46 mole l.$^{-1}$ was calculated for K. This is smaller by a factor of $\sim$ 1·7 than that (0·78 mole l.$^{-1}$) reported[7] for excitation of the same system by ultra-violet light. For this molecule at room temperature, $k_{MD} \gg k_{fD} + k_{tD}$ (see Figs. 2 and 3 and ref. 7) and thus equation (2) can be written:

$$\frac{I_D}{I_M c} = K = \frac{k_{fD}}{k_{fM}} \frac{k'_{DM}}{k'_{MD}} e^{B/kT} \qquad (3)$$

where B is the excimer binding energy and k is Boltzmann's constant. The entropy change ΔS of the system is determined by the ratio k'_{DM}/k'_{MD} and is a property of M independent of T (ref. 6). The differences in the excimer binding energy, B, for ultra-violet and X-ray excitation are within the experimental error. B is more likely to be unchanged for the two modes of excitation and the changes in K (for the two modes of excitation) are attributed to additional quenching of D^* by species formed by the ionizing radiation. It is worth noting that Berlman[8], who used a pulsed beam of electrons of 75-keV energy to excite a $\sim 2{\cdot}5 \times 10^{-3}$ M solution of pyrene in deoxygenated *cyclo*hexane, reported monomer and excimer decay times which differ appreciably from those reported by Birks, Dyson and Munro[5], who used ultra-violet light to excite the same system ($c = 2{\cdot}5 \times 10^{-3}$ M).

Fig. 1 and equation (2), however, indicate that excimers are formed subsequent to M^* formation by collision of M and M^* as suggested by Birks *et al.*[4], the process being collision controlled. It should be pointed out that triplet–triplet annihilation resulting in the formation of D^* and M^* (that is, initial formation of D^* and M^*) which is responsible for the delayed fluorescence[10] can be neglected here.

Liquids. In the liquid state of a compound, only stage (*b*) of process (1) need be considered, that is, M is excited directly by the ionizing radiation. The scintillation spectra of liquid 2-ethyl naphthalene at various temperatures (25°–142·5° C) are presented in Fig. 2. Similar spectra were obtained for liquid 1 : 2-dimethyl naphthalene for the same temperature range. Our scintillation spectra at 25° C agree well with the photofluor-

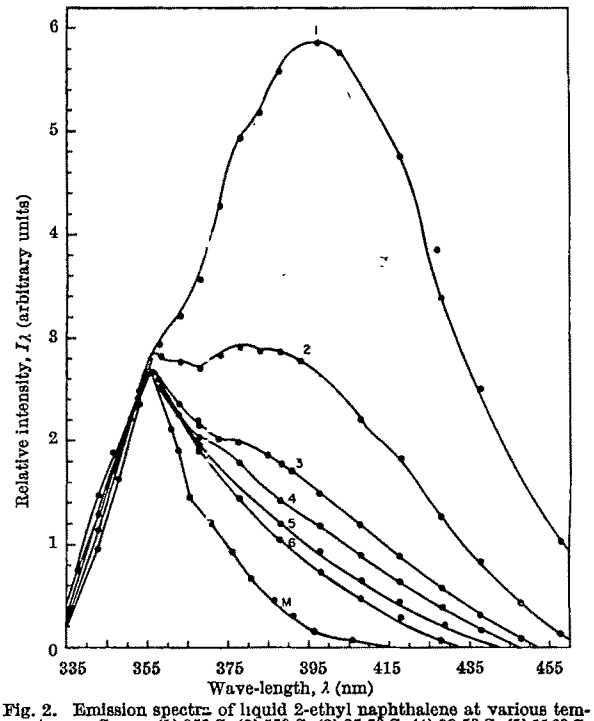


Fig. 2. Emission spectra of liquid 2-ethyl naphthalene at various temperatures. Curves (1) 25° C, (2) 55° C; (3) 85·5° C; (4) 98·5° C; (5) 116° C; (6) 142·5° C; (*M*) normalized monomer emission (solution)

escence spectra reported by Birks and Aladekomo[3]. In the case of 2-ethyl naphthalene, both the photofluorescence and scintillation spectra show a small amount of residual monomer which is more pronounced in the scintillation spectrum. As temperature increases, the excimer emission decreases and the monomer emission becomes more pronounced due to the dissociation of D^* (ref. 9). This is clearly demonstrated in Fig. 2, where curve M represents the normalized emission spectrum of M in solution (suitably shifted towards longer λ).

From equation (3), which applies also to pure liquids, B can be obtained from the slope of the $\log_{10} I_D/I_M$ versus T^{-1} curves. In Fig. 3 the observed temperature dependence of the emission spectra of liquid 2-ethyl naphthalene are plotted as $\log_{10} I_D/I_M$ versus T^{-1}. From the slope of these curves, we have calculated a binding energy equal to 0·27 eV for 2-ethyl naphthalene. A binding energy of 0·28 eV is calculated for 1 : 2-dimethyl naphthalene. For solutions excited by ultra-violet light, Aladekomo and Birks[7] have recently reported a binding energy of 0·29 ± 0·02 eV for 2-ethyl naphthalene and 0·31 ± 0·03 eV for 1 : 2-dimethyl naphthalene. They also reported identical values for the binding energy of 1 : 6-dimethyl naphthalene in solution and in the liquid state of the compound.

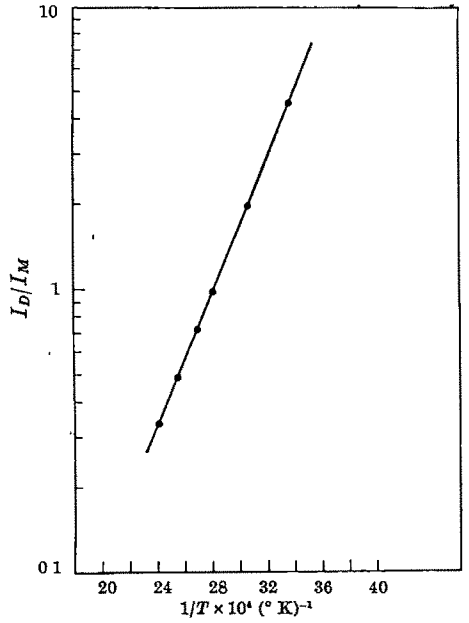


Fig. 3. Liquid 2-ethyl naphthalene I_D/I_M versus $1/T$

In conclusion, excimers in pure liquids and in solutions are formed subsequent to M^* formation by collision of M^* and M, independently of the mode of excitation. The shape and position of the monomer-excimer emission are unaffected by the mode of excitation, but the excimer-monomer quantum yield is not. The excimer binding energy for naphthalene derivatives appears to be the same for solutions and pure liquids and unaffected by the mode of excitation. From our results and the results of others[6,7] on the temperature dependence of the emission spectra of pure liquids it is also suggested that since excimers are more easily formed in pure liquids than in solutions, and since B seems to be environment independent, the excimer binding energy can be measured more easily by heating a compound in the liquid state than by cooling or heating it in a solution (where problems of solubility on cooling and solvent boiling on heating arise).

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Note added in proof. If we assume that the smaller value of the ratio I_D/I_M under X-ray excitation (as compared with its value under ultra-violet light excitation) is due to the presence of a quencher, Q, produced by the ionizing radiation, then equation (2) becomes:

$$\frac{I_D}{I_M} = \frac{k_{fD}\, k_{DM} c}{k_{fM}\,(k_{fD} + k_{iD} + k_{MD} + k_{QD}[Q])} \qquad (4)$$

Using equations (2) and (4) and taking into consideration our finding that I_D/I_M is independent of I_X and E_X within the region stated, we calculated k_{QD} for pyrene in *cyclo*-hexane from two independent sets of data: (i) from values of I_D/I_M for oxygen-free solutions excited by X-rays and ultra-violet light, and (ii) from values of I_D/I_M for oxygen-saturated solutions excited by X-rays and ultra-violet light. In both cases we obtained $k_{QD} = 6{\cdot}3 \times 10^7$ sec^{-1}.

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INVESTIGATION OF RAUSCHER VIRUS RIBONUCLEIC ACID

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WE recently reported[1] that high molecular-weight RNA (70 $S_{20,w}$) could be extracted from Rauscher leukaemia virus. The results of further investigations of the properties of this RNA are reported here.

Rauscher virus was produced *in vitro* by the chronically infected cell line *JLS V5* (ref. 2). The cells were cultivated in a medium made of casein hydrolysate in a phosphate buffer plus 15 per cent calf serum, and were seeded in aliquots of 10^7 cells in 1-l. Roux bottles. After a delay of 6 h the medium was changed and replaced with casein hydrolysate in *tris* (*tris* hydroxymethyl-aminomethane) buffer plus 10 per cent foetal calf serum, containing 5 μc. phosphorus-32 per ml. In some experiments, we used a double labelling with phosphorus-32 (at the same ratio) and algal hydrolysate protein-^{14}C (0·25 μc./ml.). The medium was changed after 3 days of incubation at 37° C; 24 h later, culture supernatants were collected and purified according to Moloney's technique[3]. The presence of virus in the final pellets was verified by electron microscopy and by inoculation of *BALB/c* mice. As controls, supernatants from non-infected *JLS V6* cell line cultures previously labelled with phosphorus-32 were submitted to the same process.

The labelled viral pellets, supplemented with unlabelled *JLS V5* cells (used as a carrier), were homogenized in 10^{-2} M sodium acetate buffer *p*H 5·1 plus 2 μg/ml. polyvinylsulphate (PVS). Following this procedure, 0·4 per cent of sodium dodecyl sulphate was added. The deproteinization was achieved by three phenolic treatments (one for 30 min with an equal volume, and two for 15 min with half volumes) at 4° C with water-saturated phenol, freshly distilled under low pressure. The RNA was precipitated with absolute ethyl alcohol in the presence of 0·1 M sodium chloride for 2 h at −10° C, washed with 67 per cent ethyl alcohol to eliminate the phenol and dissolved in 10^{-2} M acetate buffer *p*H 5·1 with 0·1 M sodium chloride.

The RNA solution was layered on top of a linear sucrose gradient (5–20 per cent w/v in acetate–sodium chloride buffer) and centrifuged in a 'Spinco *SW* 25' swinging-bucket tube. The sedimentation constant of the different RNA fractions was determined by the method of Martin and Ames[4]. A fractionation was also performed by chromatography on a methylated albumin column (MAK) according to the technique of Mandell and Hershey[5].

The base composition was achieved by the method of Katz and Comb[6] using the high molecular-weight RNA peak which was isolated in a sucrose gradient, dialysed against 10^{-3} M sodium chloride, lyophilized and hydrolysed for 20 h at 37° C with 0·3 N potassium hydroxide. The potassium hydroxide was neutralized by perchloric acid and potassium perchlorate was eliminated by centrifugation. The radioactivity in the different fractions was evaluated in a gas flow counter, after drying the planchets.

Isolation of a viral RNA with a high sedimentation constant. After a centrifugation at 20,000 r.p.m. for 15·5 h in a sucrose gradient, the following fractions were obtained (Fig. 1): by optical density three peaks were visible corresponding to the cellular RNAs of the *JLS V5* cells (28*S*, 18*S* and 4*S*) and by radioactivity two peaks, one ('light' RNA) being located at the 4*S* area, the other at the bottom of the tube. Both peaks were RNase-sensitive (50 γ/ml.).

After centrifugation at 25,000 r.p.m. for 2·5 h in a sucrose gradient (Fig. 2), the radioactivity which was previously located at the bottom of the tube constituted a well-defined peak with a sedimentation constant of about 70 $S_{20,w}$ ('heavy' RNA). All other RNA fractions remained on the top of the tube under these conditions of centrifugation. In a similar experiment with an extract from uninfected control *JLS V6* cell line culture supernatants, no peak in the 70*S* region could be detected.

Using methylated albumin column chromatography, two peaks were detected eluting respectively at 0·40 and 0·95 sodium chloride molarities (Fig. 3), in addition to unlabelled peaks of *JLS V5* cell RNAs.

Characterization of the heavy viral RNA. Such a heavy RNA fraction brings up the question as to whether the material might be a ribonucleoprotein or an aggregate. The conditions of extraction do not favour the preservation of a ribonucleoprotein-like structure. In order to exclude this eventuality, a double-labelling experiment was undertaken in which viral RNA was labelled with phosphorus-32 and viral proteins with carbon-14 (algal hydrolysate). Virus pellets were obtained by Moloney's method and divided into two aliquots; one of them was phenol-treated as above and RNA isolated by sucrose gradient, the other was purified with two consecutive centrifugations in a caesium chloride density gradient[7].

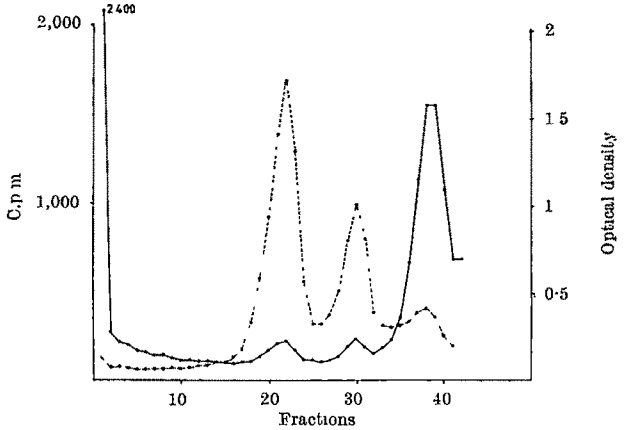


Fig. 1. Sedimentation in sucrose gradient 5–20 per cent (15·5 h, 20,000 r.p.m. in 10^{-2} M acetate, 10^{-1} M sodium chloride, *p*H 5·1) of RNA extracted from *JLS V5* cells o---o---o---o, and from the phosphorus-32 labelled viral pellet •—•—•—•, purified by Moloney's technique. The bottom tube was punctured and eight-drop samples collected to measure absorption at 2600 Å and the radioactivity in 5 per cent trichloroacetic acid precipitates

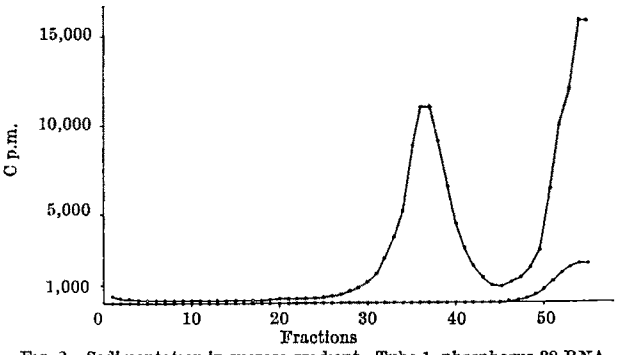


Fig. 2. Sedimentation in sucrose gradient Tube 1, phosphorus-32 RNA extracted from culture, supernatants of *JLS V5*, o---o---o---o. Tube 2, phosphorus-32 RNA extracted from culture supernatants of *JLS V6*, •—•—•—•. Running for 2·5 h at 25,000 r.p.m. Drop collection and radioactivity were made as described in Fig 1

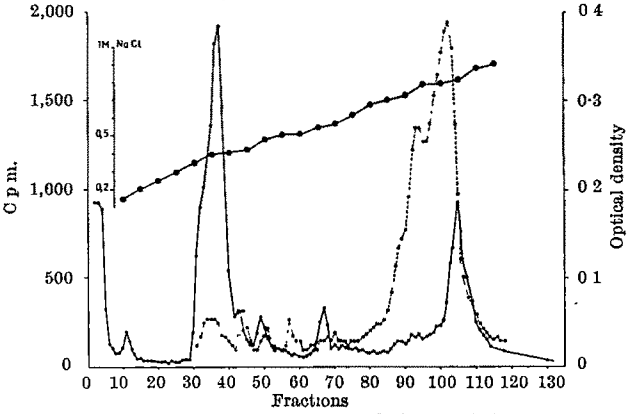


Fig. 3. Extract as in Fig. 1 chromatographed on methylated albumin column (MAK). Elution gradient from 0·2 M to 1·2 M sodium chloride phosphate buffer *p*H 7; 2·5 ml. per tube. •—•—•—•, Optical density, o----o----o, radioactivity

Both virus band and viral RNA were therefore analysed for phosphorus-32 and carbon-14 radioactivity. The results of two experiments appear in Table 1. The carbon-14 radioactivity is present in the virus and absent from the heavy RNA peak, thus excluding the possibility that this peak is a ribonucleoprotein.

Though the homogeneity and exact symmetry of the 70*S* RNA peak made it unlikely that it was a structure aggregated either by means of metallic ions or other non-covalent bonds, it was necessary to exclude this eventuality by the following experiments: RNA extraction was performed in 10^{-2} M EDTA, *p*H 7, plus PVS 2 μg/ml. Following isolation in a sucrose gradient, an RNA peak identical with the one characterized under standard conditions was obtained. Moreover, viral RNA dialysed at 4° C against solutions of various ionic strengths modified only to a small extent its sedimentation constant, as shown in Table 2. Persistent presence of the 70*S* RNA following EDTA treatment as well as in solutions with very low ionic strength indicated that this peak did not consist of an aggregated structure.

Base composition. The 70*S* peak base composition was determined for several preparations. The results are shown in Table 3. The base composition of the light RNA does not appear in the Table since it varies from preparation to preparation. These variations are probably due to very small components which were lost during the purification of this fraction, and particularly during dialysis

Table 1

Radioactivity (c.p.m.)	Virus purified by Moloney's method		Virus purified in caesium chloride gradient		Viral RNA	
Phosphorus-32	1,000	1,000	1,000	1,000	1,000	1,000
Carbon-14	167	121	170	140	0	0

Table 2

Buffers (*p*H 7)	Ionic strength Γ/2	$S_{20,w}$
0·1 M sodium chloride–0·01 M phosphate	0·160	68
0·01 M sodium chloride–0·001 M phosphate	0·0160	62
0·001 M sodium chloride–0·0001 M phosphate	0·00160	65

Table 3

UMP	GMP	CMP	AMP	Ratio
23·7 %	26·3 %	25·6 %	24 4 %	$\frac{G+C}{A+U} = 1{\cdot}07$

Table 4

RNase	0γ	0·1 γ/ml.	0·5 γ/ml.	2·5 γ/ml.	10 γ/ml.
C.p.m. phosphorus-32 precipitable by TCA	8,000	580	850	670	500

Method; 5×10^{-3} M *tris*, 2×10^{-3} M EDTA, *p*H 7·2; incubation for 20 min at 22° C.

against 10^{-3} M sodium chloride. Moreover, degraded cellular RNAs, however small their proportion may be in that peak, cannot be eliminated.

RNase sensitivity. 70*S* viral RNA was submitted to various RNase (Nutritional Biochemical Corporation) concentrations, ranging from 10 γ/ml. to 0·1 γ/ml. The results appear in Table 4. In all cases, a high degree of RNA degradation was observed. However, it was never complete, and about 7 per cent of the radioactivity remained precipitable by trichloroacetic acid (TCA).

In conclusion, the extraction of RNA from labelled Rauscher virus leads to the isolation of two radioactive fractions, one light (~4–5*S*) and the other heavy (~70*S*). These two fractions are always present, but their relative amount varies from experiment to experiment; since they are RNase sensitive and under the conditions of extraction, a completely deproteinized RNA is currently obtained, they are made of RNA. The 'light' fraction is of viral origin, since cellular RNAs are poorly labelled, as shown in Fig. 1. However, the presence of cellular RNAs (transfer RNA or degraded ribosomal fragments) cannot be excluded in this peak, as suggested by the results of extraction from uninfected *JLS V6* cells (Fig. 2). The size of this light component is too small to represent the intact viral RNA. It may represent either its product of degradation[8] or, eventually, sub-units of the viral RNA. On the contrary, the heavy fraction probably represents intact viral RNA for the following reasons: viral pellets were used for extraction and the low radioactivity found in ribosomal RNAs indicates the good quality of the purification achieved by Moloney's technique; the RNA extracted from uninfected cell culture supernatants did not give such a peak; finally, a 70*S* peak identical with that produced by the virus concentrated according to Moloney's technique was obtained following centrifugation in a caesium chloride gradient (which is known to yield an excellent virus purification). On the other hand, the possibility that the 70*S* component represents a ribonucleoprotein or an aggregate seems to be excluded. A ribonucleoprotein is unlikely because (*a*) double labelling experiments do not reveal protein and (*b*) it can be chromatographed on MAK, whereas a ribonucleoprotein would become irreversibly attached to the kieselguhr. An aggregate seems to be excluded by the results of extractions performed in the presence of EDTA and by the behaviour of the 70*S* fraction in the presence of low ionic strength solutions.

Another problem raised by this heavy fraction is that of its single or double-stranded structure. The results of base composition determinations, particularly the study of the ratios G/C and A/T, are not conclusive, but the very high sensitivity of 70*S* RNA to RNase (the significance of the small RNase-resistant fraction of the viral RNA is still unknown) is in favour of a single-stranded structure. Moreover, if such a molecule was double-stranded (*a*) it would have such a rigidity that it would be scarcely extractable without any mechanical rupture, and (*b*) its estimated molecular weight would be inconsistent with the amount of RNA reported in the related Rous sarcoma and avian myeloblastosis viruses[9,10].

These facts seem to indicate that Rauscher virus RNA is single-stranded, and its molecular weight value calculated by Spirin's expression[11] would be about 10^7. This value is close to what has recently been reported by Robinson *et al.*[12] for Rous sarcoma virus, by Harel *et al.*[13] for avian myeloblastosis virus, and by Duesberg *et al.*[14] for Newcastle disease virus. Recently, Mora *et al.*[15] have shown that the RNA extracted from the highly infectious plasma Rauscher virus is a single-stranded molecule of about 13×10^6 molecular weight. Further experiments are in progress to investigate the structure of this viral RNA. It can only be said at the present time that its behaviour under various ionic strengths at 20° C seems to be different from that of tobacco mosaic virus[16] and Rous sarcoma virus[12] RNAs.

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LARYNGEAL MYOTATIC REFLEXES

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In the course of an examination of the proprioceptive reflex innervation of the larynx, previous investigations[1–6] in this laboratory revealed the existence of articular reflexes in the larynx. These reflexes, driven by low-threshold, rapidly adapting corpuscular mechanoreceptors embedded in the capsules of all the laryngeal joints[1,2,5,6], produce transient, reciprocally related changes in the tone of the intrinsic laryngeal muscles[3,4,6] whenever the laryngeal joints are moved in appropriate directions[3–6]. The afferent impulses evoking these articular reflex responses in the laryngeal muscles are conducted rapidly into the brain stem in large diameter, myelinated afferent fibres in the laryngeal branches of the vagus nerves[2,3,5,6] —by way of the ipsilateral internal laryngeal nerve in the case of the thyrohyoid, thyro-epiglottic and cricoarytenoid joints[1,2,6], and by way of the ipsilateral recurrent laryngeal nerve in the case of the cricothyroid joint[1,2,4–6]—from which they are polysynaptically relayed to the motoneurone pools of the laryngeal muscles[4–6].

These articular reflexes, however, are essentially phasic in character, producing transient adjustments in the tone of the laryngeal muscles only at the moment of joint displacement[4–6]. The laryngeal articular mechanoreceptors thus have no sustained influence on the tone of the laryngeal muscles[6]—yet, in view of the precisely regulated,

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continuous adjustments of laryngeal muscle tone that occur unconsciously during the respiratory cycle and during phonation[6-9], a tonic proprioceptive reflex system must be present in the larynx[1,6,13-15]. Such a reflex system would require to be driven by very slowly adapting mechanoreceptors located in the larynx; and as appropriate receptors are almost entirely lacking in the laryngeal joints[2-6], we have examined the laryngeal muscles neurohistologically and neurophysiologically for evidence of the presence therein of slowly adapting mechanoreceptors capable of provoking sustained alterations in the tone of the intrinsic muscles.

Previous attempts to demonstrate such reflexes in the intrinsic muscles of the larynx in various species of animals, and in man, have either been unsuccessful[10-13] or, at best, equivocal in their results[14,15]. This is in spite of the fact that histological investigations in man, and in some animals, have suggested that certain of these muscles contain nerve endings the structure and location of which might qualify them as slowly adapting mechanoreceptors[13,16-19]. This article reports our identification of sustained laryngeal myotatic reflexes, distinct from the phasic articular reflexes that we have previously described[3-6], that are provoked from mechanoreceptors located within the intrinsic muscles of the larynx. (A later communication will describe the structural features of the receptor endings in the laryngeal muscles that we believe to be responsible for the tonic laryngeal myotatic reflexes reported here.)

Experiments were performed on twelve neurologically intact, adult cats (average weight 3·8 kg) under light, controlled (monitored) pentobarbitone anaesthesia. In eight experiments tracheostomy was not performed, and the animals were allowed to breathe an oxygen-enriched air mixture spontaneously through an intrapharyngeal tube so that air flowed in and out through the larynx in the normal manner during the respiratory cycle: in this way, the influence of the myotatic reflexes on the oscillations of laryngeal muscle tone during the various phases of the respiratory cycle could be examined. In four experiments a low tracheostomy intubation was performed, so that the same gas mixture was breathed spontaneously in the absence of air flow through the larynx, thereby enabling the influence of the myotatic reflexes to be examined in the absence of respiratory oscillations in laryngeal air pressure. The pulmonary ventilation rate, tidal volume and laryngeal resistance to air flow were monitored continuously from meters on a small-volume Drager 'Anaesthesia-Spiromat' machine, through which the animals breathed. (The 'Anaesthesia-Spiromat' was provided by the Department of Anaesthetics of the Royal College of Surgeons of England, through the courtesy of Dr. Denis Hill.)

The animals were kept warm on a thermostatically regulated, heated operating table, and were supplied with isotonic fluid through a cannula in the right femoral vein that was also used for administration of the anaesthetic agent. Operating procedures on the larynx were performed with microsurgical instruments and a Zeiss operating microscope. Throughout each experiment, all exposed tissues were submerged in a pool of warm mineral oil.

The larynx was exposed through a mid-line anterior cervical incision. After removing the extrinsic muscles on the left side, the left lamina of the thyroid cartilage was exposed, and the laryngeal nerves were identified on both sides. After freeing it from the inferior constrictor muscle and the internal laryngeal nerve (which was preserved intact), the left thyroid ala was removed up to the mid-line—except for a small vertical fragment to which the anterior end of the left thyro-arytenoid (vocalis) muscle remained attached, and a thin horizontal strip to which the upper border of the left cricothyroid muscle was left attached. These two cartilaginous strips were separated from one another, and from the remainder of the thyroid cartilage, so that they could be moved independently. Possible adventitious reflex effects from movement of the left cricothyroid joint[3-6] were precluded by disarticulating it, after dividing the inferior cornu of the thyroid cartilage and freeing the attachment of the cricothyroid muscle to the joint capsule. The lumen of the larynx was not opened at any stage. The crico-arytenoid joints, and their muscular attachments, were left intact to provide a source of articular reflexes for control purposes during traction on the laryngeal muscles attached to the arytenoid cartilages. The animals were immobilized in the supine position by fixation to a brass scaffold bolted to the operating table.

Non-elastic traction threads were attached to the cartilaginous strip along the upper border of the left cricothyroid muscle, to the cartilage fragment at the anterior end of the left thyro-arytenoid (vocalis) muscle, to the rostral end of the right sternothyroid muscle (after freeing it from its thyroid attachment), and to the proximal cut end of the left patellar tendon. The threads were passed over pulleys attached to the fixation scaffold, and graduated passive stretch was applied to each muscle in turn for varying periods through the threads, with the aid of weights ranging from 3 g to 25 g. The pulleys and threads were orientated so that traction was applied to the left cricothyroid and right sternothyroid muscles in a caudal-rostral plane, and to the left thyro-arytenoid (vocalis) muscle in a postero-anterior plane; traction was applied to the left patellar tendon in the proximo-distal plane of the leg.

Pairs of fine, stainless-steel recording electrodes (insulated except at their tips) were inserted into the left and right cricothyroid, left thyro-arytenoid (vocalis), right sternothyroid and left rectus femoris muscles in every experiment. In some experiments, additional pairs of electrodes were inserted into the right thyro-arytenoid (vocalis) muscle (after removing part of the right thyroid ala) and into the left lateral and posterior crico-arytenoid muscles (after rotating the larynx to the right). Electromyograms were recorded simultaneously from all the muscles on a Grass polygraph, together with records of the animal's respiratory movements in thorax and abdomen, and of its electrocardiogram. Recordings were run in the absence of stretch of the muscles to examine their resting activity; and continuously before, during and after the application of each stretch to each muscle, the procedure being repeated at least three times in each instance.

The effects of varying degrees of general anaesthesia on the resting activity and on the stretch responses of each of the muscles were then examined, as were the effects of serial division of each of the laryngeal nerves (internal, external, superior, recurrent, ramus communicans) on the two sides—in different sequences in different experiments.

Two types of myotatic reflex response were demonstrated in the laryngeal muscles during their passive stretch—one facilitatory and the other inhibitory. As with the laryngeal articular reflexes[4-6], these myotatic reflexes could only be elicited in the early stages of barbiturate anaesthesia: they were entirely absent at the stages of general anaesthesia customarily used for major surgical procedures.

Facilitatory myotatic reflexes. These reflexes were evidenced by a sustained increase in the amplitude and frequency of motor unit discharge in the stretched muscle that began as soon as the stretch was applied, persisted throughout its application (for periods of up to 3 min), and disappeared as soon as the stretch was removed. The threshold stretch tension required for the production of such responses in the cricothyroid and thyro-arytenoid muscles was 5 g; and the magnitude of the response progressively increased with increments in the degree of stretch, up to 15 g. But further increases in stretch tension beyond 15 g (up to 25 g) converted the facilitatory response into an inhibitory reflex response (*vid. inf.*),

evidenced by a sustained decrease in the amplitude and frequency of motor unit discharge in the stretched muscle. The threshold tension for elicitation of the facilitatory response in the sternothyroid muscle was a little lower (3 g) than in the intrinsic muscles; and the degree of stretch required to convert the facilitatory response into an inhibitory response was higher (in excess of 20 g).

Stretch applied either to the left cricothyroid or left thyro-arytenoid muscle was usually accompanied by equally sustained changes (either facilitatory or inhibitory) in the motor unit activity of other muscles on the same and on the opposite sides of the larynx, in patterns that varied with the degree of stretch and with the particular muscle being stretched. (These synergistic and reciprocal myotatic reflexes will be described in more detail in a later communication.)

An additional facilitatory reflex effect was usually associated with application of stretch to the left thyro-arytenoid muscle, in that at the moment of application of the stretch (and also at the moment of its removal) a brief (less than 1 sec) high-amplitude burst of motor unit potentials was evoked in the stretched muscle, and (at lower amplitude) also in the ipsilateral cricothyroid muscle. These bursts of activity occurred only at the beginning and end of each sustained stretch reflex response, and increased in amplitude and duration (up to 1 sec) with increasing degrees of stretch—irrespective of whether the myotatic reflex response evoked thereby was facilitatory or inhibitory. These 'on–off' transients have all the characteristics of the laryngeal articular reflex responses described previously[3–6], and were selectively abolished by section of the ipsilateral internal laryngeal nerve (which innervates the crico-arytenoid joint[1,2,6]. It is clear, then, that they are superimposed phasic reflex responses to stimulation of the rapidly adapting mechanoreceptors located in the capsule of the crico-arytenoid joint[1,2,6], resulting from movement of the arytenoid cartilage when tension is applied to, or removed from, the anterior end of the thyro-arytenoid muscle—the posterior arytenoid attachment of which is deliberately left intact in these experiments.

In those experiments in which the animal was breathing through its larynx, cyclical fluctuations in the tone of the laryngeal muscles were conspicuous in the alternating phases of respiration (they were usually absent, or barely apparent, in the tracheotomized animals). Sustained application of stretch to the cricothyroid or thyro-arytenoid muscles in these circumstances produced greater reflex effects on motor unit activity in the resting than in the active phases of the respiratory cycle. Further, the degree of stretch necessary for the production of observable effects during the active phase was substantially greater than that needed during the resting phase; and often, no facilitation of the active phase could be observed in circumstances when there was marked facilitation of activity during the resting phase. This is in marked contrast to the situation in the tracheotomized animals, in which reflex facilitation was evenly sustained throughout the period of stretch without respect to the respiratory cycle, and indicates that the myotatic reflex response to passive stretch is depressed or aborted by active contraction of the stretched muscle. This fact, which suggests that the mechanoreceptors responsible for the myotatic reflexes lie in parallel with the laryngeal muscle fibres (and not in series with them), is in accordance with an observation made elsewhere[15]; namely, that slowly adapting discharges fired into the ipsilateral recurrent laryngeal nerve by stretching the thyro-arytenoid muscle are interrupted by active contraction of the muscle in response to its direct electrical stimulation.

That these low-threshold facilitatory myotatic reflexes normally make a continuous (though varying) contribution to the regulation of the tone of the laryngeal muscles is evidenced by the fact that motor unit activity in the left cricothyroid and thyro-arytenoid muscles was substantially reduced (in frequency and amplitude) when they were detached from the thyroid cartilage, in comparison with the homologous muscles on the right side—so long as these latter were not detached as well. Relevant in this respect are some previous reports[15,13] that sustained afferent discharges can be recorded from nerve fibres supplying the cricothyroid, thyro-arytenoid and posterior crico-arytenoid muscles *in situ*; and a further observation in the present experiments that graduated stretch applied to the left (detached) cricothyroid and thyro-arytenoid muscles could, if appropriately adjusted, restore their motor unit activity to a pattern identical with that in the normally attached homologous muscles on the right side.

Inhibitory myotatic reflexes. These reflexes, manifested by a sustained decrease in the amplitude and frequency of motor unit discharge in the stretched muscles, could be elicited also from the cricothyroid, thyro-arytenoid and sternothyroid muscles—but their production required greater degrees of stretch than the facilitatory reflexes. Thus, at the stage of anaesthesia at which 5 g stretch elicited clearly detectable facilitatory responses, at least 15 g of stretch was required to convert this into inhibition in the cricothyroid and thyro-arytenoid muscles, and at least 20 g in the case of the sternothyroid muscle. Further increases in the degree of stretch above these values produced greater degrees of inhibition in each of the muscles.

As with the facilitatory responses, the reflex inhibition was sustained throughout the period of applied stretch, and showed no signs of adaptation within the periods used in these experiments. Further, the inhibitory effect was equally sustained in both the tracheotomized and the non-tracheotomized animals; and in the latter there was more marked reduction of motor unit activity during the active phases of the respiratory cycle than during the resting phases. Unlike the facilitatory reflexes, then, the inhibitory myotatic reflexes are not aborted (but are augmented) during active contraction of the stretched muscle; and this might indicate that the mechanoreceptors responsible for the inhibitory effects are different from those evoking the facilitatory responses, lying in series with the muscles (and not in parallel) and having a higher threshold.

The transient on–off bursts of articular reflex origin were still evoked in the thyro-arytenoid and cricothyroid muscles by the application of weights in excess of 15 g to the thyro-arytenoid muscle, and were more marked than with lesser weights. In these circumstances, however, they occurred at the beginning and end of a period of sustained inhibition, instead of sustained facilitation.

Effects of changing anaesthesia. Alterations in the degree of barbiturate anaesthesia had a number of significant effects on the myotatic reflex responses in the laryngeal muscles.

Such reflexes were only elicitable in the early (that is, light) stages of anaesthesia, when the pulmonary ventilation rate of the animal was in excess of 600 ml./min and the corneal and swallowing reflexes were still present. A gradual increase in anaesthesia from this stage produced a rapid reduction in, and early abolition of, the facilitatory responses; and comparison of the stretch reflex responses in the intrinsic laryngeal muscles with those simultaneously elicited in the rectus femoris muscle showed that the laryngeal myotatic reflexes were totally abolished at stages of anaesthesia at which there was very little alteration in the myotatic reflexes in the limb muscle.

During gradually increasing anaesthesia, the myotatic reflexes in the laryngeal muscles began to diminish a little before the articular reflex responses occurring in the same muscles—and the former disappeared before the latter. This relation is the reverse of that obtaining with the myotatic and articular reflexes in limb muscles[20], during increasing anaesthesia. Both types of reflex were abolished in the larynx at quite moderate degrees of anaesthesia, however, and at a point far short of that

required for abolition of the myotatic reflex in the rectus femoris muscle. With gradual recovery from established anaesthesia, the reverse phenomenon occurred in that the myotatic reflexes in the limb recovered well before those in the larynx, and in the larynx the articular reflexes returned a little before, and recovered more quickly than, the myotatic reflexes.

It was also noted that a small increase in anaesthesia abolished the facilitatory responses in the laryngeal muscles before the inhibitory reflexes elicitable in the same muscles. Moreover, in such circumstances the threshold of the inhibitory reflex system was reduced, so that 10 g (or even 5 g) of applied stretch elicited an inhibitory response in a muscle in which the same degree of stretch only produced facilitatory responses at lighter anaesthesia.

Effects of sequential section of laryngeal nerves. The various effects of section of the different laryngeal nerves on the myotatic reflexes are still being investigated, but a few observations are pertinent here.

For example, division of all the laryngeal nerves on the right side of the larynx did not significantly alter the myotatic reflex responses in the muscle being stretched on the left side of the larynx. The same applies also to the articular reflex transients. In some experiments, division of the right external (or less often, the right recurrent) laryngeal nerve diminished the resting activity in the left cricothyroid muscle, but the myotatic and articular reflexes could still be elicited therein at the same threshold. In other experiments, division of the right recurrent laryngeal nerve low in the neck (between the tenth and twelfth tracheal rings) similarly reduced the resting activity in the left thyro-arytenoid muscle; but again the myotatic and articular reflexes remained readily elicitable in the muscle. Thus, while the contralateral external (and in some cases, the recurrent) laryngeal nerve may contribute some motor fibres to the cricothyroid muscle, and the contralateral recurrent nerve may contribute motor fibres to the thyro-arytenoid muscle, in neither case does a significant proportion of mechanoreceptor afferent fibres from these muscles appear to enter contralateral laryngeal nerves.

On the other hand, section of the left internal laryngeal nerve was found in a number of instances to reduce markedly (or even abolish) the facilitatory myotatic reflex responses in the ipsilateral thyro-arytenoid (and sometimes the ipsilateral cricothyroid) muscle without altering its resting activity. The rapidly adapting articular reflex responses in these muscles were always abolished in such circumstances, whether or not the sustained myotatic reflexes were affected. This is in accordance with our earlier observation[1,2,6] that most of the mechanoreceptor afferents from the crico-arytenoid joint enter the ipsilateral internal laryngeal nerve; but, in addition, it now seems that in some (but not all) animals a proportion of mechanoreceptor afferents from the intrinsic laryngeal muscles may enter the same nerve—as has been suggested elsewhere on the basis of degeneration studies[19].

In most animals, however, bilateral section of the internal laryngeal nerves had no effect on the myotatic reflexes in the thyro-arytenoid and cricothyroid muscles—although the articular reflexes from the crico-arytenoid joint were always abolished in these muscles by this procedure. It seems, therefore, that in most circumstances the myotatic reflex afferents from these two muscles do not traverse the internal laryngeal nerve.

Additional section of the external laryngeal nerves (which contain the bulk of the motor fibres innervating the cricothyroid muscles), or proximal section of the superior laryngeal nerves close to the ganglion nodosum, in the same animals usually abolished the myotatic reflex responses in the cricothyroid muscle without affecting those in the thyro-arytenoid muscle—although the last-mentioned responses were abolished in turn by further section of the left recurrent laryngeal nerve (which, of course, contains most of the motor fibres innervating the thyro-arytenoid muscle). It seems likely, then, that the myotatic reflex afferents from the thyro-arytenoid muscle usually (though not always exclusively) enter the ipsilateral recurrent laryngeal nerve, which also receives the mechanoreceptor afferent fibres from the ipsilateral cricothyroid joint[1,2,4–6]. This would accord with previous observations[15,13] that traction on the thyro-arytenoid (or posterior crico-arytenoid) muscles provokes slowly adapting discharges in afferent fibres in the recurrent laryngeal nerve that are interrupted during active contraction of the muscle.

The myotatic reflex afferent pathway from the cricothyroid muscle has not as yet been finally delineated in our experiments. In a few instances (*vid. sup.*) some afferent fibres from this muscle may enter the ipsilateral internal laryngeal nerve, but most do not. Whether they enter the external or the ipsilateral recurrent laryngeal nerve cannot yet be decided; but the overall impression from our findings to date is (surprisingly) in favour of the latter. This impression receives support from a report[13] that no afferent impulses could be detected in fibres of the external laryngeal nerve when the cricothyroid muscle was stretched.

We noted in some animals that section of the left internal laryngeal nerve might abolish the facilitatory myotatic reflex response in the ipsilateral thyro-arytenoid muscle (and occasionally in the cricothyroid muscle as well) without affecting the inhibitory reflexes in the same muscle. Furthermore, in some of these instances the threshold of the inhibitory reflex system was reduced (as happens also with a slight increase in anaesthesia), so that the application of 10 g (or even 5 g) of stretch after nerve section evoked an inhibitory response in place of the facilitatory response that was elicited beforehand with the same degree of stretch. This observation raises the possibility that afferent fibres mediating inhibitory myotatic reflex effects on the laryngeal muscles may follow, at least in some cases, a different peripheral centripetal pathway from those mediating facilitatory myotatic reflexes in the same muscles.

The following conclusions appear to be justified by the observations described in this article:

(1) Myotatic reflexes are elicitable (by passive stretch) in the intrinsic as well as in the extrinsic muscles of the larynx.

(2) The myotatic reflexes in the intrinsic laryngeal muscles are generated from low threshold, very slowly adapting, mechanoreceptors located within the muscles themselves, whence afferent fibres pass into the ipsilateral laryngeal nerve trunks—usually the recurrent nerve, but sometimes the internal laryngeal nerve.

(3) The laryngeal myotatic reflex systems are of two types—facilitatory and inhibitory, the former being of lower threshold than the latter. Whether this dichotomy is due to the operations of two types of mechanoreceptor ending of differing threshold in the laryngeal muscles, or whether it results from the differing central effects of low- and high-frequency discharges from the same receptor endings, is uncertain as yet; but the findings in the present investigation favour the former explanation.

(4) The myotatic reflexes in the larynx are distinct from the laryngeal articular reflexes in their functional characteristics—the former being tonic reflexes provoked from very slowly adapting mechanoreceptors, whereas the latter are phasic reflexes operated from rapidly adapting mechanoreceptors.

(5) The laryngeal myotatic reflex system seems to be polysynaptic and not monosynaptic, in view of its marked sensitivity to barbiturate narcosis, and resembles the laryngeal articular reflex system in this respect. The myotatic reflex system in the larynx thus differs markedly from the monosynaptic myotatic reflex system in the limb muscles.

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SENSITIZATION OF *Escherichia coli* TO RADIATION BY BROMOURACIL: EXCESSIVE POST-IRRADIATION BREAKDOWN OF DEOXYRIBONUCLEIC ACID WITHOUT CONCOMITANT SYNTHESIS

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E. COLI is known to excise thymine dimers from its DNA during incubation after ultra-violet irradiation. This excision of thymine dimers is accompanied by extensive breakdown of the DNA with the release of the products into the acid-soluble fraction of the cell and into the medium. Moreover, both thymine dimer excision and DNA breakdown occur in wild-type strains of *E. coli* that are able to repair their DNA, but both processes are greatly reduced in certain mutants, notably strains B_{s-1} and *K*-12 *uvr*$^-$, which are unable to repair ultra-violet-irradiated *T*1 phage[1–3].

In addition to excision and breakdown, exposure of *E. coli* to moderate doses of ultra-violet light causes a lag in DNA synthesis, following which there is a resumption of synthesis at nearly the normal rate. In mutants unable to excise thymine dimers, however, the return of DNA synthesis is less than 10 per cent of that in the wild type[4,5].

According to the recently developed theories on the mechanism of the enzymatic repair of DNA following exposure to ultra-violet light[1,2], repair occurs by the following processes: (1) enzymatic release or excision of defective bases such as thymine dimers, leaving gaps in one of the two polynucleotide chains; (2) enzymatic widening of these gaps by local single-strand degradation and release of nucleotides; (3) reconstruction of the strand by insertion of nucleotides using the intact opposite strand as a template (repair synthesis); (4) completion of the DNA chain by rejoining the phosphodiester linkage.

Bacterial cells which have 5-bromouracil (BU) in their DNA in place of thymine are very sensitive to ultra-violet light[6], to X-rays[7], to mitomycin *C*[8], and possibly to other deleterious agents. It has been suggested that the presence of BU in DNA sensitizes by inhibiting post-irradiation DNA repair[9,10]. According to the foregoing model, the inhibition of DNA repair by BU might be observed as the loss of ability to break down DNA or the failure to synthesize DNA after irradiation. In the present work, radioactive thymidine was used to follow the processes of DNA breakdown and synthesis in BU-substituted *E. coli* after exposure to ultra-violet light. The results indicate that irradiated DNA containing BU is extensively degraded and that DNA synthesis is inhibited; thus, the DNA is not effectively repaired.

The bacteria used were *E. coli K*-12 strain *AB*2416 *str*^{R}*thy*$^-$*uvr*$^+$ and *AB*2419 *Str*^{R}*thy*$^-$*uvrB*, which were derived from WS 110Y25 *thy*$^-$ obtained from Dr. Rivka Rudner. Cells were routinely grown in a salts–glucose medium enriched with 2·5 mg/ml. casamino acids and thymine. This will be referred to as *EM*9. Plating medium consisted of yeast extract–tryptone agar. For the determination of post-irradiation DNA breakdown, overnight cultures in *EM*9 containing 2·5 μc./ml. ^{14}C-thymine (25 μg/ml.) were diluted five times and grown about two generations in *EM*9 containing 5 μg/ml. ^{14}C-thymine alone, or 5 μg/ml. ^{14}C-thymine + 50 μg/ml. 5-bromouracil. In a similar experiment, using ^{3}H-bromodeoxyuridine and ^{14}C-thymidine (TDR), it was calculated that about 50 per cent of the thymine in the DNA was replaced by BU. Cells were collected and suspended in *M*9 for irradiation. For ultra-violet survival curves, or determination of post-irradiation DNA synthesis, cells were prepared as stated except that non-radioactive thymine was used.

Cell suspensions in *M*9 transparent to 2537-Å light were irradiated with a low-pressure mercury germicidal lamp. The dose-rate was 10 ergs/mm^2/sec. For survival curves, irradiated samples were diluted into cold broth, plated, and colonies counted after overnight incubation at 37° C.

To determine post-irradiation DNA breakdown, samples were taken from the suspension of labelled cells at intervals during incubation at 37° C. These were acidified with perchloric acid to 0·5 N, maintained at 4° C for 30 min, and centrifuged with heat-killed cells as carrier; the radioactivity in a small sample of the supernatant was counted in a liquid scintillation counter. To measure the total radioactivity of each sample, the pellets were dispersed in the same supernatants, the tubes were heated to 90° C for 40 min, centrifuged, and the radioactivity in the supernatants again counted. After digesting the pellet in hot acid in this manner, more than 98 per cent of the radioactivity was in the supernatant.

To measure DNA synthesis after irradiation, non-radioactive cells, previously grown in *EM*9 containing 5 μg/ml. thymidine or 1 μg/ml. thymidine + 5 μg/ml. 5-bromodeoxyuridine, were suspended in *EM*9 containing 2 μc./ml. ^{14}C-TDR (1·6 μg/ml.) and incubated at 37° C with aeration for 4 h. At 30-min intervals 0·1-ml. samples of the suspension were placed on Whatman 2·4 cm filter paper disks. Immediately 0·04 ml. of 1 per cent sodium hydroxide was added to each disk and allowed to dry. Iced 10 per cent trichloroacetic acid (TCA) was then added, and when dry, the disks were washed in cold 5 per cent TCA for 1 h, re-washed for 10 min, dried and counted in a liquid scintillation counter.

Fig. 1 shows the effects of 2537-Å light on the original parental *uvr*$^+$ strain and on a non-repairing (*uvr*$^-$) mutant of *E. coli* grown in the presence and absence of BU. It is seen that in the *uvr*$^+$ parental strain, cells grown in the presence of BU for 1·5 generations are much more sensitive to ultra-violet light. In contrast, growth in the presence of BU has no effect on the fraction of *uvr*$^-$ cells surviving

after ultra-violet irradiation. These results suggest that BU inhibits DNA repair in the *uvr*$^+$ parental strain, whereas in the *uvr*$^-$ mutant, which cannot excise thymine dimers, BU has no effect on the radiation sensitivity.

Fig. 2 shows that after exposure to ultra-violet light, *uvr*$^+$ cells containing ^{14}C-thymine labelled DNA release a large percentage of this radioactivity to the medium. Both the rate and extent of this release of radioactivity are markedly greater in cells labelled with BU. These results show that the presence of BU in place of thymine does not inhibit DNA breakdown after ultra-violet irradiation, but, on the contrary, enhances breakdown.

Since BU does not inhibit DNA repair by preventing breakdown, experiments were carried out to determine the ability of normal and BU-substituted cells to synthesize DNA after irradiation. Fig. 3 shows the rate at which ^{14}C-TDR is incorporated into normal and BU-labelled cells after ultra-violet irradiation. It is seen that cells containing BU show a striking loss of ability to synthesize DNA following ultra-violet irradiation.

The foregoing results support the conclusion that in *E. coli* only normal and not BU-containing DNA is effectively repaired. This inhibition is not due to any failure in the breakdown of DNA. As evidence for this, irradiated ^{14}C-thymine-labelled cells containing BU showed a greater release of radioactive label into the medium during incubation than did normal cells. In DNA which had 50 per cent thymine replaced by BU, the probability of forming an ultra-violet-induced thymine dimer should be decreased by a factor of 4. If only thymine dimer excision initiates DNA breakdown, then it could reasonably be expected that breakdown in BU-labelled cells would be inhibited. That such is not the case suggests that BU photoproducts may also be excised. Wacker[11] has shown that BU substituted for thymine in DNA is converted to uracil after ultra-violet irradiation. Smith[12] has isolated five debrominated products after ultra-violet irradiation from *E. coli* DNA containing BU. One product was uracil; the other products remain unidentified. Thus, uracil–uracil and uracil–thymine dimers might be among the photoproducts formed in BU-containing DNA. The latter has been identified in normal ultra-violet-irradiated DNA, arising from cytosine-thymine dimerization followed by deamination of the cytosine moiety[13]. This photoproduct is excised from DNA[2], and it is likely that uracil–uracil dimers would be similarly excised.

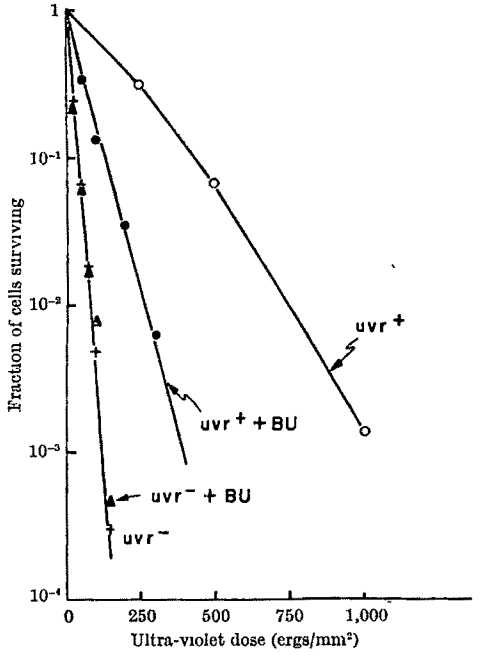


Fig. 1 Survival of colony-forming ability of *E. coli K*–12 *AB*2416 *uvr*$^+$ *thy*$^-$ and *AB*2419 *uvr*$^-$ *thy*$^-$ grown in normal and 5-bromouracil containing medium after ultra-violet irradiation. For details, see text

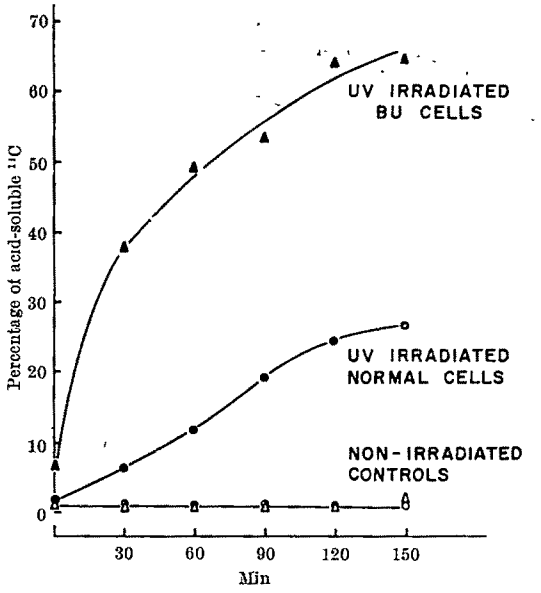


Fig. 2 Release of cold acid-soluble carbon-14 from ^{14}C-thymine labelled *E. coli K*–12 *AB*2416 *uvr*$^+$ *thy*$^-$ during incubation following ultra-violet irradiation. Washed cells grown in *EM*9, containing 5 μg/ml. ^{14}C-thymine (0·5 μc /ml.) or 5 μg/ml. ^{14}C-thymine plus 50 μg/ml. BU, were divided into two samples. One sample served as the unirradiated control, while the other was exposed to 1,000 ergs/mm^2 ultra-violet light. Both samples were transferred to *EM*9 containing 40 μg/ml. non-radioactive thymine and incubated at 37° C. At intervals the percentage of cold acid-soluble carbon-14 was determined as described in the text

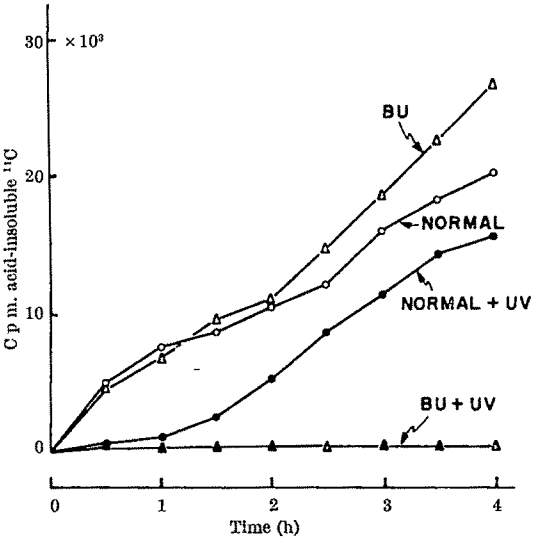


Fig 3. Incorporation of ^{14}C-thymidine into cold acid-precipitable material in normal and BU-containing *E. coli AB*2416 *uvr*$^+$ *thy*$^-$ after ultra-violet irradiation. Non-radioactive cells grown in the presence or absence of BUDR were transferred to *EM*9 containing 2 μc /ml ^{14}C-thymidine (1·6 μg/ml.) after no treatment or after exposure to 500 ergs/mm^2 ultra-violet light. The bacterial suspensions were adjusted to the same initial optical density and incubated at 37° C. At intervals cold acid-insoluble carbon-14 was determined per ml. of culture as described in the text

In comparison with normal cells, those with BU in their DNA exhibit a marked loss of ability to incorporate ^{14}C-TDR into cold acid-insoluble material after ultra-violet irradiation. There is negligible incorporation in the first 4 h after exposure to ultra-violet light, showing that neither DNA repair synthesis nor DNA replication occurs to any appreciable extent. In comparable experiments with X-rays in place of ultra-violet light, it was found that DNA breakdown in BU-containing cells was also greater than in the normal cells. After exposure to 10 krads, the ability to incorporate TDR into normal cells is decreased by only 10–20 per cent, while in BU-

containing cells, it is depressed by about 60 per cent[14]. This is in contrast to the present results obtained with ultra-violet light. Nevertheless, a similar mechanism of sensitization may be operative that involves a failure of DNA repair synthesis. The intermediate level of TDR incorporation after X-irradiation does not prove the contrary, as it may be due to DNA replication rather than repair synthesis; this point will need further work for elucidation.

In work with recombination defective mutants of *E. coli*[15], we have observed an interesting parallel in the ultra-violet-induced breakdown and synthesis in these strains and in normal cells containing BU as described here. This relationship will be pursued in future publications.

The loss of ability of BU-containing cells to synthesize DNA after ultra-violet irradiation may be closely related to the failure to repair DNA and to the abnormally high radiosensitivity. Indeed, the breakdown of 60 per cent of the DNA without concomitant DNA synthesis is indicative of the formation of two-strand breaks, which are presumably lethal. Since DNA repair synthesis follows post-irradiation breakdown in normal cells, it appears that the presence of BU primarily affects the change-over from exonuclease to repair polymerase at the sites of action in the DNA, perhaps by altering the relative affinities of the two enzymes so as to favour continued breakdown.

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PRODUCTION OF ADDITIONAL SITES OF DEOXYRIBONUCLEIC ACID BREAKDOWN IN BROMOURACIL CONTAINING *Escherichia coli* EXPOSED TO ULTRA-VIOLET LIGHT

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THE substitution of 5-bromouracil (BU) in place of thymine in the DNA of bacteria renders them highly sensitive to the lethal effects of various deleterious agents, including ultra-violet light[1,2], X-irradiation[3] and mitomycin *C* (MC)[4]. Wild-type *Escherichia coli* (henceforth called *uvr*$^+$ bacteria) that are capable of repairing damaged DNA[5] undergo considerable breakdown of their DNA after exposure to ultra-violet light or to MC[6–8]. This breakdown is thought to be initiated from single-strand gaps left by the defect excision enzymes controlled by the *uvr* genes, and to be part of the repair process[7,9]. In *uvr*$^-$ mutants of *E. coli* that lack the ability to excise thymine dimers and which are unable to repair effectively DNA exposed to ultra-violet light or to MC, there is much less DNA breakdown[8]. The presence of BU in the DNA of *uvr*$^+$ bacteria markedly enhances DNA breakdown[10]. This article provides evidence which shows that BU enhances DNA breakdown to about the same extent in *uvr*$^-$ strains of *E. coli* as it does in *uvr*$^+$ strains. This result indicates that ultra-violet light causes additional sites of degradation in DNA containing BU, which do not depend on the action of the excision enzymes. This effect occurs in addition to that already reported by Aoki, Boyce and Howard-Flanders[10], for example, that DNA degradation is uncontrolled in BU-containing bacteria. We shall also show that MC-induced DNA breakdown is enhanced in BU-containing *uvr*$^+$ bacteria, but that breakdown does not occur in *uvr*$^-$ bacteria, even if they contain BU. This observation provides additional evidence that the presence of BU in DNA of bacteria favours the continued release of nucleotides once this release is initiated by the action of the *uvr* genes.

The bacteria used were the following strains of *E. coli* K-12: *AB*2416 *Str*^{R}*thy*$^-$*uvr*$^+$ and *AB*2419 *Str*^{R}*thy*$^-$*uvrB*, both derived from *WS*110*y*25; *AB*2497 *thy*$^-$*uvr*$^+$, derived from *AB*1157 *uvr*$^+$; *AB*2498 *thy*$^-$*uvrC*, derived from *AB*1884 *uvrC*; and, *AB*2500 *thy*$^-$*uvrA*, derived from *AB*1886 *uvrA*. The last-named three thymine-dependent strains were isolated using the procedure of Stacey and Simson[11]. In referring to *uvr*$^-$ mutants, *uvrA*, *uvrB*, and *uvrC* are used to denote the genetic loci in mutant strains unable to repair ultra-violet-irradiated *T*1 phage.

Cells containing BU and labelled with ^{14}C-thymine were prepared by growth for two generations in a salts–glucose medium containing 2·5 mg/ml. casamino acids, 5 μg/ml. ^{14}C-thymine plus 50 μg/ml. BU. 30–40 per cent of the cells grown in this manner were non-viable. When only ^{14}C-thymine labelled cells were desired, the BU was omitted. In some experiments, the tritiated nucleosides of thymine and BU were used.

Cells were washed three times in Luria broth, suspended in Luria broth, and grown for 20 min at 37° to reduce radioactive precursors in the acid-soluble fraction. The cells were again washed, suspended in phosphate buffer to give solutions that were more than 55 per cent transparent to 2537 Å light, and irradiated with a low-pressure mercury germicidal lamp.

Exposure to MC was in a salts–glucose medium at 37° for 30 min, after which the MC was removed by centrifugation.

To determine DNA breakdown after exposure to ultra-violet light or to MC, cells were incubated in complete medium containing 25 μg/ml. non-radioactive thymine, and the percentage of acid-soluble carbon-14 of cells and of the medium was determined as previously described[10]. DNA synthesis was determined by incorporation of ^{14}C-thymine into cold acid-precipitable material by the filter disk method previously described[10].

Fig. 1 shows that *uvr*$^+$ bacteria grown in the presence of ^{14}C-thymine and exposed to ultra-violet light release twice as much carbon-14 into acid-soluble material as do *uvrB* bacteria. In contrast, the release of acid-soluble

carbon-14 is enhanced to about the same maximal level in either strain if they are grown in the presence of BU. These results show that ultra-violet-induced DNA breakdown in bacteria containing BU in their DNA does not depend on functional *uvr* genes.

The kinetics of DNA breakdown for several doses of ultra-violet light in both *uvr*$^+$ and *uvrB* strains of *E. coli* grown in the presence of ^{3}H-thymidine and BUDR are shown in Fig. 2. DNA breakdown becomes almost independent of dose above 240 ergs/mm^2. For doses up to 1,000 ergs/mm^2, DNA breakdown in the *uvrB* mutant strain exceeds that in the *uvr*$^+$ strain by 20–30 per cent.

The incorporation of ^{14}C-thymine into acid-precipitable material by the same cells used in the experiment in Fig. 2 is shown in Fig. 3. Non-irradiated cells of both strains containing BUDR rapidly incorporate carbon-14 into acid-precipitable material. Incorporation of ^{14}C-thymine in either strain ceases after irradiation with 240 ergs/mm^2. Although this dose greatly inhibits DNA synthesis in *uvr*$^-$ bacteria containing thymine, it scarcely affects DNA synthesis in normal *uvr*$^+$ bacteria[12,13]. These results show that during the incubation period, in which 35–60 per cent of the DNA in BU-containing cells has been degraded, no DNA synthesis has occurred. Under these conditions, double-strand breaks must occur in the DNA. These would presumably be lethal to the bacterial cell.

Fig. 4 shows that the ultra-violet-induced release of acid-soluble radioactivity from ^{14}C-thymine labelled bacteria increases the longer the bacteria are grown in the presence of BU. This result suggests that the amount of release is proportional to the amount of BU incorporated into the DNA.

Wild-type *uvr*$^+$ bacteria containing BU are considerably more sensitive to the lethal effects of MC than are *uvr*$^-$ bacteria[4]. Fig. 5 shows that the presence of BU also enhances DNA degradation in *uvr*$^+$ cells after exposure to MC. However, in contrast with the results obtained with ultra-violet light, there is no breakdown of the DNA in *uvrB* cells exposed to MC, whether or not these cells contain BU. This shows that MC-induced DNA breakdown in *uvr*$^+$ bacteria is enhanced by the presence of BU only after breakdown has been initiated by the *uvr* genes. These results also suggest that MC does not produce single-strand breaks that can serve for the initiation of breakdown in DNA containing BU.

The ability of BU-containing cells to incorporate ^{14}C-thymine into acid-precipitable material is completely depressed by exposure of the cells to 5 μg/ml. MC, as shown in Fig. 6. Under these conditions normal bacteria still incorporate about 20 per cent of the amount of ^{14}C-thymine that the untreated cells incorporate.

Uvr$^-$ mutants map at three widely spaced loci called *uvrA*, *uvrB* and *uvrC*[13,14]. Experiments so far indicate that all three genes are required simultaneously in the excision of defective bases[13]. Table 1 shows the percentage release of acid-soluble radioactivity from thymine-dependent *uvrA*, *uvrB* and *uvrC* mutants grown in ^{14}C-thymine and BU, exposed to ultra-violet light or to MC, and incubated for 2 h. All three mutant strains undergo a large amount of DNA breakdown after ultra-violet irradiation, whereas treatment with MC produces much less breakdown. The results in Table 1 confirm that all three genes are concerned with the same function.

Normal *uvr*$^+$ bacteria are thought to repair damaged DNA through the following sequence of events: (1) excision of the defect in one strand of the twin polynucleo-

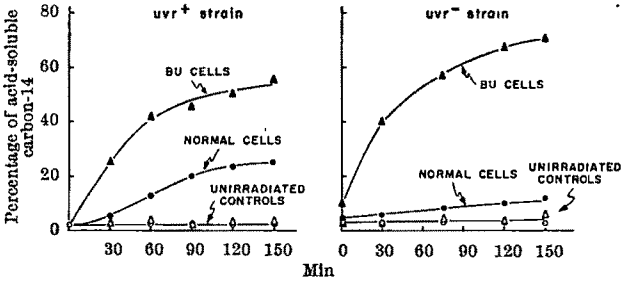


Fig. 1. Ultra-violet-induced release of cold acid-soluble radioactivity from normal and BU-containing *E. coli K*-12 *AB*2416 *uvr*$^+$*thy*$^-$ and *AB*2419 *uvrBthy*$^-$. Washed cells grown in ^{14}C-thymine with or without bromouracil, as described in the text, were untreated or exposed to 1,000 ergs/mm^2 2537 Å light. During incubation at 37° in complete medium containing 25 μg/ml. non-radioactive thymine per cent, cold acid-soluble carbon-14 was determined as previously described (ref. 10). Acid-soluble in all experiments includes the growth medium

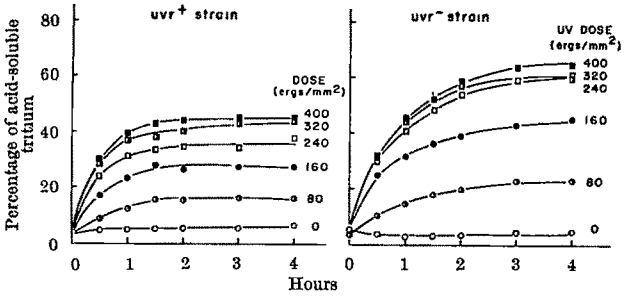


Fig 2. Release of cold acid-soluble radioactivity from BUDR-containing *E coli AB*2416 *uvr*$^+$*thy*$^-$ and *AB*2419 *uvrBthy*$^-$ for various doses of ultra-violet light. Cells were grown in medium containing 5 μg/ml. thymidine-methyl-^{3}H (^{3}H–TDR) alone or 5 μg/ml. ^{3}H-TDR plus 50 μg/ml. bromodeoxyuridine (BUDR) for two generations. Washed cells were exposed to the doses of ultra-violet light shown, incubated in complete medium containing 25 μg/ml. non-radioactive thymidine at 37°, and acid-soluble ^{3}H determined as previously described (ref. 10)

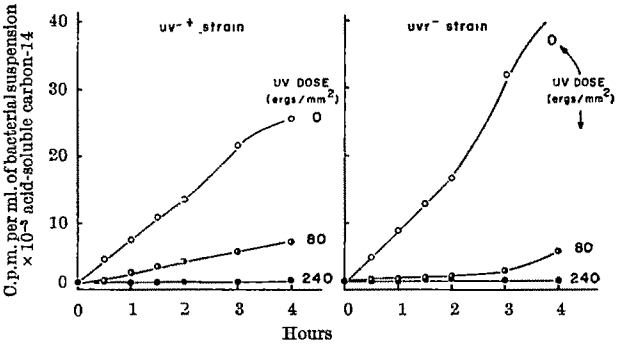


Fig. 3 Incorporation of ^{14}C-thymine into acid-precipitable material by BUDR-containing *E. coli K*-12 *uvr*$^+$*thy*$^-$ and *AB*2419 *uvrBthy*$^-$. The same cell suspensions used for the experiment in Fig. 2 were all adjusted to *O D* 0 1 after ultra-violet irradiation. Equal volumes were transferred to complete growth medium containing 2-2 μg/ml. ^{14}C-thymine and incubated at 37°. Cold acid-precipitable carbon-14 was determined by the filter disk method (ref. 10). The scintillation counter was adjusted to eliminate counts from tritium

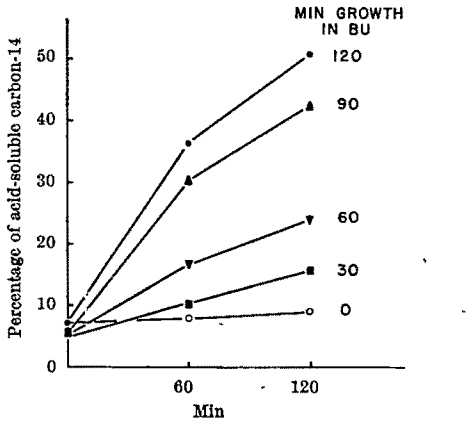


Fig. 4. Ultra-violet-induced release of cold acid-soluble carbon-14 from *E. coli* grown for various lengths of time in ^{14}C-thymine-BU medium. Overnight cultures were diluted five times into a glucose-salts-casamino acid medium containing 5 μg/ml. ^{14}C-thymine and 50 μg/ml. BU. At the times indicated, 2 ml. samples were filtered and washed on membrane filters, suspended in phosphate buffer to give a solution 80 per cent transparent to 2537 Å light, and ultra-violet irradiated with an effective dose of 1,000 ergs/mm^2. During post-irradiation incubation in complete medium containing 25 μg/ml. non-radioactive thymine, the percentage of cold acid-soluble carbon-14 was determined

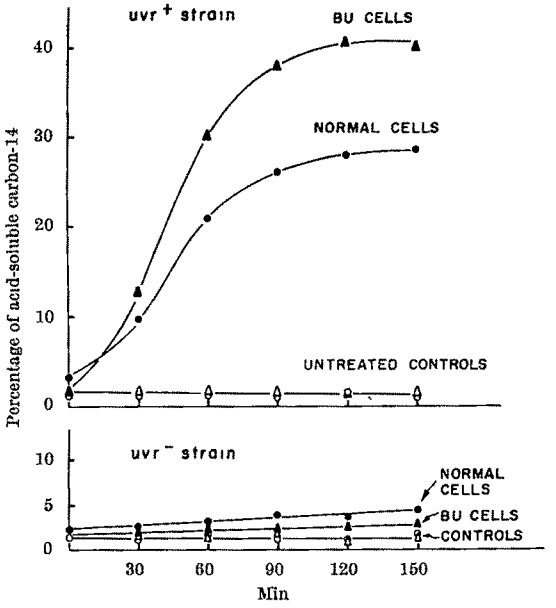


Fig. 5. Mitomycin *C*-induced release of cold acid-soluble radioactivity from normal and BU-containing *E. coli K*-12 *AB*2416 *uvr*$^+$*thy*$^-$ and *AB*2419 *uvrBthy*$^-$. Washed cells grown in ^{14}C-thymine with or without bromouracil as described in the text were exposed to 2·5 μg/ml MC for 30 min at 37° After removal of the MC by centrifugation the cells were incubated in complete medium containing 25 μg/ml. non-radioactive thymine, and the percentage of cold acid-soluble carbon-14 determined

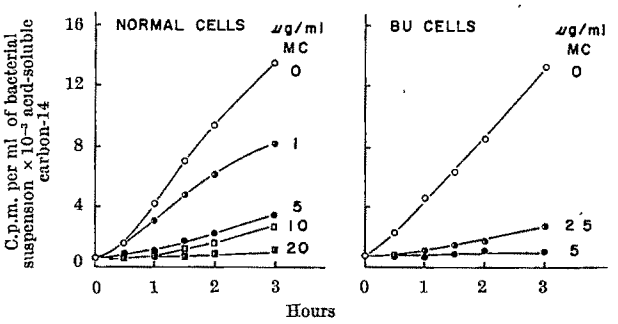


Fig. 6. Incorporation of ^{14}C-thymine into acid-precipitable material by BU-containing *E. coli K*-12 *AB*2416 *uvr*$^+$*thy*$^-$ after exposure to MC. Non-radioactive cells were grown in the presence or absence of BU and exposed to MC for 30 min at 37°. The MC was removed by centrifugation, and the cells suspended in complete medium to give the same optical density. To 1 ml. of cell suspension was added 2·2 μc /ml. ^{14}C-thymine, and cold acid-precipitable carbon-14 determined by the filter disk method (ref. 10)

tide; (2) exonucleolytic single-strand degradation, commencing at the site of excision; (3) reconstruction of the degraded strand by insertion of nucleotides into the degraded region (repair synthesis); (4) the formation of the phosphodiester link to the next section of intact polynucleotide. Implicit in this model of repair is the idea that DNA degradation gives way to DNA synthesis in some controlled manner[13]. Aoki *et al.*[10] suggested that this controlled switch-over from degradation to synthesis is altered by the presence of BU in DNA so as to favour continued degradation that ultimately produces double-strand breaks. The results of the present experiments confirm that BU enhances ultra-violet-induced breakdown

Table 1. Release of Acid-soluble Radioactivity from *E. coli* after Exposure to Ultra-violet Light or to Mitomycin C

Strain	% Acid-soluble carbon-14 after 2 h incubation following exposure to 1,000 ergs/mm² ultra-violet light	2·5 μg/ml MC
*AB*2497 *uvr*$^+$ *thy*$^-$	61	40
*AB*2500 *uvrA thy*$^-$	72	6
*AB*2419 *uvrB thy*$^-$	68	4
*AB*2498 *uvrC thy*$^-$	71	7

Bacterial cells were grown for two generations in a glucose-salts medium containing 2·5 μg/ml. casamino acids, and a 10 : 1 ratio (wt./wt.) of BU to ^{14}C-thymine. Washed cells were exposed to ultra-violet light or to MC, the MC removed by centrifugation, and incubated in complete medium containing 25 μg/ml non-radioactive thymine at 37° C.

in *uvr*$^+$ bacteria, but they also show that BU enhances to a similar degree DNA breakdown in *uvr*$^-$ bacteria. Thus, excessive breakdown in ultra-violet-irradiated bacteria containing BU is independent of the *uvr* genes, and suggests that major photo-defects exist in BU-containing bacteria after exposure to ultra-violet light, which are not removed by the excision enzymes. It is likely that these photo-defects are single-strand breaks[15], and that DNA degradation is initiated from such breaks, as also presumably occurs in normal bacteria exposed to X-rays[16].

Besides producing additional sites of DNA degradation in ultra-violet-irradiated *E. coli*, BU can also produce another effect. This is most clearly shown in the experiments with MC. Although MC induces enhanced DNA breakdown in BU-containing *uvr*$^+$ bacteria, it does not induce DNA breakdown in *uvr*$^-$ bacteria, even when they contain BU This provides direct evidence that BU can enhance MC-induced DNA breakdown only after it has already been initiated by the action of the *uvr* genes. This result supports the idea that BU alters the postulated switch-over from DNA degradation to synthesis so as to favour continued breakdown. This interpretation is strengthened by the observation that BU-containing bacteria exposed to ultra-violet light or to MC lose their ability to synthesize DNA during post-treatment incubation. In view of these results, the observed excessive DNA breakdown occurring in *E. coli* after ultra-violet irradiation can be interpreted in the following manner: the presence of BU in the DNA causes ultra-violet light to produce many single-strand breaks from which breakdown can proceed; and, independent of how breakdown is initiated—whether from the action of the *uvr* genes or whether from single-strand breaks—the presence of BU favours continued DNA breakdown by altering the normal switch-over from degradation to synthesis.

The alternative explanation, that in the *uvr*$^-$ mutants, the small amount of breakdown normally induced by ultra-violet is enhanced by the presence of BU, is rendered unlikely by the results obtained with MC. In those experiments, there was only about a third more breakdown caused by the presence of BU in *uvr*$^+$ bacteria. In contrast, ultra-violet irradiation produced a two- to three-fold increase in the DNA breakdown of BU-containing *uvr*$^+$ bacteria.

Another possible explanation for the observed extensive breakdown in *uvr*$^-$ bacteria after ultra-violet irradiation is that the presence of BU restores the action of the defective *uvr* genes, so that excision of ultra-violet photo-products can occur. This leads to uncontrolled breakdown as would be postulated in the *uvr*$^+$ strain. This explanation is not so attractive as that given here, because it is difficult to explain why the presence of BU in DNA would restore the action of the *uvr* genes for ultra-violet photo-products, but not for defects caused by MC.

The present results also show that BU-containing bacteria exposed to ultra-violet light or to MC are unable to synthesize DNA during a period in which they suffer extensive DNA breakdown. If this approaches 50 per cent of their DNA, it must result in double-strand breaks which presumably would be lethal to the cells, thus providing an adequate explanation for the increased sensitivity of BU-containing bacteria to irradiation, to MC, and possibly to other deleterious agents.

For doses of ultra-violet light of up to 1,000 ergs/mm² (the highest investigated), DNA breakdown in BU-containing *uvrB* bacteria was 20–30 per cent greater than in the *uvr*$^+$ strain. A possible explanation for this difference is that, since breakdown is proportional to the amount of BU in the DNA (Fig. 4), the *uvrB* strain incorporates more BU into its DNA than does the *uvr*$^+$ strain. Experiments using cells grown for two generations in the presence of ^{14}C-BU showed that there was 40 per cent more acid-precipitable carbon-14 in the *uvr*$^-$ strain per unit mass (as judged by optical density at 650 mμ) than in the *uvr*$^+$ strain. BU excision, controlled by the *uvr*

genes, might contribute to the cause for the relatively low amount of BU incorporated by the *uvr*$^+$ strain.

The enhancement of DNA degradation by the presence of BU in *uvr*$^+$ bacteria treated with MC may reflect the extent to which BU alters the normal switch-over from DNA degradation to synthesis in the repair process. Such an alteration could possibly arise because the presence of BU in DNA renders it an unsuitable template for repair synthesis, or because the exonuclease responsible for degradation has a high affinity for DNA containing BU. Another possibility is that BU degradation products interfere with the normal switch-over from degradation to repair synthesis. Experiments are at present being carried out to investigate these possibilities.

An interesting parallel to the experiments reported here is found in some *E. coli K*-12 mutants deficient in recombination, called '*Rec*$^-$' mutants[17]. Such mutants also manifest an apparent alteration in the controlled switch-over from ultra-violet-induced DNA breakdown to synthesis[18].

In summary, these investigations support the interpretation that sensitization of bacteria to radiation resulting from the incorporation of BU into their DNA is due to an enhancement of DNA breakdown and failure to synthesize DNA. The results also indicate that in *uvr*$^-$ mutants ultra-violet light causes strand breakage in DNA containing BU, whereas mitomycin *C* does not.

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IDENTIFICATION OF A COMPONENT OF A TRANSPORT 'CARRIER' SYSTEM: ISOLATION OF THE PERMEASE EXPRESSION OF THE *LAC* OPERON OF *Escherichia coli*

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OVERWHELMING evidence is available suggesting that certain components of cell membranes are involved in transporting across a lipid-protein barrier substances which are not ordinarily soluble in the hydrophobic structure of these membranes. It has been demonstrated by various kinetic approaches, in many different types of cells and tissues, that the transport of several classes of substances appears to be 'carrier-mediated'. Indeed, although these mobile reactive-sites (or 'carriers') have been inferred by that wealth of kinetic evidence, there remains as yet no single convincing demonstration of the characterization or identification of the elusive transport 'carrier', whether it be indigenous to the membrane, or part of the cytoplasm. It has been postulated, on the basis of certain evidence of a peripheral nature, that the system responsible for the transport of monosaccharides in the human erythrocyte is phospholipid in nature[1], or again, protein[2], but for this system the transport function has been shown to be confined to the plasma membrane[3]. The membrane-based sodium- and potassium-activated adenosine triphosphatase is clearly coupled with the transport of cations in a number of tissues[4]. Other evidence, of an even more indirect nature, suggests that the system for actively transporting β-galactosides into *Escherichia coli* has a protein, or enzymatic nature[5]. The genetic characterization of the *Lac* operon of this bacteria, which has been shown to contain genes for only three separate elements[6], and the fact that the manufacture of the products of the operon may be gratuitously induced by certain thio-substituted β-galactosides[7], have enabled us to separate and identify the gene-product of the operon mapped for 'permease' functions.

* Fellow of the U.S National Institutes of Health.

We have used the following mutant strains of *E. coli*, all of the *K*12 variety, which were kindly supplied by Prof. F. Jacob of the Institut Pasteur: 200*PS* (*FLac*$^+$), 200*P* (permease-less), and 2010 (galactosidase-less). Cultures of these mutants were re-isolated and sub-cultured on receipt, and were sub-cultured and maintained on nutrient agar slopes every 2 weeks thereafter. When prepared for experiment, the cells were cultivated in minimal salts broth[8], to which were added certain growth factors (vitamin B_1 and arginine) when required, and 0·2 per cent glycerol as a carbon source. The gratuitous inducer, β-thiomethylgalactopyranoside, was added (5×10^{-4} M) when required. The cultures were grown at 37° C for about 14 h, collected by centrifugation, washed with *tris*-succinate buffer (*p*H 7·4, 0·01 M) and the bacteria resuspended in the same buffer.

'Osmotically sensitive spheres' (OSS) were prepared according to Sistrom[9], and these were separated from the medium by high-speed centrifugation in the cold. The spheres were burst by the addition of a small volume of the dilute *tris* buffer (usually 15 ml. per g wet spheres). Bacterial membrane 'ghosts' were separated from the now very viscous supernatant (cell cytoplasm) by high-speed centrifugation in the cold. In order to separate the cytoplasmic proteins, aliquots of this viscous supernatant were applied to DEAE cellulose columns of varying dimensions. Best results were obtained when Whatman *P./DE* 100 *C.*2 DEAE cellulose (kindly supplied by Reeve Angel, Ltd.) was used. The columns were routinely eluted with a linear sodium chloride gradient (0–0·8 M) by the method of Bozk and Ling[10]. Effluent optical density (254 mμ) was continuously monitored by means of an L.K.B. (Sweden) 'Uvicord' recording system. Effluent

samples were assayed for protein (*O.D.* 280 mμ), β-galactosidase[11], thiogalactoside-transacetylase[12], and, in some cases, acid phosphatase[13].

The method used to separate the 'permease' gene product from the other cellular substituents of the crude bacterial extract involved double-isotopic labelling of the cellular proteins of a non-induced and induced culture with ^3H- and ^{14}C-labelled phenylalanine, one isotope to the former culture, the other to the latter. The uptake of ^{14}C-labelled phenylalanine by growing cultures of *E. coli*, the optimal conditions for such uptake, and subsequent separation of the proteins of a resulting cellular extract have already been reported[14]. The tracer amino-acid was supplemented with non-tracer phenylalanine to 10^{-4} M.

The cultures were grown under the aforementioned conditions, and the two mixed together before centrifugation. OSS and cell extract were prepared, and the cell extract applied to a DEAE cellulose column. 100 μl. of each effluent sample was applied to a paper disk (Whatman 3*M*) which was dried and placed in a vial containing a scintillation solution (dimethyl 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (POPOP), polyphenol oxidase (PPO)). Counting was done on a Nuclear Enterprises, G.B., Ltd., liquid scintillation counter.

Electrophoresis of certain fractions of the DEAE cellulose chromatogram was carried out on a micro-scale using cellulose-acetate strips, and the protein fixed and stained with amido-schwartz dye (3 per cent trichloroacetic acid). Conventional procedures were followed[15]. Samples were prepared by concentrating pooled samples of the column effluent with powdered 'Carbowax' (a polyethylene glycol) in a dialysis bag.

For the case with the *FLac*$^+$ mutant, a large percentage of the protein manufacturing system of the cell is devoted to producing only those proteins coded for by the induced operon[16]; thus, areas of considerable ^{14}C-enrichment in a protein-radio-chromatograph of the crude bacterial extract should be readily apparent. Fig. 1 illustrates the results of such a chromatograph. For convenience in interpreting this and subsequent figures of like nature, one line (representing counts per unit time ^{14}C alone, or ^3H alone, of the effluents) was arbitrarily forced to coincide with the second line in an area of the chromatograph where the $^{14}C : {}^3H$ ratio was minimal, and hence where no ^{14}C-enrichment was to be expected. Thus, areas of ^{14}C-enrichment can be identified by the ^{14}C-line rising above the ^3H-line, and these areas are identified by the black, filled-in part of the figures (in Fig. 4, where the radioactive labels are reversed, the filled-in part indicates an area of tritium-enrichment). Results of analysis for enzymatic activity in corresponding samples appear as block diagrams beneath the lines representing the radioactivity.

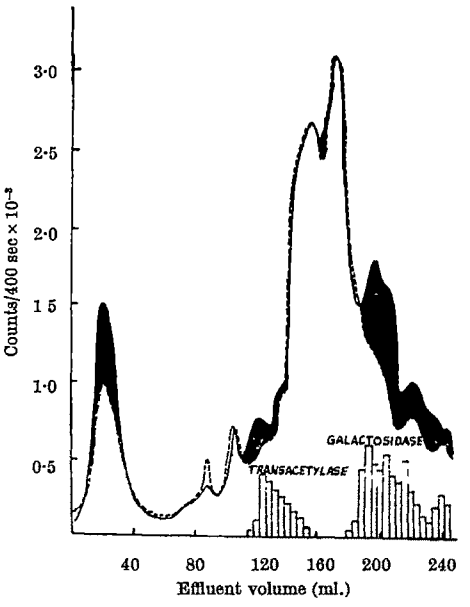


Fig 1. Effluent radioactivity and enzymatic activity of a cell extract of the *FLac*$^+$ (200*PS*) mutant of *E. coli* chromatographed on DEAE cellulose. ———, Carbon-14 content; — — — —, tritium content. Filled-in parts are carbon-14 enrichment (see text). Block diagrams represent indicated enzymatic activities drawn to an arbitrary scale. 7·5 ml. of the cell extract, from a total volume of 10 ml., was applied to the column. 2 ml fractions were collected, assayed, and counted. Tritium efficiency was 0·75 per cent, carbon-14 efficiency was 10 per cent. 0·40 mc. ^3H-phenylalanine was initially added to the non-induced culture; 0·01 mc. ^{14}C-phenylalanine was initially added to the induced culture

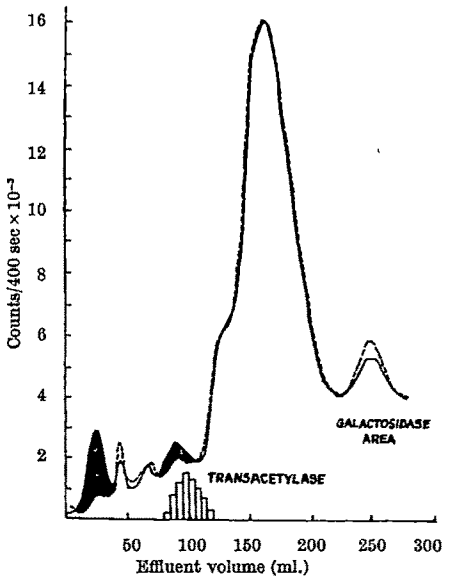


Fig. 2. Effluent radioactivity and enzymatic activity of a cell extract of the galactosidase-less (2010) mutant of *E. coli* chromatographed on DEAE cellulose. ———, Carbon-14 content; — — — —, tritium content. Filled-in parts are carbon-14 enrichment. Block diagrams represent indicated enzyme activities drawn to an arbitrary scale. *Galactosidase area* is that portion of the effluent volume which, if compared with the ultra-violet and radioactivity records of the two other mutant strains investigated, would ordinarily contain the galactosidase enzymatic activity. No such enzymatic activity was found here 7·5 ml. of the cell extract from a total of 9·5 ml. was applied to the column. 2·5 ml. eluate fractions were collected, assayed, and counted. Tritium efficiency was 0·5 per cent, carbon-14 efficiency was 10 per cent. 0·20 mc. ^3H-phenylalanine was initially added to the non-induced culture; 0·01 mc. ^{14}C-phenylalanine was initially added to the induced culture

Figs. 1, 2, and 3 illustrate the results of identical experiments conducted with each of the three mutant strains. Fig. 1 (*FLac*$^+$): there are three main areas of ^{14}C-enrichment present (the fourth, at the top of the centre peak, is created by one sample alone, has not been found in repeated experiments, and is considered by us to be probably a spurious record). Two of these areas are associated with corresponding areas of enzymatic activity. Clearly, the peak of ^{14}C-enrichment in the area of Fig. 1 associated with neither transacetylase nor β-galactosidase activity is not present in the comparable area of Fig. 3 (permease-less). But the two ^{14}C-enriched areas of Fig. 3 associated with β-galactosidase and *trans*acetylase activity both appear in areas of the chromatograph comparable with those of Fig. 1. Only the first area is missing. Fig. 2 illustrates the results for the mutant lacking galactosidase. Neither β-galactosidase enzymatic activity nor ^{14}C-enrichment appears on that part of the figure corresponding to the ^{14}C-enriched areas of Figs. 1 and 3. However, Fig. 2 does possess the ^{14}C-enriched area not associated with β-galactosidase or *trans*acetylase activity which appears in Fig. 1, but not in Fig. 3.

In order to obviate the possibility that the areas of enrichment were produced by fortuitous ^3H-quenching, the experiment was repeated with the labels reversed. Fig. 4 illustrates the results using the permease-less mutant. Again, no enrichment is noted in the 'permease'

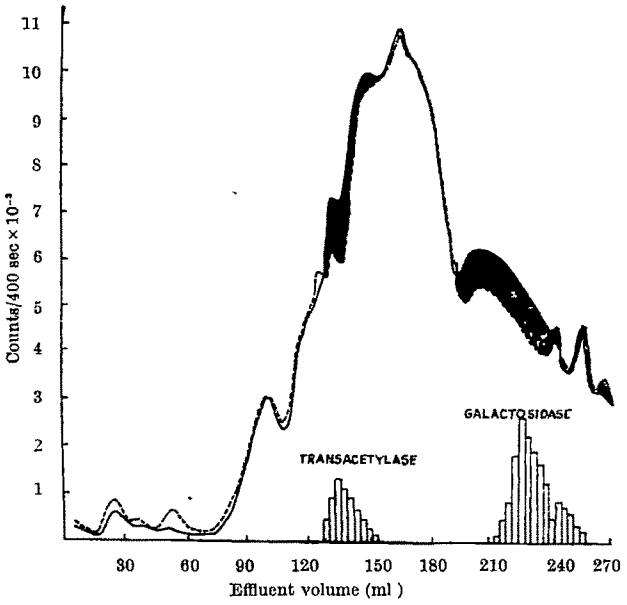


Fig. 3. Effluent radioactivity and enzymatic activity of a cell extract of the permease-less (200*P*) mutant of *E. coli* chromatographed on DEAE cellulose ———, Carbon-14 content; — — — —, tritium content. Filled-in parts are carbon-14 enrichment Block diagrams represent indicated enzyme activities drawn to an arbitrary scale. 3·5 ml. of the cell extract from a total volume of 5 0 ml. was applied to the column. Tritium efficiency was 0 5 per cent; carbon-14 efficiency was 10 per cent. 0 20 mc. ^{3}H-phenylalanine was initially added to the non-induced culture; 0 01 mc ^{14}C-phenylalanine was initially added to the induced culture

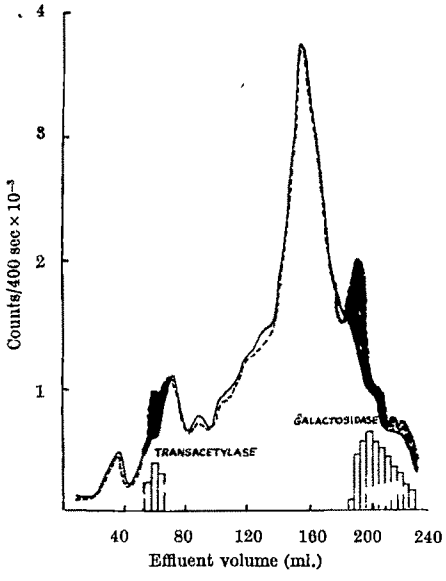


Fig. 4. Reversed-label (200*P*) experiment. Effluent radioactivity and enzymatic activity of a cell extract of the permease-less mutant of *E. coli* chromatographed on DEAE cellulose. ———, Carbon-14 content; — — — —, tritium content. The filled-in parts are tritium enrichment (see text) Block diagrams represent indicated enzyme activities drawn to an arbitrary scale. 7 5 ml of the cell extract from a total of 19 ml was applied to the column Tritium efficiency was 0·5 per cent, carbon-14 efficiency was 10 per cent. 0 02 mc ^{3}H-phenylalanine was added initially to the induced culture; 0 005 mc. carbon-14 was initially added to the non-induced culture

area, but the usual (now ^{3}H-) enrichment can be seen in each of the two areas identified with their respective enzymatic activities.

We now knew where the 'permease' area was to be found on the DEAE chromatograph, and we predicted, from the fact that the enrichment of the 'permease' peak was so much greater than that of the other two areas, that the material present in this region should be pure. With this in mind, we attempted to test the composition of effluent from this area. Fig. 5 illustrates the effluent tracing from a chromatograph of an induced culture of the *FLac*$^{+}$ mutant, while Fig. 6 is the effluent record from a chromatograph of a non-induced culture of the permease-less mutant. The figures are taken from the continuously monitored column effluent (*O.D.* 254 mμ). The absence, in Fig. 6, of the first major peak of Fig. 5 is clearly shown, and it is this area which, in the radiochromatographs, was observed to have enrichment designated as the 'permease' area.

The effluent samples from this 'permease' area of Fig. 5 were pooled, concentrated, and electrophoresed. Although there was little material present absorbing at 280 mμ, a single faint band took up stain. These investigations were

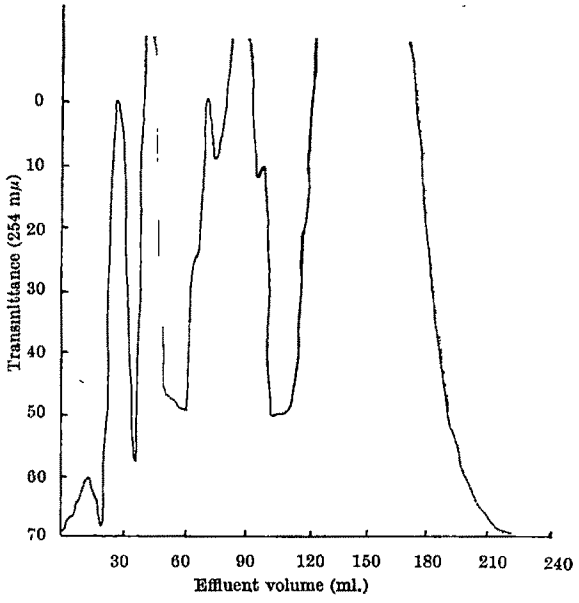


Fig. 5. Continuous tracing of the optical density (254 mμ) of the effluent from a DEAE cellulose column chromatograph of a cell extract from an induced culture of the *FLac*$^{+}$ (200*PS*) mutant of *E. coli*. 7·5 ml. of cell extract from a total volume of 17·5 ml. was applied to a DEAE cellulose column and eluted with 300 ml. of a linear gradient sodium chloride solution in 0 01 M *tris* buffer (see text)

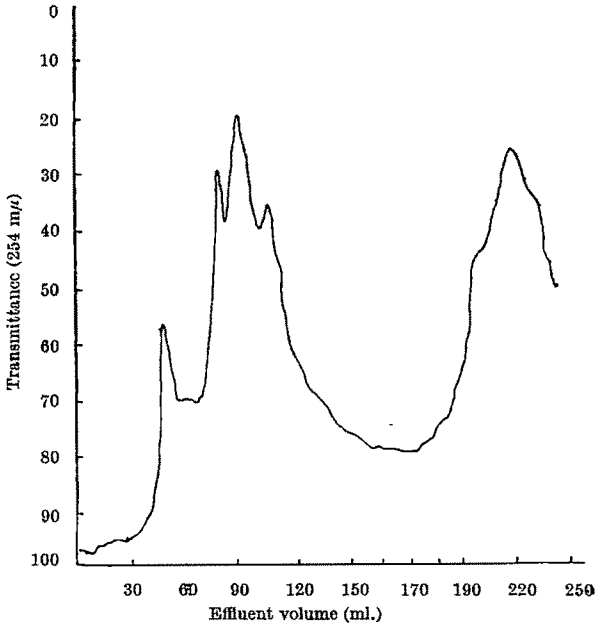


Fig 6. Continuous tracing of the optical density (254 mμ) of the effluent from a DEAE cellulose column chromatograph of a cell extract from a non-induced culture of the permease-less (200*P*) mutant of *E. coli* 7 5 ml of cell extract from a total volume of 17 ml. was applied to a DEAE cellulose column and eluted with 300 ml. of a linear gradient sodium chloride solution in 0 01 M *tris* buffer (see text)

of a preliminary nature; they are at present being pursued further.

The most direct conclusion which can be drawn from the results of the present investigations is that the enriched area of chromatographs 1 and 2, which has neither of the two enzymatic activities, is most likely to be the expression of the permease gene of *E. coli*. It would seem that the amount of permease is less than the amount of β-galactosidase formed, but somewhat greater than the amount of transacetylase formed. From the areas under the curves of Fig. 1, it can be computed that β-galactosidase is about 11 per cent of the total labelled soluble protein, the 'permease' is about 5 per cent, while the transacetylase is about 3 per cent. Nishi and Zabin[17] have shown that, in accordance with the theory of gene modulation, the amount of transacetylase formed is much less than the amount of β-galactosidase, about 0·13 per cent and 5·0 per cent, respectively, in bacteria grown at 37° C. Since the permease gene is mapped between the other two genes of the operon, it follows from this theory that the amount of permease formed should be somewhere between the amounts of the other two products, and this prediction is confirmed by our results. Our results suggest that, indeed, only three protein products are coded for by the three genes of the *Lac* operon, in accordance with the genetic predictions[18].

While we recognize the anomaly that the material present in our 'permease' area is presumably cytoplasmic, thorough extraction of the membrane fraction by vigorous methods[19] has failed to yield any enrichment. Thus, either the carrier may be extracted from the membrane by our technique into the supernatant, or what we have identified may only be some part of the complete system associated with the transport function. In such an event, it is possible that the carrier itself may not have been labelled by our technique. There is some indication that part of the cellular acid phosphatase activity may reside in this enriched area, suggesting a possible energy-supplying role for this component.

We are continuing our investigations, and at the same time are attempting to prepare large quantities of the material contained in this fraction of effluent. Moreover, we are seeking alternative procedures designed to label other classes of substances, particularly lipid and phospholipid, by a double-isotopic labelling technique.

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INDUCTION OF TUMOUR IMMUNITY WITH TUMOUR CELLS TREATED WITH IODOACETATE

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It is now clear that many lethal transplantable tumours in mice are to some degree antigenic for isogeneic or otherwise compatible hosts and that immunization of the host animal is in many cases possible with prevention or retardation of tumour growth on subsequent challenge using virulent tumour[1]. In the case of allogeneic tumours, a homograft-type reaction by the host against the foreign tissue is thought to be responsible. In the case of isogeneic tumours, 'tumour-specific antigens' to which the host has reacted are held responsible.

A major problem in the field, and in particular with isogeneic systems, has been to find an effective immunizing programme. Live, virulent tumour cells are not suitable as antigen since they may multiply and kill the host animal. Various methods have been used, therefore, to 'delethalize' tumour cells with preservation of their antigenicity. They have been processed by grinding, homogenizing, sonification, freeze-thawing, lyophilization and heat-killing, but all these techniques have led to destruction or severe loss of antigenicity[2]. One moderately effective immunizing technique has been the use, for immunization, of heavily irradiated tumour cells which, though viable, are unable to divide[3]. After an extensive course of immunization with cells thus treated, many mice will resist a lethal challenge with the corresponding tumour.

We have found that pretreatment of tumour cells with sulphydryl alkylating agents, particularly iodoacetate, suppresses their ability to induce tumours. This has occurred in all systems studied so far—allogeneic and isogeneic. The treated cells are viable; they respire at a subnormal rate. Mice treated with these cells develop intense immunity to the corresponding tumour, resisting challenge with doses of virulent tumour cells several times those which are uniformly lethal for control animals.

The initial observations were made using Ehrlich's ascites tumour in 3-month-old *A-jax* mice. In spite of its allogeneic nature, the Ehrlich's ascites carcinoma is fatal for all control mice, whatever the strain, so that a successful immunization is unmistakable. Fresh, unwashed Ehrlich's ascites tumour cells were incubated with 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M sodium iodoacetate or simply in their own ascitic fluid for 1 h at 37° C and *p*H 7·4. After incubation, the tumour was diluted with physiological saline and groups of mice were injected intraperitoneally with the tumour cells. Each mouse was given $2·5 \times 10^6$ cells in 0·05 ml. of suspending medium. All mice injected with

untreated cells or with cells incubated with 10^{-5} M sodium iodoacetate developed ascites and died. Tumour did not develop in mice given cells incubated in 10^{-2}, 10^{-3}, or 10^{-4} M sodium iodoacetate although the cells excluded the supravital stains, safranin *O* and eosin *Y*.

Experimental mice were boosted twice at 14-day intervals with $2{\cdot}5 \times 10^6$ freshly prepared, treated cells. Fourteen days after the second booster, all experimental mice plus virgin controls were challenged with $2{\cdot}5 \times 10^6$ live, fresh Ehrlich's ascites carcinoma cells (several times the uniformly lethal dose). No animal pretreated with the 10^{-2}, 10^{-3}, or 10^{-4} M iodoacetate-inactivated cells developed a tumour during the observation period of 6 weeks. All control mice developed ascites and died within 2–4 weeks. Attempts to protect mice by immunization with tumour cell homogenates, following the immunization schedule given, were unsuccessful. All mice given the equivalent amount of 10^{-2} M sodium iodoacetate in saline died when later challenged. The results are summarized in Table 1.

Table 1. Effect of Sodium Iodoacetate on Tumour-producing and Immunogenic Capacity of Ehrlich's Ascites Tumour Cells

Immunizing material	Mortality after immunization	Mortality after challenge
Untreated tumour cells	20/20	—
Homogenized tumour cells	0/10	10/10
Iodoacetate-treated tumour cells 10^{-2} M	0/20	0/20
" " " " 10^{-3} M	0/20	0/20
" " " " 10^{-4} M	0/20	0/20
" " " " 10^{-5} M	10/10	—
Iodoacetate 10^{-2} M in saline	0/10	10/10
No cells given	—	20/20

It was found that immunity developed within a few days of the initial immunization. Groups of mice were immunized intraperitoneally with $2{\cdot}5 \times 10^6$ Ehrlich's ascites tumour cells pretreated with 10^{-2} M iodoacetate and challenged with $2{\cdot}5 \times 10^6$ virulent cells 2–21 days later. All mice challenged 2 or 3 days after immunization developed ascites tumours. Three of ten in each group afterwards overcame the ascites tumours and survived, but the rest died. Sixty-seven of seventy animals challenged between 4 and 13 days after immunization were protected, but only five of twenty in which challenge was delayed for 21 days resisted challenge. Peripheral blood total and differential white blood cell counts were done on mice given iodoacetate-treated cells intraperitoneally. At 3 days there was a profound drop in lymphocytes and polymorphonuclears reminiscent of that seen in a severe systemic delayed hypersensitivity reaction[4]. By 6 days, the polymorphonuclears had recovered to baseline levels and there was a considerable lymphocytosis.

Table 2 gives our results in an isogeneic system. The *MCT* sarcoma was methylcholanthrene-induced in our own (Pondville) sub-line of *C57BL* in 1960 and converted to its ascites form in 1964. Its growth is restricted to the strain of origin in which it is uniformly fatal. It will not grow in any other strain, not even in the closely related *C57BL/6*. Inactivation of ascitic *MCT* was complete at both concentrations used: immunization was effective but incomplete. Five of twenty-two mice succumbed eventually to the challenge with intraperitoneal tumour, but these five outlived all the controls.

Several other host–tumour combinations have been studied also and these are given in Table 2. The *EL4* tumour was completely inactivated and the immunity achieved in a co-isogeneic strain was as complete as it had been with Ehrlich's ascites tumour. With Krebs-2, using the same schedule, tumour inactivation was complete but immunization was partial. In later experiments, using a single immunizing injection with challenge after 7–10 days, a complete immunization was achieved. In this latter case, as well as with *MCT* tumour, incubation with 10^{-3} iodoacetate appeared to be more effective than incubation with 10^{-4} M.

We have found it possible to influence the growth of the subcutaneously implanted solid isogeneic *MCT* tumour described above by immunization with iodoacetate-treated cells of its ascitic form. Free *MCT* tumour cells in their ascites were incubated for 1 h in 10^{-3} or 10^{-4} M sodium iodoacetate. Individual mice were immunized with $2{\cdot}5 \times 10^6$ treated cells by intraperitoneal injection. Immunized mice plus a control group received subcutaneous solid *MCT* tumour by trochar the same day. Twenty days later they were killed. The individual solid tumours were dissected free and weighed. The results are given in Table 3. The retardation of tumour growth in both treated groups was highly significant although no treated animal was completely free of tumour.

In a second experiment, immunization was done with tumour cells prewashed in physiological saline before incubation with 10^{-4} M sodium iodoacetate. Again, the depression in mean tumour weight of treated mice, when compared to untreated controls at 28 days, was highly

Table 2. Effect of Sodium Iodoacetate on Tumour-producing and Immunogenic Capacity in Isogeneic, Co-isogeneic and Allogeneic Host-Tumour Systems in Mice

Tumour	Host strain	Relation of tumour to host	Immunizing material	Mortality after immunization	Mortality after challenge
MCT	*C57BL**	isogeneic	Sodium iodoacetate 10^{-3} M	0/11	1/11
			" 10^{-4} M	0/11	4/11
			No treatment	10/10	—
			No cells	—	10/10
EL4	*C57BL/6*	co-isogeneic	Sodium iodoacetate 10^{-3} M	0/11	0/11
			" 10^{-4} M	0/11	0/11
			No treatment	10/10	—
			No cells	—	10/10
Krebs-2	Swiss	allogeneic (compatible)	Sodium iodoacetate 10^{-3} M	0/10	5/10
			" 10^{-4} M	0/10	8/10
			No treatment	10/10	—
			No cells	—	10/10
Krebs-2†	Swiss		Sodium iodoacetate 10^{-4} M	0/21	0/21

* Pondville sub-line.
† Given one single immunizing injection and challenged 9 days later. All other groups immunized three times at 14-day intervals and challenged 14 days later

Table 3. *MCT* Tumour Weights 20 Days After Challenge in *C57BL* Mice given Iodoacetate-treated *MCT* Ascitic Tumour Cells intraperitoneally on Day of Challenge

Treatment	No of mice in group	Tumour weight (g)					Mean tumour weight (g)	*S.E.* of mean	Significance of difference with respect to controls
		0 < 0·5	0·5 < 1·0	1·0 < 1·5	1·5 < 2·0	>2·0			
10^{-3} M iodoacetate*	22	9	10	3	0	0	0·58	0·06	P 0·001
10^{-4} M iodoacetate	18	4	10	3	1	0	0·77	0·11	P 0·001
None	22	0	1	7	10	4	1·77	0·14	

S.E. = standard error.
* Dialysed against Locke–Ringer's solution after incubation

significant and in three of twenty mice no tumour could be found. In two of ten mice immunized with prewashed iodoacetate-treated tumour cells 14 days after subcutaneous challenge with solid tumour, the established tumour had disappeared by 28 days. In several others, tumour growth was arrested.

Certain other reagents active on sulphydryl groups have been tested for their efficacy as inactivators. Iodoacetamide gave complete inactivation of Ehrlich's ascites tumour at 2×10^{-4} M but none at 1×10^{-4} M. With 10^{-3} and 2×10^{-4}, protection was complete; with 10^{-2}, nine of ten animals resisted challenge. *N*-Ethylmaleimide brought about an almost complete inactivation at 10^{-3} M and immunity was complete. *p*-Chloromercuribenzoate was ineffective. All animals inoculated with Ehrlich's ascites carcinoma pretreated with 10^{-3} M *p*-chloromercuribenzoate developed tumours.

We have found our alkylation technique to be a simple and effective means of inducing tumour immunity in situations where immunity has till now been difficult to produce. The mechanism by which iodoacetic acid and the other alkylating agents achieve their effect is uncertain. It would seem to be a direct effect on the tumour cell or on tumour cell antigens, rather than on the host, since iodoacetate alone failed to protect. It is well known that iodoacetate reacts primarily with sulphydryl groups; it may, in this way, inhibit enzymatic reactions essential for cell multiplication. Clearly, tumour growth was inhibited in these experiments; the mice were immunized with antigenically intact but harmless cells, and they responded to them. There is evidence that disulphide bonds are important in stabilizing the antigenic determinants of proteins[5]. Iodoacetic acid, by its action on sulphydryl groups, may stabilize tumour antigens as well.

The results with *MCT* and Krebs-2 tumours implicate some factor in addition to tumour inactivation in the immunity achieved. Treatment of either of these tumours with 10^{-4} M sodium iodoacetate led to less effective immunity despite complete tumour inactivation than did treatment with 10^{-3} M sodium iodoacetate, suggesting that iodoacetic acid affects tumour cell antigenicity directly. Why excess iodoacetic acid should interfere, even though minimally, with immunization to Ehrlich's ascites tumour cells is by no means clear. Conceivably, the excess iodoacetate might impede phagocytosis of antigen[6].

An alternative explanation would be that iodoacetate acts to thwart the production of enhancing antibodies, thus permitting a free field to the rejection mechanism. Preliminary data argue against such a view and indicate that enhancing antibody is produced normally in mice treated with lyophilized tumour exposed to iodoacetate.

Immunization with tumour cells treated with iodoacetate appears to be a powerful means of inducing tumour immunity. This is evidenced by the large proportion of animals that are made resistant to challenge, by the speed with which immunity is established, and by the ability of the treated cells to slow and in some cases reverse the course of an established solid tumour in an isogeneic system.

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PASSIVE AND ACTIVE IMMUNITY TO FRIEND VIRUS DISEASE

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Friend virus disease is characterized by proliferation of reticulum cell, erythroblastosis, hypervolaemic polycythaemia, leucocytosis, and hepatosplenomegaly[1–3]. When virus is injected into young or adult mice, the disease develops within a matter of a few days.

Earlier investigations showed that offspring from infected mothers developed the disease after a long latent period of more than 7 months[4]. This suggested to us that infected mothers are not only able to transmit virus to their offspring, but also to transmit some form of resistance to Friend virus disease. Several experiments were therefore carried out. Adult females of the *HA/ICR* and *DBA*/1 mouse strains were immunized at intervals after the onset of pregnancy (as determined by vaginal plug) with: (1) live Friend virus; (2) formalinized Friend virus vaccine; (3) heat-killed Friend virus. The offspring of these mice were in turn challenged with live virus at various intervals after birth. Offspring from non-infected females were injected with the same preparations and at the same time intervals.

Fig. 1 shows the results of challenge of offspring from Swiss mothers injected with live Friend virus on the second day of pregnancy. The 'percentage of protection' is the difference between the percentage of incidence of disease in offspring of non-infected mothers and the

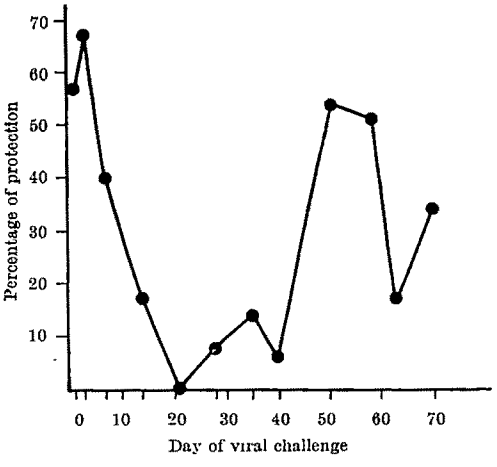


Fig. 1. Resistance of Friend virus disease in offspring of *HA/ICR* Swiss mother mice infected with Friend virus. Mothers were immunized intraperitoneally on second day of pregnancy with $10^{3\cdot1}$ $I.D._{50}$/0·1 ml. live Friend virus contained in a cell-free filtrate from spleens from Friend virus infected mice. Offspring were challenged subcutaneously, day 1, and thereafter intraperitoneally at intervals with $10^{2\cdot2}$ $I.D._{50}$/0·1 ml. live Friend virus. The same preparation was used throughout. Offspring from non-infected mothers were injected simultaneously

percentage of incidence in offspring of infected mothers. There is evidence of protection against Friend virus disease when offspring are challenged 1–15 days after birth. There is very little evidence of protection when mice are challenged 20–40 days after birth. However, there is renewal of protection when mice are injected 45 or more days after birth. We attribute the initial wave of protection to passive immunity, and the second wave to active immunity.

Fig. 2 shows the results of challenge of offspring from Swiss mothers that were injected with live Friend virus on the twelfth day of pregnancy. Offspring show very little resistance to the development of Friend virus disease when injected in the early period after birth. However, mice injected 20–28 days after birth are highly resistant. The delay in the initial passive immunity may be due to the lower antibody-level at delivery, since the mother was injected on the twelfth day of pregnancy. However, as the antibody rises in the mother, the offspring are protected by way of lactation. The second wave of active immunity occurs at approximately the same time as it did in the previous experiment.

Fig. 3 shows the results of challenge of offspring from *DBA*/1 mothers that were injected with live Friend virus on the twelfth day of pregnancy. The results are similar to those obtained from offspring of Swiss mice (Fig. 2), but the degree of protection as the result of passive immunity is much less in *DBA*/1 mice. This may be due to the fact that *DBA*/1 mothers received one log dose less virus than the Swiss mothers. However, protection due to active immunity, although delayed by about 20 days, is just as effective as in Swiss mice.

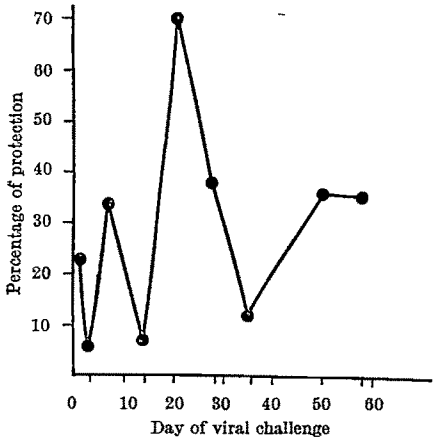


Fig. 2. Resistance of Friend virus disease in offspring of *HA/ICR* Swiss mother mice infected with Friend virus. Mothers were immunized intraperitoneally on twelfth day of pregnancy with $10^{3\cdot4}$ $I.D._{50}$/0·1 ml. live Friend virus. Offspring were challenged subcutaneously, day 1, and thereafter intraperitoneally at intervals with $10^{2\cdot9}$ $I.D._{50}$/0·1 ml live Friend virus

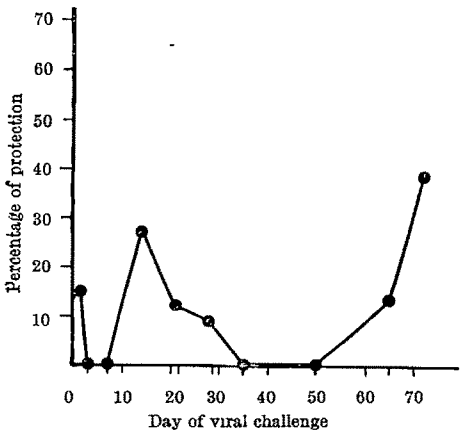


Fig. 3 Resistance of Friend virus disease in offspring of *DBA*/1 mother mice infected with Friend virus. Mothers were immunized intraperitoneally on twelfth day of pregnancy with $10^{2\cdot4}$ $I.D._{50}$/0·1 ml. live Friend virus. Offspring were challenged subcutaneously, day 1, and thereafter intraperitoneally at intervals with $10^{2\cdot5}$ $I.D._{50}$/0·1 ml. live Friend virus

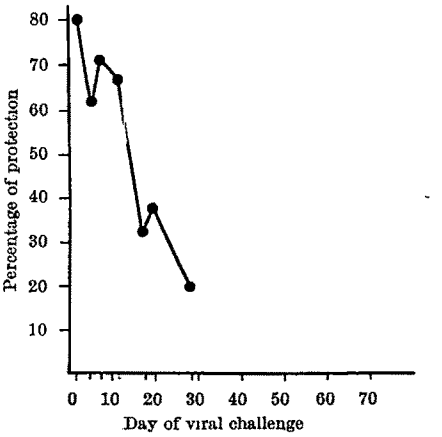


Fig. 4. Resistance of Friend virus disease in offspring of mother mice receiving formalinized Friend virus vaccine. Swiss mother mice were injected three times, on the third, seventh and tenth days of pregnancy, with a formalinized vaccine prepared with $10^{3\cdot8}$ $I.D._{50}$/0·1 ml. live Friend virus. Enough formalin was added to cell-free filtrate from spleens to give a final concentration of 1 : 500. The mixture was kept at 4° C and shaken manually daily for 30 days. Offspring were challenged subcutaneously, day 1, and thereafter intraperitoneally at intervals with $10^{2\cdot9}$ $I.D._{50}$/0·1 ml. live Friend virus

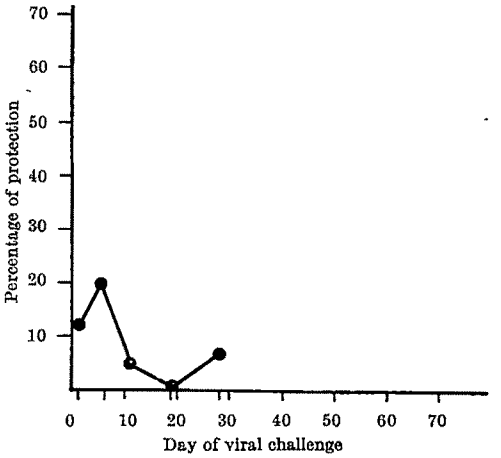


Fig 5. Resistance of Friend virus disease in offspring of mother mice receiving heat-killed Friend virus vaccine. Swiss mother mice were injected three times, on the third, seventh and tenth days of pregnancy, with a heat-killed vaccine prepared with $10^{3\cdot1}$ $I.D._{50}$/0·1 ml. live Friend virus. Cell-free filtrate was inactivated in a water bath for 2 h at 100° C. Offspring were challenged subcutaneously, day 1, and thereafter intraperitoneally at intervals with $10^{2\cdot8}$ $I.D._{50}$/0·1 ml. live Friend virus

In order to demonstrate that we are dealing with passive immunity, Swiss females were immunized with formalinized Friend virus vaccine. Mothers were injected three times, on the third, seventh and tenth days of pregnancy, and offspring were challenged at intervals after birth with live virus.

Fig. 4 shows the results. Protection against the development of Friend virus disease by passive immunity was essentially the same as when mothers were injected with live virus on the second day of pregnancy. The experiment is still in progress. However, protection should not occur in the later time intervals after birth, since live virus was not present in the formalinized vaccine.

Fig. 5 shows the results with heat-killed vaccine. Mothers were injected three times, on the third, seventh and tenth days of pregnancy, and offspring were challenged with live virus at intervals after birth. Very little protection was obtained against development of the disease during the period of passive immunity. This suggests that the heat-killed virus was an inadequate antigen and did not elicit a good immune response in the mothers.

Table 1. Resistance to Development of Friend Virus Disease

Week of challenge*	% protection	Offspring from infected mothers† Unprotected animals Average mortality (days)	Average spleen wt. (g)	Offspring from non-infected mothers Average mortality (days)	Average spleen wt. (g)
1	46 8	53 6	2 83	39 8	2 95
2	33 5	64 5	2 85	36 0	3·4
3	5·0	47 5	3 15	39·0	3 65
4	13 0	37·5	3·5	41 5	3·5
5	7 0	48 0	2 9	42 0	3 75
6	6 0	48·0	3·1	39 0	2 7
7	45·0	57 5	3 5	35 0	3·1
8	47·5	55·0	2 7	43·5	3 7
9	28 5	60 5	2·65	36 0	3 05
10	45 5	54·5	2 3	37·5	3·2
Average		52 6	2 94	38 9	3 3
Range		(38–65)	(2·3–3 5)	(35–44)	(2·7–3 8)

* Data pooled at weekly intervals.
† Mothers injected on second day of pregnancy.

Table 1 summarizes the data from offspring of Swiss mothers injected with live virus on the second day of pregnancy. Data are pooled at weekly intervals. As seen in the previous figures, the early period of passive immunity and the later period of active immunity are clearly seen in the column "% protection". The next column shows the average age of death of those animals that were not protected; spleen weights are shown in the third column. The average age at death and spleen weights of infected offspring from non-infected mothers are shown in the last two columns. Offspring from this group showed virtually no resistance to the disease. It should be noted that, although spleen weights are relatively uniform throughout, there is a longer interval to death of offspring from infected mothers during periods of passive and active immunity than of offspring from non-infected mothers. Although these animals were not fully protected against development of the disease, they must have had some degree of resistance to progression of the disease.

The data from all experiments are presented in Table 2. Data are pooled at weekly intervals. In all instances, protection due to passive immunity was evident. The best protection was obtained with formalinized vaccine. In all instances, passive immunity is short-lived and disappears at 3–5 weeks after birth, or at about the time the animals are weaned. As shown in the previous figures, there is a period when protection is essentially absent, following the period of passive immunity. This is followed later by protection which is presumably due to the development of active immunity.

Table 2. Percentage of Protection against Challenge with Friend Virus

Pre-treatment of pregnant mothers	Week of challenge* 1	2	3	4	5	6	7	8	9	10
(I) Live virus										
a, Swiss, early pregnancy (2)†	46	34	5	13	7	6	45	48	29	46
b, Swiss, late pregnancy (12)†	24	7	70	28	12	–	50	45	—	—
c, *DBA*/1, late pregnancy (12)†	15	27	12	9	0	0	—	13	38	—
(II) Formalinized virus										
a, Swiss (3, 7, 10)†	71	45	36	20						
(III) Heat-killed virus										
a, Swiss (3, 7, 10)†	16	5	0	7						

* Data pooled at weekly intervals.
† Day of pregnancy

A young mammal derives its immunity passively from the mother either before, after, or both before and after birth[5]. It is interesting to note in the results presented here that transmission of passive immunity continues for about 28 days after birth in the mouse, and stops abruptly. Our data seem to fit with those of others[6], indicating that the transmission of passive immunity in the mouse is most effective during the period of suckling. This is unlike the situation in the guinea-pig, rabbit and man (Table 3). However, passively acquired antibodies are transmitted postnatally, not prenatally, in the ox, goat, sheep, pig and horse, but for a period of only 36 h; in the dog, mouse and rat the time interval is longer.

Table 3. Time of Transmission of Passive Immunity in Mammals

Species	Transmission of passive immunity Prenatal	Postnatal
Ox, goat, sheep	0	+ + + (36 h)
Pig	0	+ + + (36 h)
Horse	0	+ + + (36 h)
Dog	+	+ + (10 days)
Mouse	+	+ + (16–28 days)
Rat	+	+ + (20 days)
Guinea-pig	+ + +	0
Rabbit	+ + +	0
Man	+ + +	0

In all instances, passive immunity is short-lived. The mechanism (or mechanisms) which causes the cessation of this capacity of the organism to respond is not well understood.

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POSSIBLE EFFECT OF MITOTIC RECOMBINATION ON GENE CONVERSION AND NEGATIVE INTERFERENCE

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In recent years it has become recognized from work with a variety of ascomycetes that segregation of gene loci may occur in ratios differing from 1 : 1. Extensive tetrad analysis[1–5] has established the occurrence of all the possible ratios—namely 5 : 3, 6 : 2, 7 : 1 and 8 : 0. The explanations usually put forward to account for these ratios, which imply gene conversion, have been based on copy-choice[6,7], although the 7 : 1 and 8 : 0 ratios are difficult to account for in this way.

We here examine the possibility that these ratios can be explained by the occurrence of a mitotic recombination immediately before meiosis. The model proposed assumes that mitotic recombination occurs by breakage and reunion after reduplication of DNA in a diploid nucleus and, furthermore, that each chromatid contains at least four DNA strands. Recombination of two strands between two chromatids differing in a gene could account for the 6 : 2 ratio (Fig. 1). The remaining 5 : 3, 7 : 1, and 8 : 0 ratios would result from recombination of one, three and all four strands respectively. If the initial break were between one double helix of each chromatid, then separation of the chromatids could result in further breakage of the same strand or perhaps—less frequently—of the sister strands (Fig. 2). An event of the former kind would lead to gene conversion for a limited number of closely positioned loci, while the remainder of the genes would segregate normally. This is in fact the result usually observed.

The exchange postulated here has an interesting effect on negative interference in that, provided that the breaks span a marker, a quarter of the strands in a nucleus affec-

ted by the mitotic exchange will enter meiosis in the double recombinant state. Clearly the effect of such previous recombination on the frequency of the double recombinant class will be considerably diluted by meiosis in unaffected cells. Consideration of the expected frequency of double crossing-over with different dilution factors shows that previous mitotic division could have a marked effect on coincidence, particularly where the genes are close together, and could give values greater than 100 (Tables 1 and 2). It therefore appears possible that previous mitotic exchange could both determine gene conversion and contribute to negative interference.

The proposed model is open to many obvious criticisms. The four-stranded chromatid was proposed because four is the smallest number which can account for the 5 : 3 and 7 : 1 ratios. Yet recent evidence[8] has shown that ascospores may be heterozygous for their DNA which suggests that more than four strands of DNA may be present in each chromatid. In higher organisms there is,

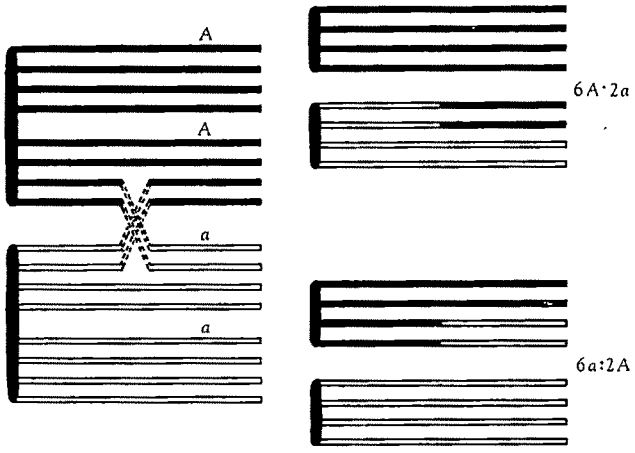


Fig. 1. Derivation of 6 , 2 ratios for alleles *A*, *a*, from the breakage of only two strands of a four-stranded chromatid. The figure shows only the daughter cells from the appropriate segregation of the chromatids at the mitotic division

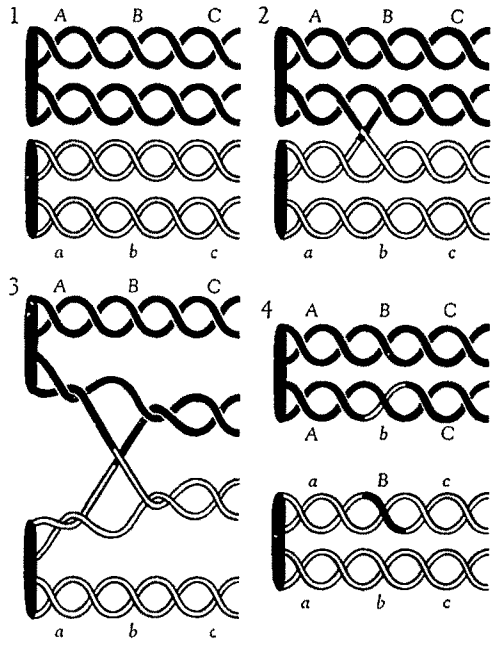


Fig 2 The derivation of double recombinant strands at a mitotic division. Each chromosome is composed of two chromatids, each of two double helices of DNA (1 chromatid = 4 DNA strands) Each helix, which should also have spiral form, is represented by a single black or white line 1, The initial state. 2, Showing the point of breakage of two strands of DNA in each chromatid. 3, The separation of the chromatids causes break-backs for each chromatid 4, The double-recombinant *AbC* and *aBc*

indeed, doubt as to the number of DNA strands in each chromatid. Electron micrographs have indicated that chromatids in the fern *Todea barbara* may have four strands[9], with the two helices formed into a major spiral. Alternatively they may be multi-stranded[10]. Experimental evidence from sea-urchin eggs[11] shows that the zygote nucleus is capable of dividing directly into four nuclei after only one replication of DNA, thus indicating that its chromatids consist of at least four strands. It is also well established that sub-chromatid breaks may occur[12]. There is clearly no certain evidence that fungal chromatids are four-stranded, but it is reasonable to assume that the level of organization of the DNA may lie between that of the bacterial cell and that of higher organisms.

The majority of ascomycetes do not have a true diploid mitosis, fusion of unlike haploid nuclei only occurring immediately before meiosis. It is possible that fusion may occur earlier, though infrequently, or that the diploid nucleus in the ascus initial may occasionally divide again before meiosis. Yet another possibility exists, for it has been shown that in the basidiomycete *Schizophyllum commune* recombination between haploid nuclei apparently occurs[13]. Evidence has also been presented for haploid recombination in *Neurospora crassa*[14], where it was found to occur between non-homologous chromosomes and would seem to be related to their organization during mitosis. There is, in fact, a growing body of evidence which is difficult to reconcile with the generally accepted processes of recombination[15]. Thus it seems possible to assume that mitotic recombination of the type assumed in our model may occur between overlapping homologous chromosomes, particularly if division of the nuclei is synchronized. This can happen only after the disappearance of a nuclear membrane at a time when DNA synthesis has already occurred.

The frequency of aberrant ratios is low and ranges from approximately 0·1–0·38 per cent in *Neurospora*[4], 0·042 per cent in *Aspergillus*[16], and 0·01–0·07 per cent in *Sordaria*[5] to 0·002 per cent in *Ascobolus*[17]. In yeast, on the other hand, which may have a true diploid phase, the apparent frequency of conversion is higher[2] although other explanations have been put forward for the abnormal spore ratios[18].

The model proposed here assumed that meiosis follows immediately after mitotic recombination. If meiosis is delayed by further mitotic divisions without recombination, then potential 6 : 2 ratios may become 8 : 0 ratios, while potential 5 : 3 ratios may become first 6 : 2 and then 8 : 0 segregations. The majority of aberrant ratios appear to be 6 : 2 or 5 : 3 events, with 8 : 0 less frequent and 7 : 1 extremely rare. It would, therefore, appear that if mitotic recombination is related to gene conversion, it must occur soon before meiosis.

Mitotic recombination within diploid nuclei is generally regarded as rare. If the results given here for the effect of mitotic recombination on conversion and negative interference are to be valid, then the ratio of affected to unaffected meioses should accord with previous evidence. The frequency of exchange has been estimated to be approximately 1 in 500 mitoses[19], but this is based on the exchange of whole chromatids. It may be that the part chromatid exchanges would be more difficult to recognize genetically, although there is no reason to assume that they would be more frequent than whole chromatid exchanges. It is probable that in Tables 1 and 2 the dilution ratio of one affected to ten unaffected is too low, and that the ratio 1 to 100 is more likely. It will be noted that the greater dilution still gives coincidence values of 3·54 and 26·0 for genes 0·1 and 0·01 units apart respectively. On the other hand, in *Fusarium oxysporum* a frequency as high as one recombination in forty nuclei has been found[20]. On this basis a coincidence value of about 100 might be obtained with genes 0·01 units apart, and this result would be similar to those already reported[21,22]. It will be noted,

Table 1. Coincidence Values, Percentage of Expected Aberrant Ratios and Variation in Percentage of Parental, Single and Double Recombinant Classes for Genes 1 Unit Apart, based on a Two-strand Exchange Model

Genotype	Frequency from unaffected meioses	Frequency from meioses affected by previous mitotic exchange	1 Mitosis : 10 meioses dilution factor 1 . 1,000	1 Mitosis : 100 meioses dilution factor 1 : 10,000
Parental	98 01	73·51	97 985	98 0075
Recombinant region 1	0·99	0·99	0 990	0·9900
Recombinant region 2	0·99	0·99	0·990	0 9900
Recombinant region 1+2	0 01	24·51	0·035	0·0125
	Coefficient of coincidence		3·333	1·244
	Aberrant ratios		0·05	0·005

Note: The dilution factor is calculated on the assumption that a mitotic recombination will occur with (1/10)th or (1/100)th of the frequency expected for a meiotic recombination per unit length.

Table 2. Effect of Gene Distance on Coincidence Values and Percentage of Aberrant Ratios with Ratios of 1 Mitosis per 10 or 100 Meioses, based on a Two-strand Exchange Model

Dilution factor calculated as in Table 1

Distance between genes		1 Mitosis : 10 meioses	1 Mitosis : 100 meioses
10 units	Dilution factor	1 : 100	1 : 1,000
	Coincidence	1·2	1·02
	Aberrant ratios	0·5	0·05
1 unit	Dilution factor	1 : 1,000	1 : 10,000
	Coincidence	3·333	1·244
	Aberrant ratios	0·05	0·005
0·1 unit	Dilution factor	1 : 10,000	1 : 100,000
	Coincidence	25·15	3·54
	Aberrant ratios	0 005	0·0005
0 01 unit	Dilution factor	1 : 100,000	1 : 1,000,000
	Coincidence	250·0	26·0
	Aberrant ratios	0·0005	0·00005

occurs before the first locus, the result of such an event in association with normal meioses, is some increase in negative interference. In fact, the more the cells are affected in this way, the more similar the situation becomes to that envisaged by Pritchard[24]. Thus some cells show meiotic recombination while others fail to do so because they are homozygous and not because pairing is ineffective. Positive interference is brought about when single mitotic recombination occurs between the sites, since these events change a proportion of the parental strands into one or other of the single recombinant classes. Finally, the less likely occurrence of multiple exchange would lead to positive interference for recombination before *A*, *B* and *A*, *C* and negative interference for the remaining possibilities. The negative interference values would not be high, for although the double recombination frequency rises, so also does the frequency of single recombinants. The results contrast with those obtained for part-chromatid exchange, where the double recombinant group increases without alteration of the single recombinant values.

It appears that other workers have not considered the possibility that mitotic recombination could affect meiotic segregation. This has probably been due not only to an almost exclusive interest in meiosis but also to the belief that mitotic recombination is too rare to be of interest. It is apparent from the foregoing discussion that mitotic recombination does offer explanations alternative to those

Table 3. The Effect of Prior Mitotic Recombination occurring in Different Regions of a Three-gene Model on the Frequency of the Parental (*a*), the Single Recombinant (*b*, *c*) and the Double Recombinant Class (*d*) obtained at Subsequent Meiosis

Genetic classes obtained at meiosis		Site before which prior mitotic recombination occurs						
	None	*A*	*B*	*C*	*AB*	*AC*	*BC*	*ABC*
Parental	*a*	$a+\frac{1}{2}(b+c+d)$	$\frac{1}{2}(a+b)+\frac{1}{2}(c+d)$	$\frac{1}{2}(a+c)$	$\frac{1}{2}(a+b)+\frac{1}{2}(c+d)$	$\frac{1}{2}(a+c)+\frac{1}{2}(b+d)$	$\frac{1}{2}(a+b+c+d)$	$\frac{1}{2}(a+b+c+d)$
Recombinant in region 1	*b*	$\frac{1}{2}b$	,,	$\frac{1}{2}(b+d)$	,,	$\frac{1}{2}(b+d)$	,,	,,
Recombinant in region 2	*c*	$\frac{1}{2}c$	$\frac{1}{2}(c+d)$	$\frac{1}{2}(a+c)$	$\frac{1}{2}(c+d)$	$\frac{1}{2}(a+c)+\frac{1}{2}(b+d)$	,,	,,
Recombinant in region 1+2	*d*	$\frac{1}{2}d$	,,	$\frac{1}{2}(b+d)$	,,	$\frac{1}{2}(b+d)$	,,	,,

however, that the frequency of aberrant ratios found for genes 0·01 unit apart at this dilution is at the lower limit of the distribution given above.

Kitani *et al.*[5] found a high frequency of exchange between the markers on either side of conversion event which was, however, more evident in 5 : 3 rather than in 6 : 2 asci. They believed that this disparity was difficult to reconcile with previously postulated explanations of conversion. In the model under discussion the mitotic recombinations leading eventually to 5 : 3 and 6 : 2 ratios produce at meiosis either a 'heterozygous' chromatid with two dissimilar DNA duplexes, or a 'heterozygous' chromosome with two dissimilar chromatids. It is possible that the high frequency of recombination associated with gene conversion is due to instability between the strands, the effect being greatest where the chromatids are of mixed genetic constitution. This may, therefore, represent a return to Pontecorvo's suggestion[23] that a high frequency of recombination may be associated with heterozygosity even though, in this instance, the heterozygosity is within the chromosomes rather than between them.

The foregoing discussion has been concerned largely with part chromatid exchange. Pontecorvo[19] was concerned with the exchange of whole chromatids rendering genes distal to the point of recombination homozygous in half the affected cells. In order to examine the possible effect of recombination before and between three genes on the coefficient of coincidence, an estimate was made of the results from such events (Table 3). In making the estimates it was assumed that recombination could initially affect any chromatid at random and that the separation of the chromatids after mitotic recombination was also at random. The results of the subsequent meiosis are presented in Table 3. Where mitotic recombination already proposed for gene-conversion and negative interference. The model is essentially simpler than many and it offers a real advantage over mechanisms which involve copy-choice. For, while the proposed model involves only one process of recombination, copy-choice also requires breakage and reunion to explain tetra-type asci.

The hypothesis could be supported by further evidence and in particular from estimates of conversion in higher organisms where the longer diploid phase of growth should allow greater evidence of the effect of part or whole chromatid exchanges.

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LETTERS TO THE EDITOR

ASTRONOMY

Possible Luminescence Effects on Mercury

A GROWING body of evidence leads to the conclusion that luminescence effects occur on the lunar surface. Large intensity fluctuations on the Moon have been observed photographically by Kopal and Rackham[1], spectroscopically by Spinrad[2], and photometrically and polarimetrically by Gehrels *et al.*[3]. If the luminescence phenomena are induced by the solar wind (see Nash[4]), we might do well to look to other bodies in the solar system, which are similarly unprotected by an atmosphere, for evidence of luminescence.

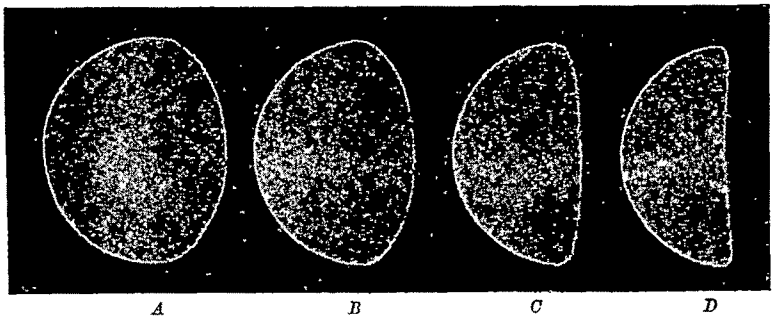
Fluctuations in the intensity of portions of the surface of the planet Mercury were recorded by Antoniadi[5–7], who regarded the apparent obscuration of dark areas as the result of variable cloud cover. From his extensive observations with the 33-in. Meudon refractor, Antoniadi concluded that the density and extent of the 'clouds' varied with the changing distance of Mercury from the Sun, probably reaching maximum at perihelion and minimum after aphelion.

The newly derived rotation period of Mercury reported by Pettengill and Dyce[8] casts some doubt on the long-term changes reported by Antoniadi because his conclusions were based on the assumed 88-day period. Antoniadi[5] also noted, however, that changes occur on Mercury in a few tens of hours, and these cannot be simply explained in terms of the shorter rotation period. Other observers have also reported short-period changes on Mercury[9].

In the interval 1958–64, I made an extensive series of observations of Mercury with telescopes up to 82 in. in aperture. Visual studies of Mercury are difficult owing to the proximity of the planet to the Sun, but my observations generally confirm that variations in the intensity of certain of Mercury's markings can occur in a matter of several hours or days.

Fig. 1 shows four drawings of Mercury made in a nine-day period in 1963. Drawing *A* was made with a 36-in. reflector and the remainder with a 12-in. reflector, all in fairly good seeing conditions. Between the first and last drawings in Fig. 1, a darkening at the south cusp is apparent. Later drawings made as the crescent waned show the south cusp darkening further until it was the darkest region on the planet. The apparent change in the interval of three days between *A* and *B* may be related in part to the higher resolving power of the 36-in. telescope used for *A*.

The great similarities between the Moon and Mercury that have been pointed out by many investigators and the probable luminescence phenomena on the Moon may make it possible to interpret changes on Mercury as the effects of luminescence of the surface materials.



A B C D

Fig. 1. Four drawings of Mercury. *A*, April 12, 1963, 2200 U.T ; *B*, April 16, 1963, 2030 U.T., *C*, April 20, 1963, 2330 U.T.; *D*, April 21, 1963, 2330 U.T. South is at the top

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GEOPHYSICS

Night-time Electron Temperatures in the Upper *F* Region

SATELLITE observations indicate that night-time electron temperatures in the upper *F* region are larger than the neutral gas temperatures. Willmore[1] estimates that an energy input $Q/n_e = 1{\cdot}5 \times 10^{-6}$ eV sec^{-1} is required at 650 km to maintain the temperature difference, and he suggests that bombardment by electrons of kilovolt energies might provide a suitable source. This communication shows that his suggestion is consistent with an upper limit on the precipitated flux of fast electrons set by optical observations[2].

The rate of electron heating due to bombardment by fast electrons is:

$$Q = p\frac{\mathrm{d}E}{\mathrm{d}x} v n_{ef} \text{ eV cm}^{-3} \text{ sec}^{-1} \qquad (1)$$

where $\mathrm{d}E/\mathrm{d}x$ is the energy loss in eV per cm path, p is the fraction of this loss converted to kinetic energy of the ambient electrons, and v is the velocity and n_{ef} the number density of the fast electrons. The energy loss due to collisions of fast electrons, of energy E eV, with ambient electrons is[3]:

$$\left(\frac{\mathrm{d}E}{\mathrm{d}x}\right)_e = \frac{2 \times 10^{-12}}{E} n_e \text{ eV cm}^{-1} \text{ for } E > 3 \text{ eV}$$

Using (1) and (2) and taking $p = 1$, $Q/n_e = 1{\cdot}5 \times 10^{-6}$ and $E = 10^3$, Willmore[1] obtained:

$$v n_{ef} = 7 \times 10^9 \text{ cm}^2 \text{ sec}^{-1}.$$

The precipitated flux of fast electrons is:

$$J = \frac{1{\cdot}6 \times 10^{-12}}{\varphi} E v n_{ef} \text{ ergs cm}^{-2} \text{ sec}^{-1} \qquad (3)$$

where the factor $1{\cdot}6 \times 10^{-12}$ converts eV to ergs and where φ is equal to 1 if the fast electrons are incident vertically and all effects of the magnetic field are neglected. With $\varphi = 1$, $E = 10^3$ and $v n_{ef} = 7 \times 10^9$ we obtain $J = 10$ ergs cm^{-2} sec^{-1}. This cannot be correct, since[2] the optical observations of N_2^+ emissions indicate that J cannot exceed some 10^{-2} ergs cm^{-2} sec^{-1}. We consider two effects which greatly reduce the value of J which is consistent with the suggested mechanism of ionospheric heating.

The energy loss due to excitation and ionization of neutral atoms of number density n_a is:

$$\left(\frac{dE}{dx}\right)_a = \frac{2 \times 10^{-12} \ln (E/80)}{E} n_a \text{ eV cm}^{-1} \text{ for } E > 500 \text{ eV} \quad (4)$$

We are interested in altitudes for which, at night, $n_a \sim 100\, n_e$. Comparing (2) and (4), we see that the fast electrons lose most of their energy in collisions with neutral atoms. About a quarter of this loss is accounted for by the kinetic energy of secondary electrons produced in ionizing collisions. The secondary electrons have $E \simeq 10$ eV. Dalgarno, McElroy and Moffett[4] estimate that:

$$\left(\frac{dE}{dx}\right)_a \simeq 10^{-16} n_a \text{ eV cm}^{-1} \text{ for } E \sim 10 \text{ eV} \quad (5)$$

Comparing (2) and (5), we see that the secondary electrons lose most of their energy in collisions with the ambient electrons. We may therefore use (4) in (1) with $p = 1/4$. This gives $vn_{ef} \simeq 10^8$ cm^{-2} sec^{-1}.

The second effect we consider is the reduction in J due to magnetic field trapping of fast electrons. Assuming that kilovolt electrons are injected at equatorial latitudes with isotropic pitch angle distributions, the factor φ in (3) has been calculated on solving a Fokker–Planck type of diffusion equation, allowing for scattering and energy degradation processes. This calculation, which will be described in detail elsewhere[5], shows φ to be of order 100. We therefore conclude that the electron heating can be explained with precipitated fluxes J as small as 10^{-3} ergs cm^{-2} sec^{-1} and hence that the discrepancy with the optical observations is removed.

It is of interest to consider the ionization which would be produced by fast electrons. Since each ionization provides the ambient electrons with about 10 eV of kinetic energy, the electron production rate is $q = (Q/10)$ electrons cm^{-3} sec^{-1}. Thus, with $(Q/n_e) = 1{\cdot}5 \times 10^{-5}$ eV sec^{-1} and $n_e = 10^4$ cm^{-3} we obtain $q = 0{\cdot}015$. It may be noted that the value of Q determined from observations of electron temperatures sets a limit on q which is more stringent than the limit set by optical observations.

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Argon Retentivity of Nephelines

Nepheline[1] is the most characteristic feldspathoidal mineral in alkaline rocks and occurs in volcanic, plutonic and metasomatic rocks. In particular, these nepheline-bearing rocks occur fairly widely in the Canadian Shield.

The idealized formula of this framework silicate is $Na_3K(Al_4Si_4O_{16})$, which corresponds to a potassium concentration of 6·69 weight per cent. Reported values[1] range from 3 per cent to 10 2 per cent, rendering nepheline potentially suitable for dating by the potassium–argon method. An investigation has therefore been begun to test this possibility by examining the retentivity of nephelines for argon. Some of the preliminary findings are presented here.

All the samples have been collected from the Grenville province of the Canadian Shield, where muscovites, amphiboles and biotites are well known[2,3] to yield potassium–argon ages in the range 800–1,200 m.y. Samples of at least this age are necessary, since some minerals, for example some feldspars[4,5] and glauconite[6,7], while apparently reliable in the 0–100 m.y. range, are definitely unreliable when found in older rocks. The retentivity has been tested by comparing the potassium–argon age obtained on the nepheline with that given by a co-existing biotite or amphibole, these two minerals being generally accepted as exhibiting high argon retentivity[8,9].

Table 1 shows the initial findings of this programme. The immediate result which is clear from the data is that nepheline appears to retain argon extremely well over periods of at least 10^9 years, since in no instance is the nepheline age less than that of the associated mineral. The nepheline and biotite from Princess Quarry agree exactly in age. In the four other examples reported, the nepheline actually yields a slightly higher age than the corresponding index mineral. The age of the Goulding-Keene nepheline exceeds the average biotite age by 4·6 per cent, and the replicate analyses performed would seem to indicate that this small difference is real. In the three remaining samples, the nepheline unambiguously indicates a higher age. Thus, for samples from Gill Quarry and Blue Mountain, the nepheline ages are greater than those of the biotites by 16 per cent and 12 per cent, respectively. The potassium–argon age of the nepheline from the Bigwood Township nepheline syenite exceeds that of the amphibole (extremely fresh hastingsite) by 18 per cent.

Table 1. Comparison of Potassium–Argon Ages of Nephelines and Coexisting Biotites and Amphibole

Locality	Mineral	Potassium (%)	$^{40}Ar^*/^{40}Ar$ (total)	$^{40}Ar^*$ p p m.	Age (m y)
Princess Quarry, Ontario	Biotite	7·55	0 92	0 6205	900
	Nepheline	4·85	0 87	0 4011	904
Goulding-Keene Quarry, Ontario	Biotite (coarse)	7 66	0 95	0 5904	855
			0 77	0 5742	836
	Biotite (fine)	5 96	0 87	0·4658	865
	Nepheline	4 76	0 95	0 3805	879
			0 76	0·3927	903
Gill Quarry, Ontario	Biotite	6 99	0 92	0 5422	859
			0 88	0 5410	858
	Nepheline	4 83	0 94	0 4556	1,003
			0 93	0 4494	992
Blue Mountain, Ontario	Biotite	7 60	0 94	0 6240	900†
	Nepheline	4·85	0·87	0 4597	1,007
Bigwood Twp, Ontario	Amphibole	1·37	0 77	0·1260	983
			0 99	0 1256	981
	Nepheline	4 71	0 86	0 5438	1,169
			0 98	0 5277	1,144

* Radiogenic component
† Measured by the Geological Survey of Canada (ref. 17).
λ_ε equals $0\ 584 \times 10^{-10}$ yr.$^{-1}$. λ_β equals $4\ 72 \times 10^{-10}$ yr^{-1}. ^{40}K equals 0 0119 per cent potassium, atomic.

It could be argued that the data indicate that potassium–argon ages determined with nephelines are anomalously high. For example, argon may have been trapped by the framework structure as a result of metamorphism of the nepheline syenites during which nepheline reacts with feldspar[10]. However, this is not considered likely at present since in no case does the nepheline age exceed the typical values found by potassium–argon dating in the Grenville. Evidence is accumulating that uranium–lead and whole-rock rubidium–strontium ages are systematically higher than potassium–argon mineral ages in complex metamorphic terrains[11,12]. It is of more than passing interest, therefore, that the nepheline ages come closer to concordance with those measured by other means. However, at the present time, it is not at all clear whether the divergences between the results of different methods are the results of errors in the various decay constants[13], the differing responses of different mineral types and decay systems to later metamorphism, or whether the measured ages represent the different times of closure of the systems during uplift and cooling[14,15]. The high argon retentivity of nephelines having been established, however, it does seem that a systematic potassium–argon investigation of nephelines will shed some light on this fundamental problem in geochronology.

In this work, potassium concentrations have been determined by flame photometry, using lithium as an internal standard and sodium as a buffer. Radiogenic argon-40 analyses were performed on an '*MS*10' mass

spectrometer after dilution with argon-38. Inter-laboratory comparisons have shown that any single age obtained is accurate to ± 5 per cent[16].

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METALLURGY

T-phase Precipitation induced by the Addition of Silver to an Aluminium–Copper–Magnesium Alloy

RECENTLY attention has been directed to the effects of small amounts (approximately 0·1 atomic per cent) of silver on the structure and properties of certain age-hardening aluminium alloys, since the changes that occur are thought to have both fundamental and practical significance[1–7]. As part of this work, a detailed investigation is being made of silver additions to a wide range of aluminium–copper–magnesium alloys and some interesting results have been obtained for aluminium–2·5 per cent copper–1·5 per cent magnesium (contents expressed in weight percentages). As reported previously[5], the addition of 0·5 per cent silver to this alloy both accelerates the rate of ageing and causes increased hardening in the medium temperature range (120°–240° C) (Fig. 1). Reasons for these changes have now been sought using X-ray diffraction and transmission electron microscopy to investigate alloys which were solution-treated at 500° C, cold-water quenched, and aged at 200° C in a salt bath. Results are shown in Figs. 1 and 2.

The ternary alloy age hardens in two stages. The first stage occurs very rapidly and is due to the formation of a uniform dispersion of G.P.B. zones throughout the matrix. The second rise in hardness is associated with the growth of clusters of the precipitate *S* (possibly *S′*), which is lath-shaped and nucleated on dislocation lines generated during or immediately following quenching. The appearance of *S* at peak hardness is shown in Fig. 2*a*, and other evidence indicated that G.P.B. zones were also present. Softening occurs by the growth of *S* and the disappearance of G.P.B. zones. These results are in accord with earlier observations made on similar alloys by Silcock[8] and Wilson and Forsyth[9].

In the silver-containing alloy, two-stage ageing was again evident, but the morphology and dispersion of the precipitate particles were completely changed. For example, at peak hardness, the microstructure (Fig. 2*b*) was more characteristic of an aluminium–zinc–magnesium alloy. In most regions precipitate particles were small (average size 100 a.u.) and uniformly dispersed; dislocations generally had only a minor effect on precipitation (*A* in Fig. 2*b*). X-ray work showed that G.P.B. zones were formed at very short ageing times as for the silver-free alloy, but the second rise in hardness was associated with

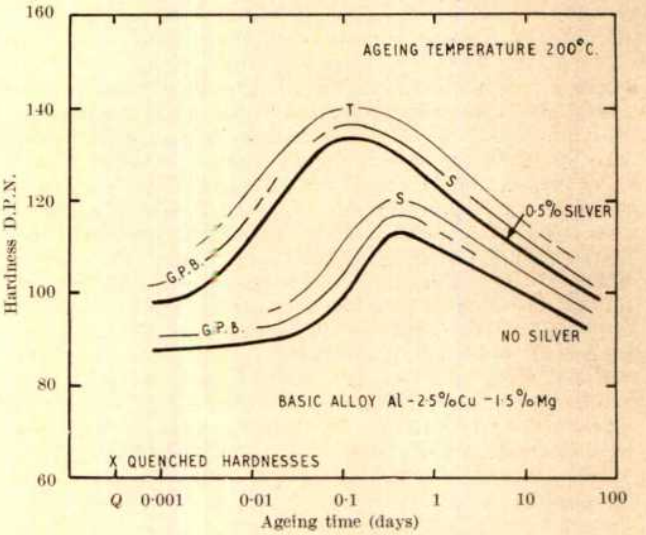


Fig. 1. Precipitate structures occurring at different parts of the hardness/time curves of aluminium–2·5 per cent copper–1·5 per cent magnesium, with and without 0·5 per cent silver, aged at 200° C

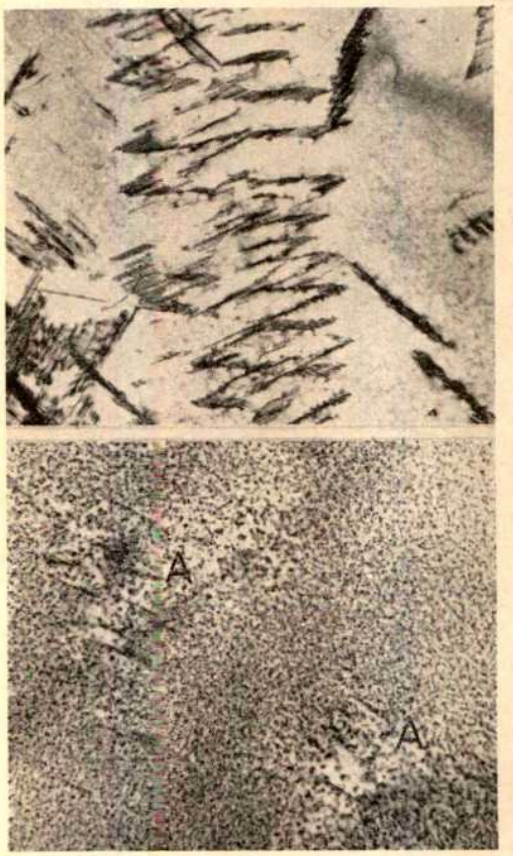


Fig. 2. Transmission electron micrographs showing the effects of 0·5 per cent silver on the microstructure of aluminium–2·5 per cent copper–1·5 per cent magnesium aged to peak hardness at 200° C: (*a*) No silver, aged 0·3 days; (*b*) 0·5 per cent silver, aged 0·15 days (× 37,500)

a T-phase structure. At peak hardness this T phase predominated and a small amount of S was detected. As softening occurred, the S phase increased as a uniform dispersion of lath-shaped particles and the T phase gradually decreased in amount. The S particles appeared to be nucleated homogeneously and were much finer than those present in the ternary alloy.

The effect of silver in promoting T-phase precipitation may be related to the fact that small amounts of silver stimulate hardening in aluminium–5 per cent magnesium by precipitating an equilibrium T phase, approximately Al_6 Ag Mg_4, as we have found, and which was reported recently by Wheeler *et al.*[10]. In the present work, using molybdenum radiation, the X-ray reflexions from the small particles were too diffuse to enable us to decide whether the T phase is this compound or Al_6 Cu Mg_4.

Another observation was that the peak hardnesses of the silver-free and silver-containing alloys were closer than might be expected from the marked contrast in the appearance of the precipitates. This hardness difference was even less at longer ageing times when there was still a marked disparity between the size and dispersion of the lath-shaped S particles. This suggests that the ability of dispersions of this kind to retard dislocation movements during plastic deformation is relatively insensitive to the size of the particles. The results may also have practical implications in view of the fact that aluminium–2·5 per cent copper–1·5 per cent magnesium is the age-hardening component of several commercial alloys used for elevated temperature applications. All these aspects are being investigated in more detail.

We thank Mr. B. E. Williams for his contribution to the experimental work.

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Enhanced Diffusion Rates during the Fatigue of Metals

Enhanced diffusion rates during plastic straining or fatigue testing have been the subject of controversy for some time[1]. Conclusive evidence of this phenomenon, notwithstanding an estimate of the effect, has not yet been achieved. However, an investigation recently conducted on the fatigue properties of certain copper alloys at room temperature under unique conditions has allowed confirmation of changes in diffusion rate and also a measure of this change.

Fatigue tests have been conducted on oxygen-free high-conductivity copper specimens possessing a surface-alloyed layer of silver to a depth of 90 μm; the silver concentration varied from approximately 4 per cent at the surface to zero at 90 μm. Specimens were tested in a Wohler-type fatigue machine, operating at 2,880 c.p.m. at a stress-level giving a total life of 10^7 cycles. After failure, sections were taken to allow measurement of the radial distribution of the silver by means of a 'Cameca' electron-probe microanalyser. Comparison with blanks prepared under identical conditions, but not fatigue tested, revealed a significant difference in the silver concentration and penetration and showed the diffusion rate during fatigue to be considerably increased. Calculation shows that the diffusion activity in the fatigue specimen was equivalent to that which would have occurred had the specimen been simply heated to 300° C for the duration of the test, namely, 60 h. Several microanalyser traces were taken in different grains to allow for the orientation dependence of the diffusion process and the averaged results are displayed in Fig. 1.

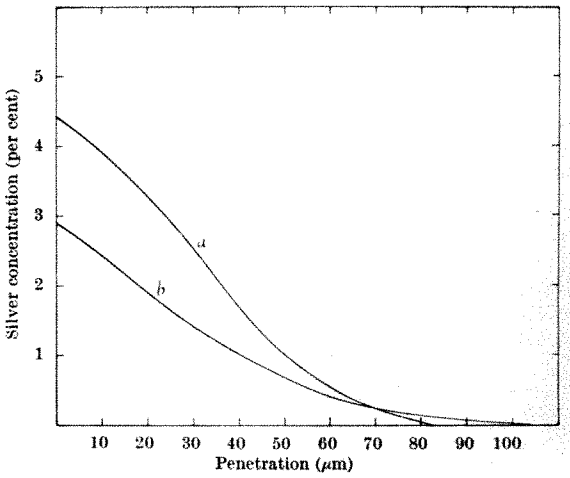


Fig. 1. Penetration curves for silver in copper before and after fatigue testing. *a*, Normal; *b*, after fatigue testing

Normally, long-range diffusion phenomena do not occur in copper at room temperature because the concentration of vacancies in thermal equilibrium in the crystal lattice and also the jump frequency are too low to facilitate appreciable movement of the atoms. The explanation of this phenomenon would appear to lie in the abnormally high vacancy concentration being produced during fatigue. The presence of a concentration gradient of silver in the surface layer of the copper has given directionality to the increased atomic movement and enabled a measure of this effect to be made.

Previous attempts[2] to measure an enhanced diffusion rate due to plastic deformation have been pursued in tests at temperatures well above room temperature where normal thermal diffusion rates have been appreciable. It is considered that the much larger rates of diffusion in these tests overshadowed the changes produced due to excess vacancy concentration and led to the confusion regarding the occurrence of this phenomenon.

The results obtained here are in keeping with observations made in irradiated materials[3] where diffusion changes have been detected and the cause been attributed to excess vacancy concentration. These measurements were achieved by detecting changes in short-range phenomena, such as ordering in certain alloy systems, and showed a similar equivalent temperature dependence.

The importance of this type of measurement lies in the fact that this is the first method yet realized where measurement of the mobility of atomic defects, important to the fundamental mode of fatigue failure in metals, has been made possible and should allow eventually a physical description of the process leading to crack initiation during fatigue.

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CRYSTALLOGRAPHY

Crystalloluminescence

RECENT advances in the analytical techniques applied to the study of faint light sources have made it possible to investigate the nature of the light emitted during processes as diverse as crystallization, nucleation, cracking and various chemical reactions.

The crystalloluminescence observed during the nucleation of alkali halides is now well understood as an impurity phenomenon associated solely with three-dimensional, that is, homogeneous, nucleation[1]. Each pulse, several hundreds of photons in size, is emitted as the result of a single nucleation event. A nucleus of critical size, which forms as a disordered, glassy aggregate, eventually 'clicks' over into crystalline form within a few nanoseconds. The energy released during this phase change is converted into fluorescent light with high efficiency provided that suitable heavy-metal impurities are present to act as fluorescent centres. The development of nanosecond pulse techniques has enabled this mechanism to be examined in great detail. Crystalloluminescence, which thus accompanies the birth of a crystal, however, is a comparatively rare phenomenon, and it confirms the validity of Volmer's equation of the rate of homogeneous nucleation as will be shown in a forthcoming publication.

Another phenomenon, described as triboluminescence, is known to occur in numerous solid systems[2]. It is associated with electrical discharges across freshly formed gaps when certain solids are subjected to mechanical or thermal stress, the best-known examples, perhaps, being chlorates, bromates and mixed sulphates, all of which emit light on crystallization, and also ordinary sugar and saccharin, which both show luminescence when crushed. The energy released on cracking need not, of course, be emitted entirely as the long, irregular pulses characteristic of triboluminescence, but may also be converted to fluorescence and phosphorescence (random emission of single photons) in the presence of suitable impurities. Triboluminescence and the crystalloluminescence described in the foregoing are thus basically not related in any way to each other, the first being due to a corona discharge while the second is caused by a molecular rearrangement in which the released energy is converted into light by a mechanism akin to piezoluminescence. Crystalloluminescence shows marked regularities in time and pulse-size distribution depending on supersaturation whereas triboluminescence is invariably random in all respects.

There is a further source of light emission, which sometimes accompanies crystallization events and which appears to have caused some confusion in the past, namely, chemiluminescence. It is usually associated with free-radical recombinations (for example, it is easily observed during the homolytic oxidations of lucigenine, luminol and phenol) and it is also present in many autoxidations (for example, of benzaldehyde, aniline and oleic acid). Thus, the light observed many years ago during the crystallization of molten acetates[2] was not crystalloluminescence but it was due to free-radical activity caused by the thermally produced acetyl radical and its fragments[3]. Furthermore, chemiluminescence has more recently been detected[4] in the course of some heterolytic reactions from which oxygen emerges in the diamagnetic singlet state. As a rule, chemiluminescence can readily be distinguished from both triboluminescence and crystalloluminescence since the light is emitted randomly as single quanta. Nevertheless, it is often more difficult to separate chemiluminescence and low-level phosphorescence.

During the crystallization of non-ionizing organic compounds, the energy changes involved are insufficient to cause genuine crystalloluminescence and the light that is observed occasionally in such systems can be shown to be due to either triboluminescence or chemiluminescence

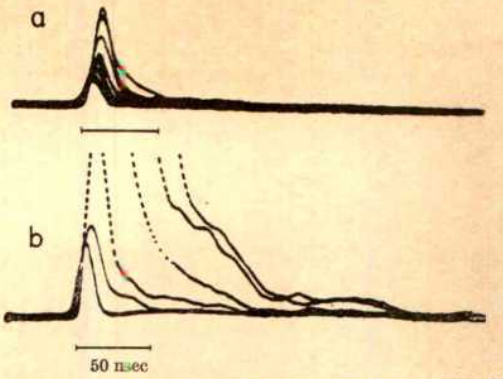


Fig. 1. Triboluminescence pulses observed during the crystallization of benzanilide from alcohol

or both. Thus we have found that the recently reported luminescence during the formation of benzanilide from aniline and benzoylchloride[5] is essentially caused by the cracking of those crystals appearing first (cf. Fig. 1), which form colourless phosphorescent leaflets and which, since Tschugaeff's time[6], have been known to be triboluminescent. The groups of triboluminescent pulses shown in Fig. 1*a* and *b* were photographed on 'Polaroid' 10,000 ASA using an oscillograph and associated amplifiers with a bandwidth of 75 Mc/s. The composite rise-time of the apparatus, including that of the multiplier photocell, was 9 nsec. The cell possessed a tri-alkali photocathode with a flat response from 1800 Å to 6500 Å and was cooled to −60° C.

Fig. 1*a* and *b* indicate, respectively, the lower and upper limits of the size range of the triboluminescent pulses obtained during the crystallization of benzanilide from various organic solvents. The larger pulses seen in *b* exhibit the irregularities typical of triboluminescence. Owing to a masking effect, much of the triboluminescent light is converted into phosphorescence which does not appear in the photographs but which can readily be observed at higher sensitivities. The fact that both types of pulses shown were due to triboluminescence (rather than to chemiluminescence, phosphorescence, or thermal emission from the photocathode) was confirmed by the detection of the electrostatic discharges, which are invariably associated with triboluminescence, by a method similar to that previously used by Belyaev *et al.*[7]. During both crystallization of benzanilide from solutions and its formation from benzoyl chloride and aniline in ether the multitudes of pulses observed varied randomly in size from 10 to 10,000 quanta.

The very high sensitivity of the techniques used in these laboratories allowed also the observation of a faint glow from the ethereal solutions of the two reagents as well as from pure ether itself. This weak light is, no doubt, due to very slight autoxidation of ether vapour, which may in turn induce secondary oxidation of the solutes.

The many pitfalls for the casual observer, who is likely to confuse one type of light with another, can be avoided by the use of apparatus of sufficient sensitivity and the means of distinguishing between pulses arising from single quanta and those of a more composite nature.

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CHEMISTRY

Kinetics of the Reaction of Nitric Oxide with Molecular Oxygen

The results of detailed kinetic investigations of the reaction of nitric oxide with oxygen at a temperature of 25° C and partial gas pressures of 1–50 mm mercury demonstrate that this reaction does not exactly follow an equation of third order[1]. The formal third-order rate constant $k_{exp} = (d[NO_2]/dt)/[NO]^2 \cdot [O_2]$ calculated from reaction rates near the starting point of the reaction ($[O_2]$, $[NO] \gg [NO_2]$) depends on the composition of the sytesm: it follows from experimental data that k_{xp} does not depend only on the concentration of nitric oxide ($[NO]$) or that of oxygen $[O_2]$, but on their ratio $[O_2]/[NO]$ (Fig. 1). The relation $k_{exp} = k_{exp}$ ($[O_2]/[NO]$) decreases monotonously: within $[O_2]/[NO]$ values of three orders $dk_{exp}/d([O_2]/[NO]) < 0$, that is, $\partial k_{exp}/\partial[O_2] < 0$ and $\partial k_{exp}/\partial[NO] > 0$, which formally corresponds with a reaction order higher than 2 with respect to the concentration of nitric oxide and lower than 1 with respect to that of oxygen. This conclusion is in qualitative agreement with the results published by Treacy and Daniels[2]. For $[O_2]/[NO] \ll 1$, the limiting value of k_{exp} of $1{\cdot}48\ (\pm 0{\cdot}05) \times 10^4$ l.²/mol² sec was found; for $[O_2]/[NO] \gg 1$ this value is $0{\cdot}80\ (\pm 0{\cdot}05) \times 10^4$ l.²/mol² sec.

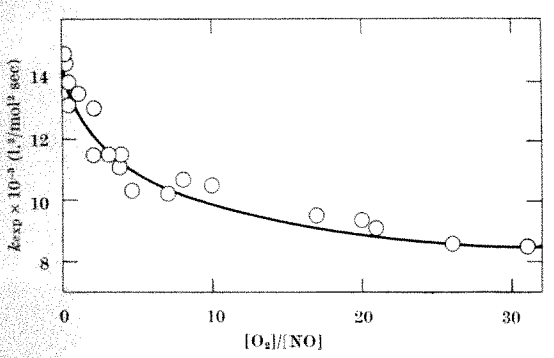


Fig. 1. Dependence of the formal third-order rate constant on the ratio of oxygen and nitric oxide concentrations

Heterogeneity of the reaction cannot be the cause of these deviations from third-order kinetics, as it has been established by differential calorimetry that the reaction under the conditions considered here is homogeneous[3]. The following mechanism of the reaction, agreeing with the relation of k_{exp} to $[O_2]/[NO]$ already mentioned, has been considered:

$$\text{(I)}\quad NO + NO \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} N_2O_2^*$$

$$\text{(II)}\quad N_2O_2^* + M \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} N_2O_2 + M$$

$$\text{(III)}\quad N_2O_2 + O_2 \xrightarrow[k_3]{} 2NO_2\ (\rightleftharpoons N_2O_4)$$

$$\text{(IV)}\quad N_2O_2^* + O_2 \xrightarrow[k_4]{} 2NO_2\ (\rightleftharpoons N_2O_4)$$

In reaction (I) the quasi-molecule of the dimer $N_2O_2^*$ is formed, which is stabilized in reaction (II) by molecule M, represented in this case only by a molecule of oxygen, O_2, or nitric oxide, NO. Assuming a stationary concentration of the stabilized dimer N_2O_2, the following equation can be derived for k_{exp}:

$$k_{exp} = 2K_1\left[k_4 + k_3\frac{k'_2[NO] + k''_2[O_2]}{k'_{-2}[NO] + (k''_{-2} + k_3)[O_2]}\right] \quad (1)$$

where $K_1 = k_1/k_{-1}$; k'_2, k'_{-2}, and k''_2, k''_{-2} are the rate constants of reaction (II) with $M = NO$, or $M = O_2$, respectively. According to this equation, k_{exp} is only really a function of the ratio $[O_2]/[NO]$; it follows also from equation (1) that the values of the constants k''_{-2} and k_3 must be comparable if deviations from a third-order kinetics are observable. It can be shown that this condition can be fulfilled if certain assumptions concerning the quasi-molecule of the dimer are made; however, the relatively low value of the probability factor of the reaction (I) must be considered. It follows from the quantitative evaluation of experimental data on the basis of the mechanism suggested that reaction (IV) may be neglected. The efficiency of the nitric oxide molecule in reaction (II), that is in stabilizing the quasi-molecule, is approximately six times greater than is that of the oxygen molecule.

According to the proposed mechanism, in the presence of the inert component M the constant k_{exp} would depend on the concentration of M and, in the case of a sufficiently high concentration, k_{exp} should be equal to $1{\cdot}48 \times 10^4$ l.²/mol² sec, regardless of the value of the ratio $[O_2]/[NO]$. This means that in a system with $[O_2]/[NO] \gg 1$ the value k_{exp} should rise with increasing $[M]$ to the value of $1{\cdot}48 \times 10^4$ l.²/mol² sec, whereas in a system with the ratio $[O_2]/[NO] \ll 1$ it would be actually independent of the concentration of the inert component. This assumption has been verified for $M \equiv CO_2$; the efficiency of the carbon dioxide molecule in reaction (II) is approximately forty times lower than that of the nitric oxide molecule. Therefore the positive effect of the inert component may only actually be observed in the systems with $[O_2]/[NO] \gg 1$ and $[M] \gg [O_2]$. The high efficiency of the nitric oxide molecule compared with those of oxygen and carbon dioxide is probably due to the ease of energy transfer in the exchange collision $N_2O_2^* + NO \rightarrow NO + N_2O_2$.

The proposed mechanism is therefore in qualitative agreement with the experimental data and does not lead to obvious discrepancies with known facts. An analogous mechanism with the intermediate ONOO instead of N_2O_2 leads to a kinetic equation which does not correspond with the experimentally found dependence of k_{exp} on the ratio of $[O_2]/[NO]$.

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Phase Determination in Flame-sprayed 'Nickel Aluminide' Coating

'Nickel Aluminide' coatings ('Metco 404', Metco Inc., Westbury, New York, patents pending on the material and process) are manufactured by introducing aluminium particles jacketed with nickel into the flame of a spray gun. This powder then undergoes an exothermic reaction, and as a result of this heat evolution these coatings are self-bonding to many other materials. The producers claim[1,2] that the coating, flame-sprayed according to their instructions, consists mainly of NiAl, Ni_3Al and mixtures of these two. They claim further that they also have found some 'black oxide' of aluminium in the pores and voids of the coating.

The investigation reported here has, however, produced quite another result. An electron-probe X-ray microanalysis of such a coating revealed five distinct areas: (1) nickel metal phase; (2) aluminium metal phase; (3) a phase containing aluminium 12–17 weight per cent and nickel 83–88 weight per cent; (4) a phase containing aluminium 17 weight per cent and nickel 83 weight per cent (Ni_3Al); (5) a phase consisting of aluminium 55·2 weight per cent and no nickel (Al_2O_3) (especially around the edges of the pores as white 'needles'). In the micrograph (Fig. 1) the different phases are shown.

An X-ray diffraction analysis has further shown the presence of the phases Ni_3Al, nickel and aluminium with

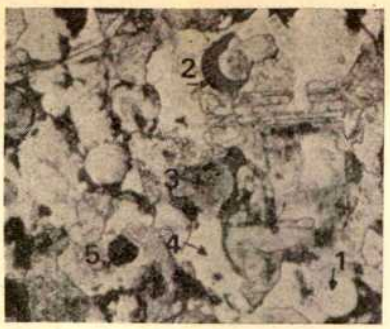


Fig. 1. 'Nickel Aluminide' coating after treatment 30 sec in 'Carapellas' reagent (×200)

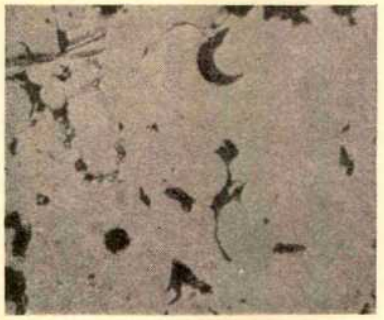


Fig. 2. 'Nickel Aluminide' coating before etching (×200)

traces of Al_2O_3 only. The lattice gratings of nickel have, however, considerably widened and lead to the result that apart from the Ni_3Al phase there exist phases consisting of a solid solution of aluminium in nickel.

It has further been claimed[1] that 'Carapellas' reagent (ferric chloride and hydrochloric acid in ethyl alcohol) attacks only NiAl and leaves Ni_3Al (see Figs. 1 and 2). In this investigation it has, however, been found that 'Carapellas' reagent attacks part of the areas with compositions up to the neighbourhood of Ni_3Al with the result that this etchant attacks phases with aluminium in solid solution in nickel but not crystallized Ni_3Al phases.

It can thus be seen that the exothermic reaction between nickel and aluminium in flame spraying guns is far from complete and gives no NiAl phase. It does, however, produce some Ni_3Al phase and also to some extent nickel phases with aluminium in solid solution to a concentration of up to 17 weight per cent.

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Detection of Magnetite as a Surface Impurity on Thin Foils of Ferrous Materials

FURSEY[1] recently reported that oxide films stripped from the surface of a specimen of mild steel immediately after electropolishing and washing were of the form Fe_3O_4. These films were shown to be barely visible and featureless when observed under high magnification in the electron microscope.

Over the past few months we have found that this oxide occurs on thin foils from a variety of low-alloyed steels and has also been observed on pure iron foils[2], but not on stainless steels. Although the oxide is not visible its presence is quite definite from the diffuse electron diffraction spot patterns that it gives. Subsequent examination of a 0·1 per cent niobium, 0·1 per cent carbon steel which was accidentally overheated in the electron microscope showed discrete particles on the surface of the ferrite matrix (Fig. 1). Diffraction analysis of these revealed the same pattern of reflexions as observed with the other steels but this time the reflexions were much sharper (Fig. 2).

We did not encounter the difficulty experienced by Fursey[1] regarding the identity of the diffracting medium. Although Fe_3O_4 and γ-Fe_2O_3 have virtually identical

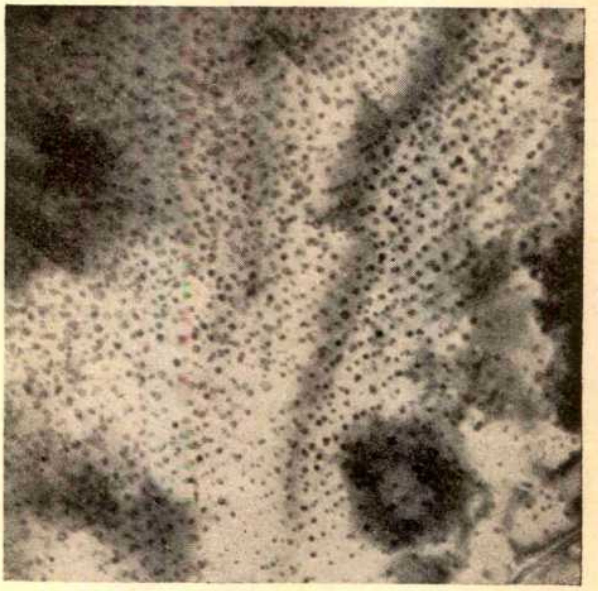
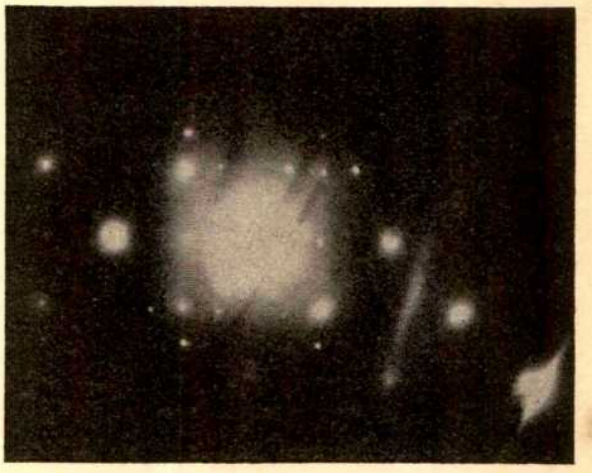


Fig. 1. Microstructure of a 0·1 per cent niobium, 0·1 per cent carbon steel after overheating in the electron microscope showing discrete oxide particles (×60,000)



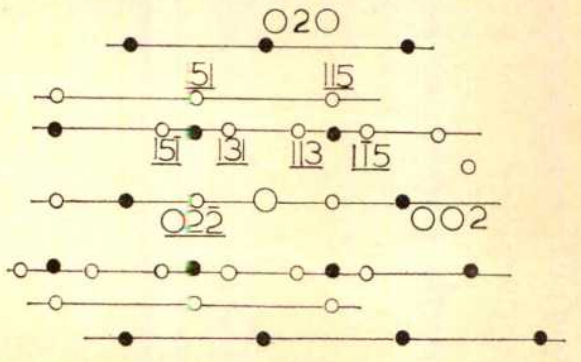


Fig. 2. Electron diffraction pattern taken from the area shown in Fig. 1. ○, *hkl*-oxide; ●, *hkl*-ferrite

lattice parameters and crystal structures, since the latter has a defect spinel structure, reflexions forbidden by the true spinel lattice will occur. Of these the {011} should be approximately 4/9 as intense as the {220} according to the *X-ray Powder Diffraction File* of the American Society for Testing and Materials[3]. Fig. 2 shows no evidence of this.

The oxide pattern comes from more than one crystallographic zone, namely $[\bar{6}11]$ and $[\bar{4}11]$. The zone axis of this is thus some 15° from the [100] zone observed by Fursey[1], and this pattern shows four of the {311} reflexions observed by her. The other four {311} reflexions are observed when the zone axis is tilted further away from the [100] so that the $[\bar{2}11]$ zone diffracts. Diffraction patterns are most commonly obtained from a range defined by the zone axes $[\bar{6}11]$ and $[\bar{2}11]$ (ref. 4).

It is likely that this oxide forms by atmospheric oxidation, the shortest exposure to air being sufficient to produce small areas. The diffuse spot patterns from it, the invisibility of it and its appearance on the overheated foil, suggest that it forms initially as a thin layer or as small disk-shaped particles, and that it will preferentially precipitate on ferrite grains which have a cube edge almost normal to the foil surface. The orientation relationship with the matrix will be published elsewhere[4]. It is reasonable to assume that the niobium steel already had a surface oxide layer before overheating in the microscope and that the effect of the heat was to cause growth of the oxide to give the discrete particles shown in Fig. 2.

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Anodic Photocurrent and the Primary Process in Electrochemical Photoeffect

Anodic photocurrents are often produced when polarized metallic electrodes covered with oxide or other semiconducting material are illuminated in solutions[1]. The primary process is here the generation of a pair electron-hole, which results from the absorption of a light quantum in the surface layer.

A different kind of anodic photocurrent has been found in the case of a dropping mercury electrode illuminated by light not absorbed in the solution. This was observed in solutions of glyoxal, diacetyl, sodium oxalate, dimethyl oxalate, oxalic acid, pyruvic acid, glycine, Cl-acetic acid and a series of α-hydroxy acids. The condition of activity is apparently the presence of a $\rangle C{=}O$ group in the molecule with a negative substituent on the neighbouring carbon atom. In solutions of compounds giving polarographic anodic currents due to formation of complexes with mercury, such as ethylenediamine tetraacetic acid or ethylenediamine, the anodic photocurrent does not appear.

In a similar fashion to cathodic photocurrents on the dropping mercury electrode[2,3], anodic photocurrents are directly proportional to the surface of the electrode and to the intensity of light; the relation between the energy of the red limit of photocurrent and the potential of the electrode is linear (Fig. 1). However, unlike their effect on cathodic photocurrents, aqueous and ethanolic solutions yield anodic photocurrents of the same order of magnitude.

Since the absorption of light in the bulk of the solution has been excluded in the present experiments and since the absorption by the metallic surface should lead to cathodic photoemission of electrons, there is only one

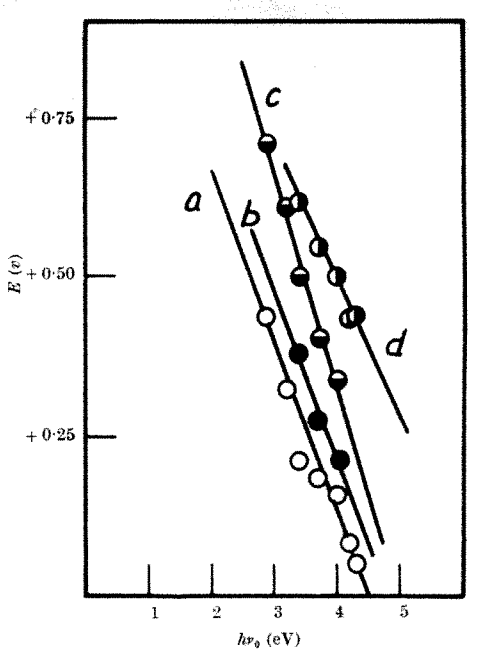


Fig. 1. Relation between the energy of red limit of photocurrent and the electrode potential in: (*a*) 0·1 M sodium tartrate in water; (*b*) 0·1 M oxalic acid in ethanol; (*c*) 1 M oxalic acid in water; (*d*) 0·1 M glycine in water. Potentials measured from the potential of zero charge

possible explanation—that the light-absorbing species responsible for the anodic photocurrent is a complex between the adsorbed organic compound and the surface of the electrode.

The mechanism of photoexcitation of such a complex can be interpreted in terms of the charge-transfer–no-bond theory applied to adsorption[4]. The electrode plays the part of an electron acceptor, the organic molecules are electron donors, and the adsorption bond corresponds to the ground state of the complex. On absorption of light quantum the electron is transferred from the adsorbed substance to the electrode. If the electron returns to the ground state, no direct current can be observed. The occurrence of a photocurrent indicates, therefore, that the complex in its excited state has undergone some secondary irreversible change. In the example given here, this change is probably an electrolytic oxidation of the dative state of the donor.

In solutions of sodium oxalate, for example, judging from the photochemical behaviour of oxalate complexes of mercury[5], it can be assumed that from the oxalate anion in the dative state the oxalate radical-anion $C_2O_4^-$ is formed which, as a strong reducing agent[6,7], is readily oxidized on the electrode to carbon dioxide, thus yielding the anodic current.

The photoexcitation of a charge-transfer complex between the electrode and an adsorbed substance might also explain the primary processes in the cathodic photocurrent in cases where direct emission of electrons is not likely to occur, as it has been observed in aqueous solutions[2,3].

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BIOCHEMISTRY

Anomalous Dispersion of the Faraday Effect in Haemoglobin and Myoglobin

THE Faraday effect, namely, the magnetic rotation of the plane of polarization of light, was discovered in 1846 (ref. 1). However, except for Shashoua's studies[2,3] the anomalous dispersion of the Faraday effect in the polyatomic molecules has not been extensively investigated. Valuable information concerning the nature of electronic levels of molecules and macromolecules, and their configuration and conformation, can, in fact, be obtained from the anomalous dispersion of the Faraday effect[4–9]. The Faraday effect is closely connected with the Zeeman effect (see, for example, ref. 9); it is well known that the Zeeman effect plays an important part in spectroscopy, in the investigation of the structure of atoms and other polyelectronic systems. There are good reasons for suggesting that the anomalous dispersion of the Faraday effect is very susceptible to any change of the molecular structure.

We used as the first substances for our investigation horse haemoglobin (Hb) and the dolphin myoglobin (Mb). The ultimate purpose of this work was to study the co-operative properties of Hb considered as the model of an allosteric enzyme. Earlier we studied the anomalous dispersion of the natural optical activity in the absorption bands of Hb, Mb and their oxygenated forms[10].

We used a spectropolarimeter with an autocollimative quartz monochromator, utilizing polarizing prisms made from Iceland spar with a layer of molten quartz. The modulation was performed in a Faraday cell with the molten quartz rod. A magnetic field strength of 11,500 gauss was used, and the path length in the substance was 100 mm. The slit width was 0·2 mm. The light source used for emission in the long wave-length region was an incandescent lamp, and in the short-wave-length region a xenon lamp. The mean standard deviation in the measured rotation angles was ± 0·001°.

The substances were studied in 0·1 M phosphate buffer, *p*H 7·0. The concentrations for the measurements in the long wave-length region varied from 0·09 to 0·16 per cent, and for the short wave-length region from 0·018 to 0·031 per cent.

In Fig. 1 are shown the curves for the anomalous dispersion of the Faraday effect together with absorption curves for Hb and oxygenated Hb (HbO_2) in the long wave-length region. In Fig. 2 analogous curves are shown for Mb and MbO_2. In Figs. 3 and 4 the same data are given for the short wave-length region—for the Soret band. (The natural optical activity[10] has already been subtracted.) Hb is characterized by a complex curve of the anomalous dispersion of the Faraday effect with two minima in the absorption band 556 mμ. It is difficult to make any definite conclusions concerning the type of the two components of this complex curve before a more

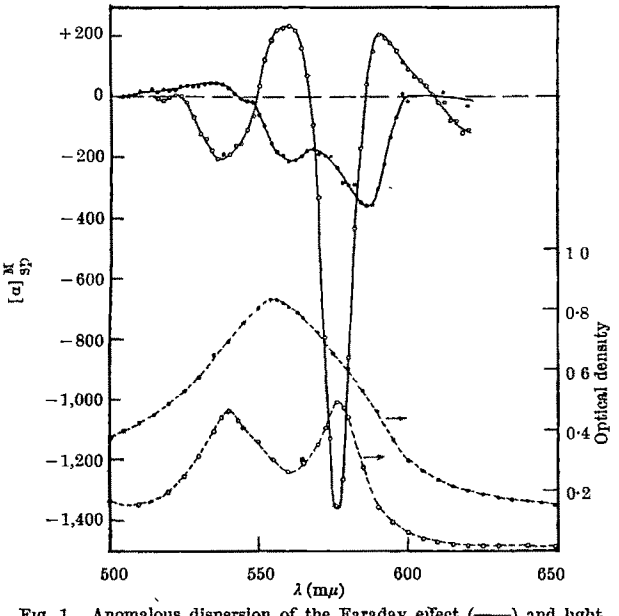


Fig. 1. Anomalous dispersion of the Faraday effect (——) and light absorption (- - - -) for Hb (●) and HbO_2 (○). $[\alpha]^{M}_{sp}$—specific magnetic rotation at 10,000 gauss

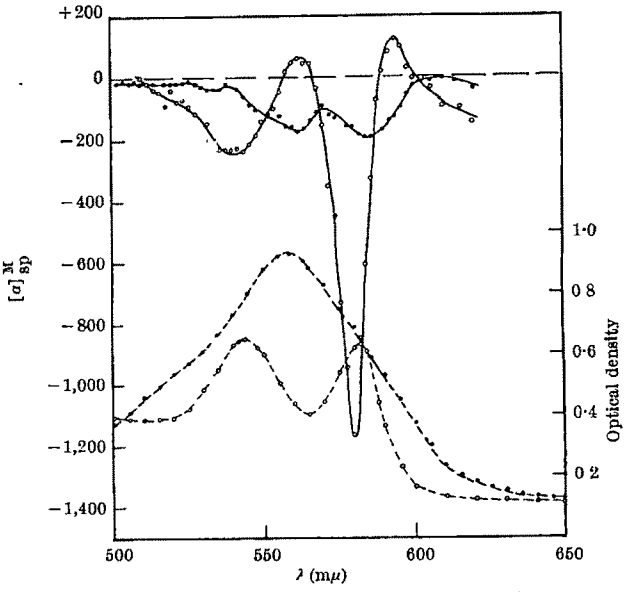


Fig. 2. Anomalous dispersion of the Faraday effect (——) and light absorption (- - - -) for Mb (●) and MbO_2 (○)

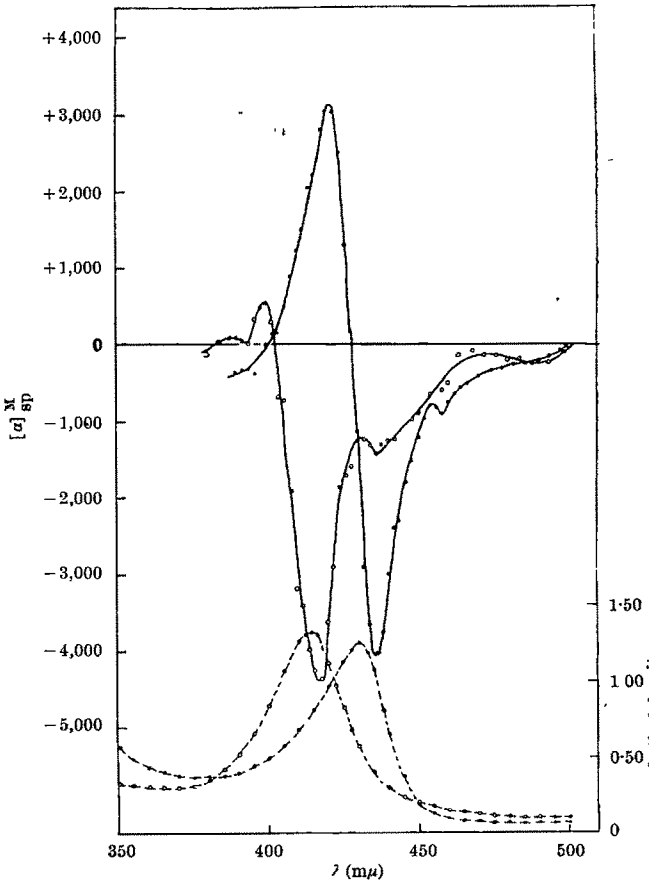


Fig. 3. The same curves as at Fig 1 but for the Soret band

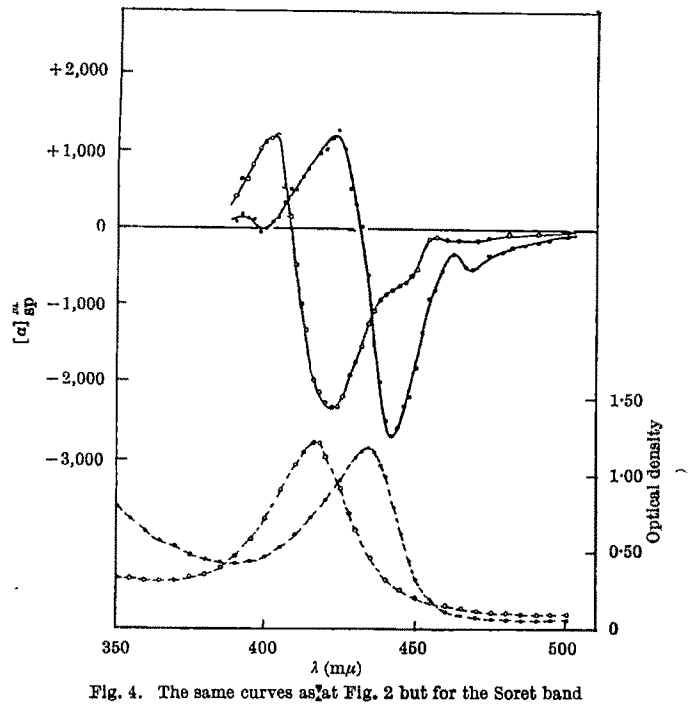


Fig. 4. The same curves as at Fig. 2 but for the Soret band

detailed analysis has been performed. However, the negative value of rotation in the whole region from 500 to 600 mμ suggests that both simple curves correspond to the symmetrical type, that is:

$$\theta_i = \frac{A_i}{(\nu_i^2 - \nu^2)^2} \qquad (1)$$

In the Soret band a much stronger effect is observed—an asymmetrical curve of anomalous dispersion of the Faraday effect corresponding to:

$$\theta_j = -\frac{|B_j|}{\nu_j^2 - \nu^2} \qquad (2)$$

The amplitude of the anomalous dispersion of the natural optical activity in this band is also much greater than in the long wave-band[10].

Without carrying out investigations on the temperature dependence of the anomalous dispersion of the Faraday effect it is difficult to say whether this kind of dispersion is determined by the paramagnetism of the molecule or by the electronic transition corresponding to the paramagnetism, independent of the temperature (the Van Vleck term in the diamagnetic susceptibility[5]). Investigations of the anomalous dispersion of the Faraday effect makes it possible, in principle, to determine the magnetic properties of the electronic levels of the molecules.

The oxygenation of Hb means the transition from the paramagnetic into the diamagnetic form. In all three absorption bands—α, β and Soret band—of HbO_8 the symmetrical curves of type (1) are observed. This is the Macaluso–Corbino effect[9,11], which corresponds to the simple Zeeman effect and therefore to the usual diamagnetism[5,9].

The curves of the anomalous dispersion of the Faraday effect for Mb and MbO_2 are qualitatively similar to the corresponding curves for Hb and HbO_8; however, well-pronounced quantitative differences are observed. It is possible that their interpretation, which will be done later, will give important information concerning the difference of the properties of the haem group included in the molecules of Mb and Hb, and MbO_2 and HbO_8.

If Mb is transformed by oxidation into the ferri-compound, the anomalous dispersion of the Faraday effect in the Soret band changes in a regular way. On one hand, the mininum near 440 mμ becomes smaller, while on the other the minimum near 410 mμ increases. In this way investigations of the anomalous dispersion of the Faraday effect make it possible to study the kinetics of the oxidization of the haem-containing protein and also of the oxygenation. Similar results were obtained by Shashoua[3], who studied cytochrome *c*.

We thus see that the anomalous dispersion of the Faraday effect is really very susceptible to changes in the molecular structure.

Further quantitative studies of the anomalous dispersion of the Faraday effect in Hb and Mb are in progress.

We thank Prof. M. I. Kornfeld and Dr. B. P. Atanasov for valuable help.

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Nature of the Free Amino-acid Pool in Avian Tissues

In an earlier report from these laboratories[1] we demonstrated significant diurnal changes in protein, nucleic and seven free amino-acids of liver during the active involvement stage of a mild Newcastle disease virus (NDV) infection in chicks. The investigations have now been extended to include a comparison of liver and serum levels of these parameters during the incubation stage of NDV.

Procedures for the care and NDV inoculation of some 400 4-week-old chicks, fed a standard diet, and biochemical determinations of the tissues were the same as those previously described[2]. Killing and tissue sampling of control and infected chicks started at the time of inoculation and continued at specific intervals over the 72-h incubation period of the NDV. Statistical analyses were those of Snedecor[3].

All values for the liver were first computed in terms of μmoles/mg DNA as an estimate of cellular levels. For comparative purposes, the sum total of the seven liver free amino-acids determined were designated the 'free amino-acid pool' and the percentage of each free amino-acid in the pool was calculated. In the case of the sera, the percentage of each free amino-acid in the total of the seven was computed from values expressed in terms of μmoles/ml.

Fig. 1 shows the data for the liver. Within and between the control and infected groups there were significant ($< 0{\cdot}05$–$0{\cdot}01$) changes in free amino-acid pool size which were related to sampling hour and to infection. In spite of these fluctuations in pool size, there was a remarkable constancy in the percentage of each of the individual components. For example, while pool size for the controls changed as much as 40 per cent, the value for lysine remained between 10 and 12 per cent of the pool. However, these slight changes were significant ($< 0{\cdot}01$). Examination of the values for individual birds showed that variability in pool size was greater between chicks within sampling periods than variability in percentage of the individual amino-acids in the fluctuating pools. It

is of interest that the two non-essential amino-acids had somewhat higher variability.

Except for the first sampling period, 7 h after NDV inoculation, pool sizes of the control and infected groups showed significant ($< 0{\cdot}05$–$0{\cdot}01$) divergence at certain sampling hours. The daily average of the pool size over the 72-h period in the controls showed no trend, whereas in the infected groups there was a linear decline.

Similar data on the free amino-acids in the sera of the control and infected chicks are shown in Fig. 2. Here again there were significant ($< 0{\cdot}01$) within-day changes in the total quantity of free amino-acids. The curve of the infected groups was slightly higher than the controls, but fluctuations in both curves were related to sampling time and not to infection. As in the liver, the percentage of the individual amino-acids in relation to the total of the seven determined in the sera was remarkably constant.

Roberts and Simonsen[4] showed that in a given healthy species at a particular stage in its development, each normal tissue has a highly specific pattern of free amino-acids. The data presented here support these findings and, in addition, indicate that in spite of wide daily fluctuations in pool size not only the pattern but the quantity of free amino-acids in the pool is very uniform.

The data are further evidence that most phases of metabolism are maintained in balance by a homeostatic mechanism(s). This would be expected unless unusual stress occurs, since the free amino-acids reflect the average of the various pools which provide the *prima materia* to the different codes governing the synthesis of proteins. However, calculation of previously reported data[2] obtained during the active involvement stage of a severe NDV infection showed that NDV can change the make-up of the free amino-acid pool(s). In this case there were significant changes in the percentages of some amino-acids in the pool(s) which correlated with an increase in degree of disease involvement and a significant depression of body-weight.

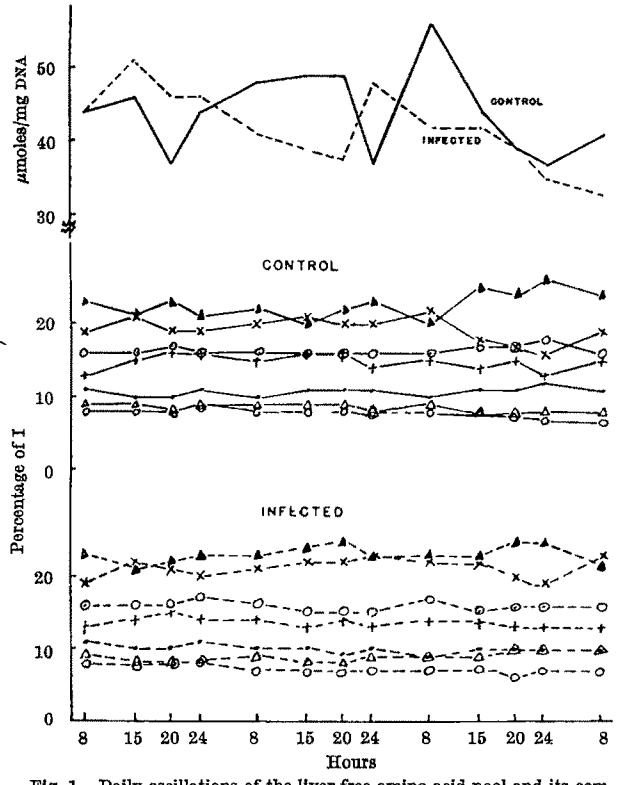


Fig. 1. Daily oscillations of the liver free amino-acid pool and its components during the incubation stage of a Newcastle disease virus infection. ▲, Alanine; △, arginine; ×, aspartic acid; O, histidine; O, leucines; ●, lysine; +, valine

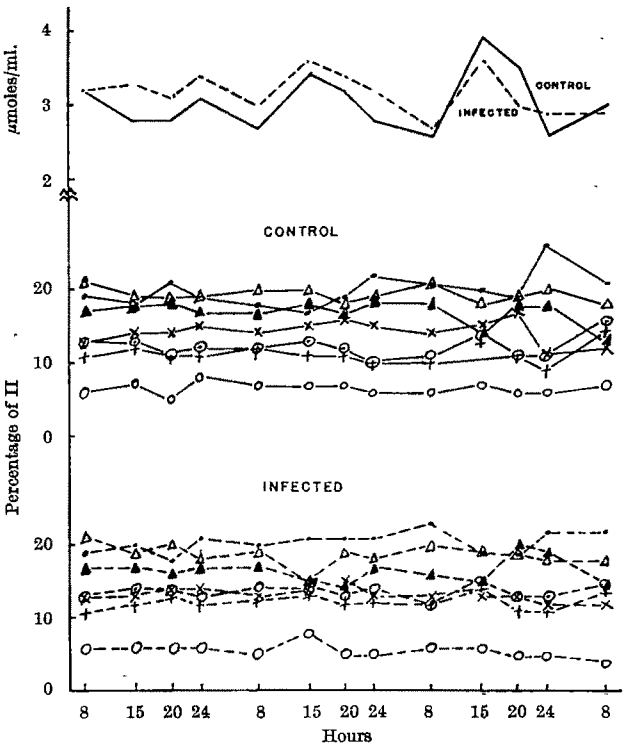


Fig. 2 Daily oscillations of total quantities and individual serum free amino-acids during the incubation stage of a Newcastle disease virus infection. ▲, Alanine; △, arginine; ×, aspartic acid; O, histidine; O, leucines; ●, lysine; +, valine

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Ubiquinone (Coenzyme Q) and the Respiratory Chain

UBIQUINONE (coenzyme *Q*, Co *Q*), a lipid soluble benzoquinone which is localized principally in mitochondria[1,2], has been shown to undergo cyclic oxidation-reduction during the oxidation of substrates of the citric acid cycle[3]. Kinetic investigations[4,5] of the redox reactions, however, have shown that its rate of reduction is insufficient to account for the total electron flux through the system. Treatment of mitochondrial particles with acetone, which extracts ubiquinone, has been claimed to result in a loss of electron transport activity which could not be restored without the addition of ubiquinone or one of its homologues[6]. These experiments showed that ubiquinone functions as an electron carrier in the respiratory chain, but while the kinetic data suggest that it has a position in a branch pathway[7], the extraction-reactivation experiments have been taken as proof of its obligatory involvement in a single sequence of electron transfer components[8]. In the course of a detailed investigation of the acetone extraction technique we have now obtained direct evidence that electron transport can occur in mitochondrial particles from which all the ubiquinone has been removed. This provides additional support for the concept

that electron transfer occurs through a branched chain system with one pathway dependent on ubiquinone and at least one other pathway in which ubiquinone does not participate.

The mitochondrial preparations used in these investigations were of the Keilin and Hartree type and were prepared from the pig heart-muscle by a method previously described[9]. In some cases the preparations were also made deficient in cytochrome *c* (ref. 10). The particles were suspended in 0·1 M Na/K phosphate buffer, *p*H 7·4, and extracted with acetone. Dry acetone was cooled to −15° and 75 ml. measured into a 100 ml. centrifuge tube. The mitochondrial particle preparation (3 ml.) was then added by drops to the acetone and the mixture stirred for 1–5 min. After centrifugation (3,000*g* for 10 min) the residue was extracted again with a further 75 ml. portion of acetone. The centrifugation and extraction were repeated and the residue was washed four times with 0·02 M Na/K phosphate buffer, *p*H 7·4. The final residue was suspended in 0·1 M Na/K phosphate, *p*H 7·4, using a Potter–Elvehjem homogenizer. Although it was found that the two extractions with acetone were sufficient to remove all the detectable ubiquinone in most experiments three extractions were made.

The complete absence of any detectable ubiquinone in these preparations was demonstrated. The lipids were extracted by the methods of Pumphrey and Redfearn[11] (including the treatment with sodium hydroxide to ensure complete dissociation of any tightly bound ubiquinone) or of Folch *et al.*[12] and then examined spectrophotometrically. The extracts were also subjected to a thin-layer chromatographic procedure[13] capable of detecting amounts of ubiquinone of the order of 0·25 μg, but in large-scale extractions the lipid extract was chromatographed on alumina before the spectrophotometric analysis and thin-layer chromatography. Although no trace of ubiquinone could be detected by any of these procedures, a small amount of a substance with absorption maximum at 276 mμ was isolated (Fig. 1). The slow disappearance of this peak on treatment with sodium borohydride and the lack of the appearance of a maximum at 290 mμ, coupled with its thin-layer chromatographic behaviour, ruled out the possibility that this substance was ubiquinone.

The reliability of the extraction and assay procedures was tested by adding to an acetone-extracted preparation an amount of ubiquinone equivalent to 0·18 μg per mg protein; it was found that more than 90 per cent of the added ubiquinone was recoverable. In a typical determination a three-times acetone-extracted preparation equivalent to 3 g of protein and containing originally 10 mg of ubiquinone was extracted but no ubiquinone was found. Since the method used was capable of detecting 0·1 per cent of the 10 mg of ubiquinone originally present and assuming that there is normally a ten-fold stoicheiometric excess of ubiquinone over the other respiratory chain components,

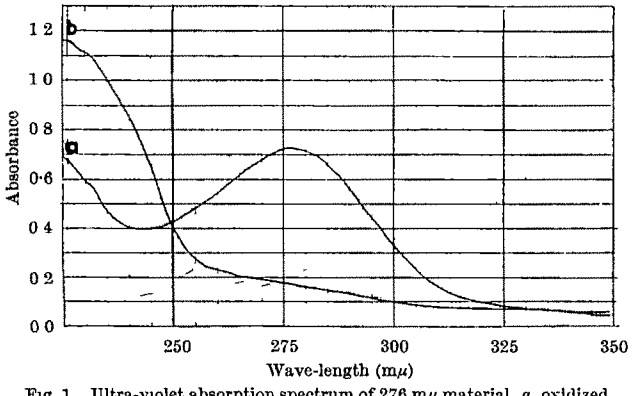


Fig 1. Ultra-violet absorption spectrum of 276 mμ material *a*, oxidized form; *b*, after reduction with sodium borohydride. Solvent, ethanol

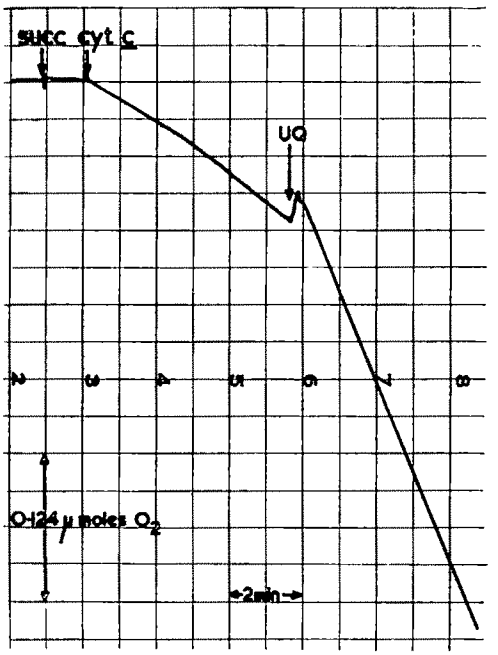


Fig. 2. Succinate oxidase activity of a three-times acetone-extracted heart-muscle preparation. Effect of addition of ubiquinone-15 and cytochrome *c*. Oxygen uptake measured polarographically with a Clark electrode at 20°

if any ubiquinone remained after acetone-extraction, it would be less than one-hundredth of the stoicheiometric equivalent amount.

Measurement of the succinate oxidase activity of the acetone-extracted preparations showed there was little or no activity. On the addition of cytochrome *c*, however, an appreciable activity was obtained (Fig. 2). The activity was further stimulated by the addition of a ubiquinone homologue such as ubiquinone-15 (*Q*-3). The oxygen uptake obtained in the presence of cytochrome *c* alone was completely sensitive to antimycin *A* and was equivalent to up to about 40 per cent of the activity after the addition of the ubiquinone homologue. These results are a clear indication that electron transport activity is proceeding in heart-muscle particles which are devoid of any detectable ubiquinone. D. E. Green and his colleagues have already shown that acetone-extraction induces a requirement for added cytochrome *c*, but in their experiments succinate oxidase activity could only be recovered by the addition of both cytochrome *c* and a ubiquinone homologue.

The demonstration that electron transfer could occur in ubiquinone-free mitochondrial particles raised the question as to which of the respiratory chain components were involved in this process. Spectrophotometric determinations indicated that both cytochrome c_1 and *b* become reduced on the addition of succinate to the acetone-extracted preparation (Fig. 3). There is, however, little or no reduction of cytochromes *a* and a_3. Addition of ubiquinone-15 to the preparation increased considerably both the rate and extent of reduction by succinate of cytochrome *b*. The results of an experiment on a three-times extracted preparation are given in Table 1. In this experiment, 13 per cent of the chemically reducible cytochrome *b* has been reduced by succinate. In the presence of ubiquinone-15 the extent of reduction increases to 26 per cent. Cytochrome c_1 appears to be much less affected by the addition of ubiquinone-15, but occasionally the rate and extent of reduction are increased. In the same experiment, the amount of cytochrome c_1 reducible by succinate in the absence and presence of added ubiquinone were 63 and 71 per cent, respectively. Cytochromes *a* and a_3 were reduced very slowly in the presence of added ubiquinone-15.

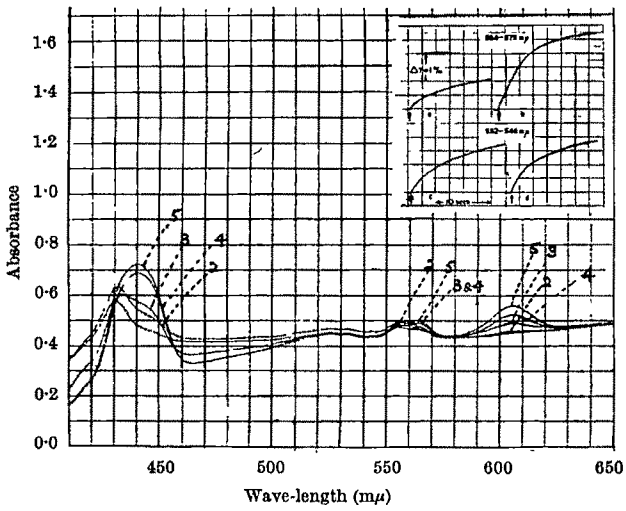


Fig. 3. Spectrophotometric determination of cytochrome reduction in a three-times acetone-extracted heart-muscle preparation. Difference spectra measured in a Unicam *S.P.* 800 recording spectrophotometer at 20°. 2, Spectrum after addition of succinate, 3, 1·25 min after addition of ubiquinone-15, 4, 6·25 min after addition of ubiquinone-15, 5, after addition of sodium dithionite. Inset shows rates of reduction of cytochrome *b* and cytochrome c_1, before (*a* and *c*) and after (*b* and *d*) addition of ubiquinone-15. Rates measured using Aminco-Chance dual wave-length spectrophotometer at 20°

Measurement of succinate–cytochrome *c* reductase activity showed that the rate of reduction of added cytochrome *c* was not the limiting step in the succinate oxidase system since the rate was higher than the overall oxygen uptake (Table 1). This suggests that the treatment with acetone has also caused some damage or disorganization in the cytochrome oxidase region of the chain.

In a recent article, D. E. Green[8] has stated that "the extraction and reactivation experiments provide unequivocal evidence for the obligatory participation of Co *Q* in the succinoxidase electron transfer chain", and he assigns a position for ubiquinone mediating electron transfer between the non-haem iron associated with the flavoproteins and the cytochrome *b* of the *b*-non-haem iron-c_1 complex. The present experiments, which have clearly indicated that electron transfer can occur in mitochondrial particles which are devoid of ubiquinone, invalidate this conclusion. It is clear from the present investigations that although ubiquinone is undoubtedly involved in the electron transfer process, it cannot be regarded as a component of a single sequence of components. In fact, there is already a considerable body of evidence from kinetic investigations[4,5] that ubiquinone is on a branched pathway of the electron transfer system. The activity obtained in the presence of cytochrome *c* alone probably represents a ubiquinone-independent pathway that is operating in the intact physiological system. It could be argued, however, that the acetone extraction has brought about an extensive structural disorganization so that artificial pathways are created. If the latter were the case, it is likely that the more drastic extraction procedure used by the Madison group would produce greater structural damage. The fact that they were unable to obtain activity with cytochrome *c* addition alone, and that no cytochrome *b* reduction could be observed in some of their extracted preparations[14], would suggest that the ubiquinone-independent pathway had been destroyed.

Table 1. Effect of Added Ubiquinone on Maximum Rates of Cytochrome Reduction and Oxygen Uptake in a Three-times Acetone-extracted Preparation

Cytochrome	Rate of reduction (μmoles/min/mg protein) In absence of Co *Q*	In presence of Co *Q*
b	0·00077	0·0019
c_1	0·0024	0·0039
c	0·08	0·26
Oxygen uptake*	0·014	0 046

The rates of reduction of cytochromes *b* and c_1 were measured in the Aminco-Chance dual wave-length spectrophotometer using the wave-length pairs shown in Fig. 3. Cytochrome *c* reduction was measured by the rate of increase of extinction at 550 mμ of added cytochrome *c*. Oxygen uptake was measured polarographically with a Clark electrode. All rates were determined at 20°.

* Expressed as μatoms oxygen/min/mg protein.

These experiments have again raised the question of the precise function of ubiquinone. The relative abundance of the quinone, its lipid solubility, the kinetics of its redox reactions and now the evidence that electron transfer can occur in its absence, suggest the possibility that ubiquinone may have another major function in addition to its role as an electron carrier.

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Collagenase Activity of Immature *Fasciola hepatica*

An essential phase in the life-cycle of many animal parasites involves extensive migration through the host tissue, and the concept of enzymatic mechanisms playing some part in this process is now generally accepted. This subject has been extensively reviewed by Lewert[1].

The mechanism of helminth migration is very similar to that following bacterial invasion, particularly *Clostridium welchii*, the toxin of which was shown[2] to contain a collagenase enzyme. The method for detecting collagenase activity developed by these authors is based on the release of bound dye from azocoll and has been applied successfully by Lewert and Lee[3] in their search for this type of enzyme in helminths, such as *Schistosoma mansoni* and *Strongyloides rattii*.

Until the present investigation a collagenase-type enzyme had not been demonstrated in the actively migrating immature fluke, although Boscardi and Coltorti[4] were able to show that the mature fluke does not possess mucinase, an enzyme sometimes associated with collagenase in helminths. However, from the evidence advanced by Dawes[5] on the tissue-feeding habits of young migrating flukes, it might be expected that these would have a need for a powerful external tissue-liquefying mechanism, which would not be necessary for the adult flukes since they are non-migratory residents of the liver bile ducts.

The need for immature flukes, in the work reported here, required a host animal from which they were readily available and, therefore, one in which they could be detected easily at a suitable stage in their development. For this reason the mouse was selected as the host, since it was found by Taylor and Parfitt[6] that this animal satisfied such requirements for short-term infections. A series of experiments was therefore performed in which mice were each infected orally with 10 metacercariae fo

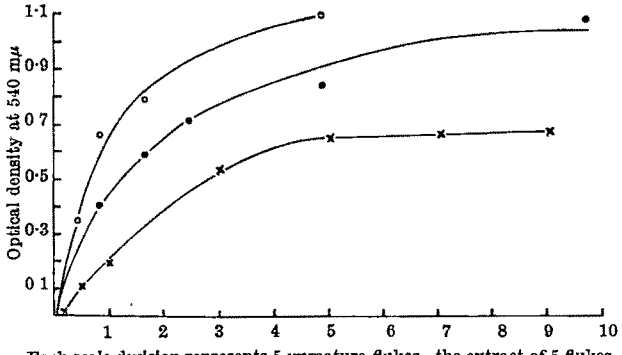
varying periods of time ranging from 14 to 28 days. It was found that with the longer period of infection mice started to die after 23 days, very few surviving for more than 26 days. After 17 days most of the flukes had reached the liver and at this stage were approximately 2–3 mm in length; this appeared to be an ideal time for removing the flukes, since a shorter period of infection resulted in a reduction in the number recoverable.

After the mice were killed the livers were removed immediately and transferred to warm saline, where they were dissected by pulling the tissue apart rather than cutting, so as to minimize damage to the flukes. All those immediately visible were removed and washed with saline and in the meantime, provided that the residual liver tissue was maintained at 37° C, any flukes which remained unexposed initially soon migrated into view.

Originally, immature flukes collected in this way were extracted in 0·1 M sodium phosphate buffer *p*H 7·4 in an M.S.E.–Mullard ultrasonic apparatus, at 0° C for a period of 4 min. After removal of the fluke debris by centrifugation, the clear supernatant was tested for collagenase activity by incubating at 37° C with the azocoll substrate, prepared according to the method described by Oakley *et al.*[2]. Experiments were later carried out in which living immature flukes were incubated with azocoll immediately after removal from the host to determine whether enzyme activity was demonstrable without any previous physical extraction. This was an attempt to decide whether or not collagenase was actually excreted by the live organism into its environment.

The amount of dye released into solution from azocoll is proportional to the amount of collagenase activity present and Fig. 1 illustrates such a relationship resulting from the action of sonic extracts from varying numbers of immature flukes and similarly by living immature flukes on azocoll. The release of approximately equivalent amounts of dye brought about by known amounts of a *Cl. histolyticum* collagenase preparation (Sigma Chemical Co.) is also shown for comparison.

These results indicate that although an increase in either the amount of fluke extract or the number of whole flukes used gave a corresponding increase in dye release, this relationship was not linear and may be due in part to the amount of substrate being a limiting factor, since 24 whole immature flukes were sufficient to completely lyse 10 mg of azocoll in 2 h. The finding that an ultrasonic extract from a corresponding number of flukes did not produce such a high level of activity may be due to direct stimulation of the secretory glands of the living fluke by the azo dye, resulting in a more active enzyme complex being secreted into solution, as compared with physical extraction. Stirewalt[7] was able to demonstrate that immersion of *Schistosoma mansoni* cercariae into 'Pelikan' india ink induced a similar secretion of material from the acetabular gland complex.



Each scale division represents 5 immature flukes, the extract of 5 flukes, or 10 μg of *Cl. histolyticum* collag enase

Fig. 1. Activity against azocoll: 10 mg azocoll in 3·8 ml. 0 1 M phosphate buffer at *p*H 7·4, incubated 3 h at 37° C. ○, Live flukes; ●, sonic extract of flukes; ×, *Cl. histolyticum* collagenase

On the other hand, the reduced level of enzymatic activity when sonic extracts were used may have been brought about by enzyme inactivation as a result of oxidation effects produced by the ultrasonic vibrations, a phenomenon which has been investigated by Santamaria *et al.*[8] with mucinase activity, and which they prevented by including reducing agents.

The demonstration of collagenase activity in immature flukes would seem to indicate the presence of an efficient enzymatic penetrating mechanism, at a stage in the life-cycle when it requires to actively burrow through tissue. From the time it emerges from a cyst in the host's intestine until it reaches the bile ducts, the fluke first penetrates the intestinal wall and finally the liver mass, a journey which is accompanied by considerable tissue damage and inflammatory reaction[9]. There is evidence that the collagenase-like enzymes of *Schistosoma mansoni* cercariae are inhibited by substances present in the host's serum[10] and whether such inhibitors are produced as a result of a definite antibody response towards the parasite or, more simply, are non-specific substances arising from tissue damage remains uncertain. However, from his observations on the invasiveness of *Fasciola hepatica* weakened by X-irradiation, Dawes[11] is inclined to the view that local tissue reactions may be directed more towards the repair of damaged tissue than against the invading parasite. Further investigations on the anti-collagenase effect of such tissue reactions might help to elucidate whether this is antibody in nature or non-specific and, in addition, may reveal any significant reduction in the collagenase activity of the immature fluke, thereby preventing, or at least retarding, its migration.

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Liquid Ion-exchange Extraction of some Physiologically Active Amines

Some long-chain alkyl phosphoric acids and long-chain alkylamines can be used as liquid ion-exchangers in a process analogous both to conventional ion-exchange with solid resins[1] and liquid-liquid extraction. The liquid ion-exchanger is dissolved in a water-immiscible liquid and partitioned with an aqueous solution of the ions to be separated. Extensive use has been made of this technique in the separation and recovery of inorganic cations, particularly heavy metal ions such as uranium.

Khim[2] has shown that nucleic acids and nucleoside phosphates can be extracted from aqueous buffer solutions by high molecular weight alkylamines in solvents such as amyl acetate and dibutyl ether. Dolinsky and Stein[3] have used a similar technique in an analytical procedure for extracting colouring matters from foods. This communication is concerned with the extraction of some organic bases from aqueous media using di-(2-ethylhexyl) phosphoric acid (DEHPA) in chloroform solution. There

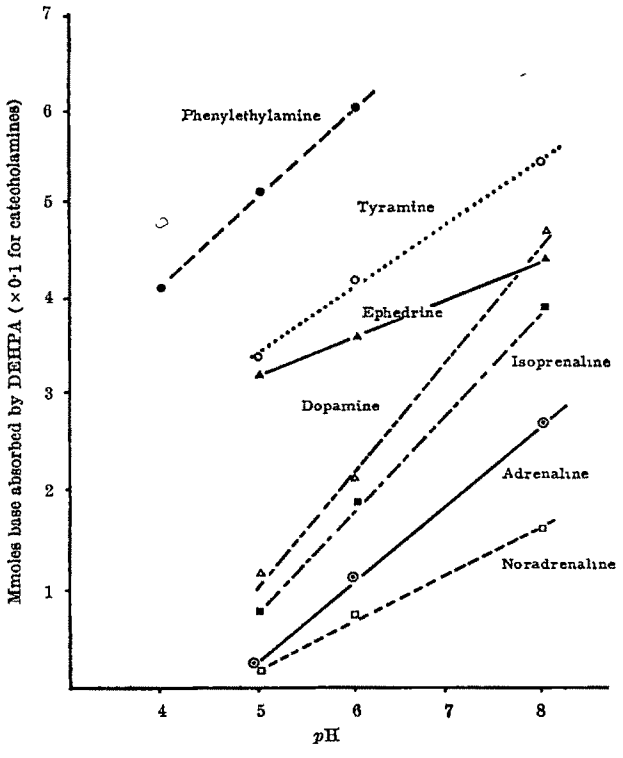


Fig. 1

appear to have been no reported separations of organic bases by this technique.

We have examined a series of physiologically active amines dissolved in citric acid/sodium phosphate buffer solutions[4] using 0·1 M DEHPA in chloroform. The results indicate that by appropriate variation of the *p*H of the buffer solution, mixtures of similar amines could be separated by the liquid ion-exchange process. The *p*H range of 4–8 was chosen to fall between the *p*K*a* values of the bases (9–11) and that of DEHPA (3·2), thus ensuring almost complete ionization of all the molecular species involved in the reaction.

The concentration of each amine in the aqueous solution before and after the ion-exchange reaction was compared by measuring the optical density at the λ_{max} for each compound. Each amine was dissolved in buffer solutions (diluted 1 : 10 to ionic strength 0·033) previously equilibrated with the DEHPA/chloroform solution, at three different *p*H values. The concentrations used were 0·1 mg/ml. for compounds having phenolic hydroxyl substituents and 1 mg/ml. for non-phenolic phenylethylamines, which produced optical densities in the range 1·0–2·0 at the λ_{max} for each amine (a Perkin–Elmer 350 ultra-violet spectrophotometer was used). An aliquot of each of these solutions, usually 5 ml., was shaken for 30 sec with an equal volume of 0·1 M DEHPA in chloroform, the phases separated by centrifuging for 5 min, and the change in concentration of the amine in the aqueous phase determined spectrophotometrically. Thus the concentration of amine extracted into the organic phase could be calculated over a range of *p*H values. The results (Fig. 1) indicate the existence of a linear relationship between the *p*H of the aqueous solution and concentration of amine extracted into the organic phase. It is possible at *p*H 5 to separate phenylethylamines, *p*-hydroxyphenylethylamines and catecholamines from each other by a very few stepwise partition reactions.

The amines can be readily converted to the aqueous phase by changing the *p*H; for example, more than 95 per cent of a sample of ephedrine was recovered from DEHPA solution by a single extraction with buffer at *p*H 2, and similarly noradrenaline at *p*H 3.

Table 1. PERCENTAGE OF BASE EXTRACTED BY DEHPA

*p*H	5	6	8
Dopamine	4	51	83
Isoprenaline	19	48	98
Adrenaline	8·5	39	89
Noradrenaline	6	24	52

The general tendency toward greater ease of extraction into DEHPA solution from catecholamines through tyramine to phenylethylamine (Fig. 1) may be explained by the possibility of intermolecular hydrogen bonding between phenolic hydroxyl and amino nitrogen groups which would sterically hinder the extraction of catecholamines and decrease the effective electron density on the amino nitrogen atom. The degree of extraction of each of the four catecholamines investigated was very *p*H-dependent, these drugs being not appreciably extracted at *p*H 5 and almost completely at *p*H 8 (Table 1).

The greater affinity for DEHPA of tyramine compared with dopamine may be further explained by the slight base-weakening effect of the meta-hydroxyl group discussed by Lewis[5] and Clarke and Perrin[6]. The addition of an alcoholic β-hydroxyl group in the side-chain reduces the basic strength of the amino group[5], which explains the relative ease of extraction of phenylethylamine (*p*K*a* 9·86) compared with ephedrine (*p*K*a* 9·49) and dopamine (*p*K*a* 10·63) compared with noradrenaline (*p*K*a* 9·78).

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PHYSIOLOGY

Responses of Thalamic Neurones to Iontophoretically Applied Drugs

CLINICAL observations concerning the role of anticholinergic drugs[1] and ventrolateral thalamotomy[2] in the treatment of Parkinson's syndrome, together with histochemical investigations of the distribution of acetylcholinesterase in the thalamus[3], particularly in those nuclei relaying ascending reticular afferent fibres, suggest a possible transmitter-function for acetylcholine (ACh) in the ventrobasal thalamic nuclei.

To examine this question five-barrelled micropipettes were used to study the responses of 1,038 cells in the thalami of eleven cats. The centre barrel was filled with 4 M sodium chloride and recorded the electrical activity of single thalamic neurones extracellularly. One of the other four barrels usually contained 0·1 N hydrochloric acid for inducing lesions[4] and the remainder contained saturated solutions of the drugs to be tested by iontophoretic release into the immediate vicinity of individual neurones.

Cats were anaesthetized with pentobarbital sodium, fixed in a stereotaxic frame and the overlying brain tissue of one cerebral hemisphere was removed by suction to expose the fornix and floor of the lateral ventricle. Localization of the thalamic recording sites was accomplished by using as markers two stereotaxically oriented micropipettes inserted just beyond the lateral-most extent of the thalamus in the frontal Horsley-Clark planes *F*11 and *F*8 (ref. 5). These pipettes were inserted to a depth of 10

mm below the surface of the fornix, were cut, and left *in situ* to serve as markers during the experiment and for subsequent histology. Serial sections of the brain between and in the plane of the markers permitted precise localization of recording sites in relation to small hydrochloric acid lesions (Fig. 1*b*) placed at critical situations along most of the 63 micropipette tracks traversing the thalamus in these experiments.

The responses of thalamic neurones to iontophoretic application of ACh, using currents ranging from 10 to 120 n.amp, are summarized in Fig. 1*a*. The number of neurones fired by ACh in this way ranged between 1 out of the 43 cells identified in the n. lateralis dorsalis (LD) and 63 out of 101 neurones in n. ventralis posteromedialis/ventralis medialis (VPM/VM). It can be seen in Fig. 1*a* that neural excitation by ACh predominantly occurs in the ventrobasal thalamic nuclei, particularly in the ventral half of n. ventralis lateralis (VL), the medial part of n. ventralis postero-lateralis (VPL), and in VPM/VM. With the cell totals observed in each nucleus (see legend to Fig. 1) differences of approximately 10 per cent are statistically significant. The latency of the ACh-induced excitation was usually less than 15 sec (Fig. 1*c*), even in the case of the least sensitive neurones in which larger currents (60–120 n.amp) were used to apply the drug. There were many cells especially in the deepest thalamic nuclei which responded with high-frequency discharge at very short latency even to the minute amount of ACh released when the backing current was switched off. ACh-excited units generally occurred in vertical chains of several sensitive cells, but not infrequently ACh-insensitive neurones were encountered so close to ACh-sensitive cells that two clearly separable unit spikes of these opposite ACh sensitivities were recorded from one site. A type of response frequently found in cells excited by ACh comprised rhythmic burst discharges—similar to those described by Andersen *et al.*[6] as characteristic of the majority of thalamo-cortical relay neurones when stimulated either ortho- or anti-dromically.

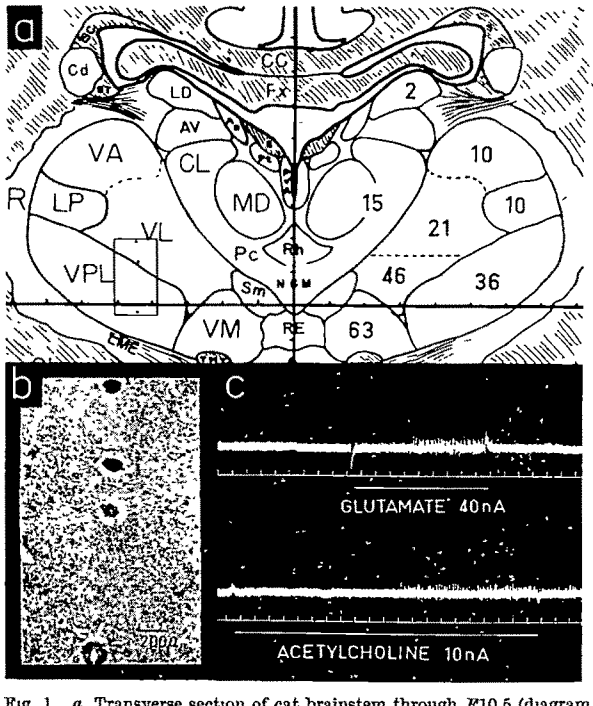


Fig. 1. *a*, Transverse section of cat brainstem through *F*10 5 (diagram and abbreviations after Jasper and Ajmone-Marsan (ref. 5)) Numbers on right-hand side are percentages of cells sensitive to ACh in various nuclei of the thalamus. Data from n centralis lateralis (CL) and n medialis dorsalis (MD) and also from n ventralis postero-medialis (VPM) and n ventralis medialis (VM) have been combined (since these last two nuclei occupy approximately corresponding ventro-medial locations through contiguous a.p. sections from *F*8 to *F*11). A broken line 1 5 mm above the Horsley-Clark zero has been used to divide the VL nucleus. Percentages are based on the following total number of cells in each case: LD–43; VA–116; LP–73, CL/MD–142; VL (above)–195, VL (below)–191; VPL–122; VPM/VM–101. *b*, Photomicrograph of VL and VPL nuclei in the region indicated by the boxed outline in *a*. The four lesions were formed by passing hydrochloric acid from the micropipette by iontophoresis. *c*, Recording of electrical activity of a unit 300μ below third lesion in *b*, showing response to 40 n.amp glutamate (upper) and 10 n amp ACh (lower) applied iontophoretically. The white line beneath unit records indicates the period for which the drug was applied. Time mark in sec

Other drugs were used in assessing the nature of the excitant action of ACh on thalamic neurones. These included carbaminoylcholine, acetyl-β-methylcholine, propionylcholine, butyrylcholine, nicotine, eserine, prostigmine, 'Tensilon', dihydro-β-erythroidine, hexamethonium, atropine and hyoscine. The most effective cholinomimetics were carbaminoylcholine and acetyl-β-methylcholine, and rather less excitation was produced by propionylcholine and butyrylcholine. Prostigmine and 'Tensilon' potentiated the effects of ACh, and in large doses they often excited. Excitation of most cells by ACh was effectively blocked by atropine or hyoscine; but dihydro-β-erythroidine was effective in preventing ACh-excitation in only six out of thirty cells tested. Hexamethonium also blocked the ACh-excitation of some cells.

From the pharmacological properties already described, most of the thalamic neurones excited by ACh seemed to be predominantly muscarinic in their responses. The few neurones which exhibited a more nicotinic response were often found to be less deeply situated in the ventrobasal thalamic nuclei.

It seems clear that a high proportion of neurones in the ventral basal thalamic nuclei VL, VPL and VPM/VM are excited by ACh. Andersen and Curtis[7] have also described the excitation of cells in the VPL nucleus by ACh and suggested that this might be related to a synaptic process.

The data presented in this report would be consistent with the presence of cholinergic synapses on cells in the ventral thalamic nuclei. Cholinergic afferent fibres may be derived from the ascending projection of cholinesterase-staining reticular fibres described by Shute and Lewis[3]. In addition, the presence of cholinergic cerebellar efferents in the thalamic nuclei is suggested by the observation that potentials evoked in the VL nucleus by stimulation of the brachium conjunctivum, which contains cholinesterase-staining fibres[8], have been abolished by atropine[9].

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Prolonged Survival of Isolated Frog Muscle and its Sensitivity to Acetylcholine

In adult muscle fibres the membrane sensitivity to acetylcholine is restricted to the neuromuscular junction and its immediate surrounding[1]; but if the muscle is deprived of its motor innervation the entire muscle fibre surface becomes sensitive to acetylcholine[2,3].

It has recently been shown[4] that mammalian muscle can survive in organ culture, and that the fibres develop the generalized chemosensitivity even when kept in a completely synthetic medium. It was desirable to find out if similar results could be obtained from frog muscle as, for many experimental purposes, frog fibres are more convenient than mammalian fibres. One important drawback is that the development of acetylcholine-sensitivity

in denervated frog muscle kept *in situ* is very slow, several weeks having to elapse before the whole fibre becomes reactive to acetylcholine[3]. Our first step, therefore, was to find if frog muscle could survive in isolation for such a long period.

Frog sartorius muscles were dissected under aseptic conditions and mounted lightly stretched on nylon holders. Immediately after mounting, the muscles were placed vertically in individual culture chambers and immersed in about 25 ml. of culture fluid. The medium used was the chemically defined *TC*-199 (Difco) diluted to 50 per cent normal strength and with cation concentrations adjusted to: Na^+ 115 mM, K^+ 2·67 mM, and Ca^{++} 1·8 mM. Antibiotics added were chloromycetin 2 mg/100 ml. or penicillin 50,000 u./100 ml. and streptomycin 50 mg/100 ml. The medium was not oxygenated or stirred, and it equilibrated with air by way of a sterile cotton wool filter. The whole fluid was changed at approximately weekly intervals to counteract the effects of evaporation. The temperature was maintained near 20° C.

Muscle fibres kept in the culture medium were examined with intracellular microelectrodes, and were found to maintain normal resting and action potentials for about 2 weeks (Fig. 1*A*). A small segment of the motor nerve was usually left attached to the muscle, in order to test the state of the nerve-muscle junction. Neuromuscular transmission ceased after approximately 8 days: *in situ* controls kept at the same temperature failed at about the same time. After 3 weeks of culture the resting potential began to decline, reaching about 75 mV 1 month after isolation. The amplitude of the action potential also declined, but it still had an 'overshoot' and many fibres propagated action potentials and gave twitches (Fig. 1*B*) even at the longest culture period attempted so far (1 month). The muscles looked normal when examined under the light microscope and showed well-preserved fine structure under the electron-microscope.

The spatial distribution of acetylcholine-sensitivity was tested by micro-iontophoretic application of the drug to

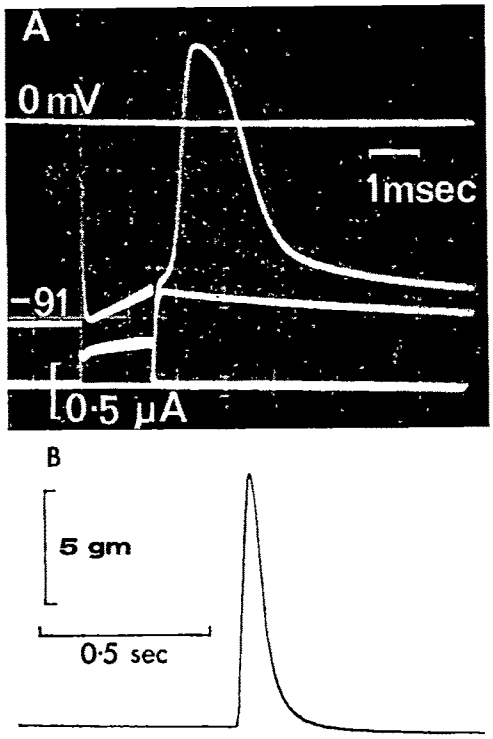


Fig. 1 Action potential and twitch recorded from frog sartorius muscle maintained in organ culture. *A*, Top trace, action potential evoked by intracellular stimulation, after 12 days of maintenance. Two pulses of different intensity were given. The first was just subthreshold, while the second evoked an action potential, bottom trace, stimulating current monitor. *B*, Muscle twitch after 27 days of maintenance

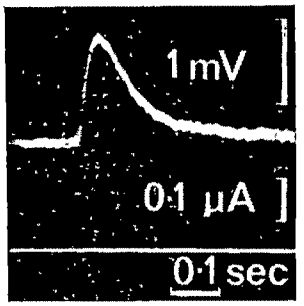


Fig. 2. Depolarization produced by micro-iontophoretic application of acetylcholine to a fibre in a muscle maintained in isolation for 27 days. The upper trace records intracellular potential while the lower trace monitors the current which flows through the acetylcholine pipette and releases the drug. Acetylcholine was applied at a point about 3 mm from the pelvic tendon, a region not normally sensitive to the drug

different parts of the fibre surface. The end-plate remains highly sensitive throughout the culture period. The regions, away from the end-plate, which normally are very insensitive to acetylcholine[5] developed the generalized sensitivity characteristic of denervated muscle. For example, in two muscles cultured for 27 days at 20° C, all the fibres tested at about 3 mm from the pelvic tendon were sensitive to acetylcholine (Fig. 2). The corresponding region had very low sensitivity in the normal control muscles. The mean sensitivity in the muscle kept in the culture medium was not very different from that of the corresponding region in denervated muscle kept *in situ* for the same time, and at the same temperature.

In contrast to the widespread distribution of acetylcholine sensitivity the cholinesterase remained visible with histochemical techniques, and had a normal distribution, being highly concentrated at the end-plate and myotendinous regions.

These experiments confirm the earlier observation[4] that muscles are intrinsically capable of developing acetylcholine sensitivity and that the induction of acetylcholine receptors is not dependent on the participation of other organs in the body.

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Effect of a 'Fast' Nerve on 'Slow' Muscle Fibres in the Frog

Kuffler *et al.*[1–3] have shown that in the frog there are two very distinct types of skeletal muscle fibres; one, the more common, is capable of generating spikes and giving twitches; while the other is not. Further differences are that fibres of the spike type are innervated by fast nerve fibres and give only a transient contraction to maintained depolarization. On the other hand, when similarly stimulated, the non-spike type of muscle fibre produces a prolonged contraction. These 'slow' muscle fibres are multiply innervated by slow nerve fibres.

The question arises whether the different characteristics are imposed on the muscle fibres by the type of nerve fibre they receive. Buller, Eccles and Eccles[4] have already shown that the contractions of slow and fast mammalian muscles alter when their fibres are cross-innervated with nerves of the opposite type. In contrast, Feng *et al.*[5] have recently reported that a fast muscle of the chick does not change its speed of contraction when innervated by the nerve of a slow muscle.

The muscle fibres in the frog sartorius are fast (twitch) fibres, while the iliofibularis contains some slow fibres mixed among the twitch ones. In the experiments reported here, the mixed iliofibularis muscle was connected to the sartorius nerve, which normally innervates only fast fibres. To accomplish this, the sartorius muscle was removed from the left leg. The iliofibularis muscle from the right leg was then dissected, together with a small segment of its nerve, and placed in the left leg instead of the removed sartorius. The proximal stump of the sartorius nerve was then joined to the distal segment of the iliofibularis nerve in the transplanted muscle. With this operative procedure the possibility of innervation by aberrant motor nerve fibres is reduced to a minimum since the only muscle nerve cut in the left leg is the small sartorius branch. The muscles were examined 6–18 months after the operation. At this time the transplanted iliofibularis muscle contracted when the sartorius nerve was stimulated, and with intracellular microelectrodes most fibres examined were found to be innervated: they responded with an end-plate potential or an action potential to stimulation of the sartorius nerve. The regenerated neuromuscular junctions showed the properties previously described[6].

The normal iliofibularis and the remaining sartorius muscle were also isolated, and together with the transplanted iliofibularis they were set up for isometric tension recording. Prolonged depolarization of the normal iliofibularis muscle, with acetylcholine, produced the usual well-maintained contraction. On the other hand, in the iliofibularis innervated by the sartorius nerve, only a transient contraction was evoked. In two cases, the time course of this contraction was similar to that obtained in the normal sartorius muscle. In four others, as in Fig. 1, the contraction in the transplanted iliofibularis was somewhat more prolonged than in the fast sartorius muscle; but still clearly shorter than that of the iliofibularis innervated by its original nerve.

The absence of maintained contraction in these experiments could be interpreted as being due to a transformation of the slow fibres into twitch-like fibres due to the influence of the fast sartorius nerve; however, it could also be that the slow muscle fibres degenerate when the iliofibularis is innervated by the sartorius nerve. This second possibility is ruled out because electron-microscopic investigations of the transplanted muscles revealed normal muscle fibres which show the lack of *M* line in the thick filaments, and the special structure of the *Z* line both characteristic of slow fibres[7–8]. It is interesting to note that these structural features were still observed even 15 months after innervating the iliofibularis with the sartorius nerve.

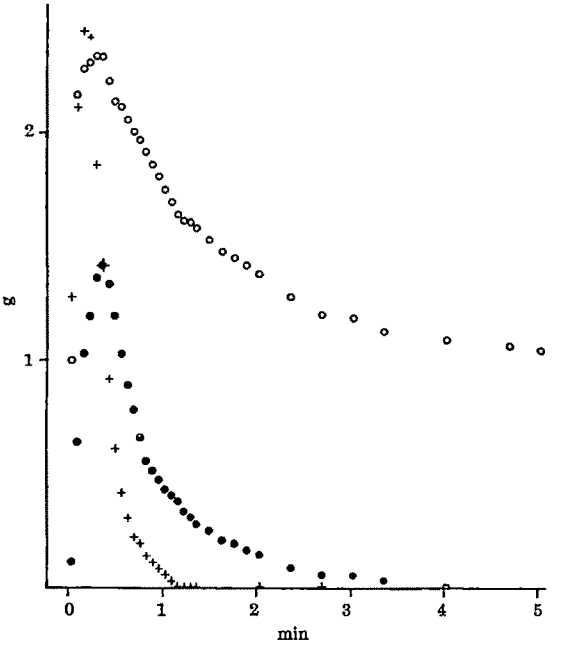


Fig. 1 Isometric contraction of transplanted iliofibularis innervated by sartorius nerve (dots); normal iliofibularis (circles), and normal sartorius (crosses). 13 months after operation done in August 1962. Abscissa: time after immersion of the muscle in a solution containing acetylcholine 2×10^{-5} g/ml. Initial tensions were 0·6 g and 0·65 g in transplanted and normal iliofibularis, respectively

In conclusion, the iliofibularis muscle can be transplanted and innervated by the fast sartorius nerve. The contraction due to prolonged action of acetylcholine is reduced in duration, but the electron-microscopical appearance of the *M* and *Z* lines in the originally 'slow' muscle fibres is not altered. Thus the ability of slow muscle fibres in maintaining tension for long periods does not seem to depend on these structural features.

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PHARMACOLOGY

Synergism of Three Antimicrobial Drugs

Combined therapy with two or more antibacterial drugs may be useful in the treatment of an individual case, and in discouraging the development of resistance[1]. A formula was previously presented which could be used in evaluating the combined effect of two drugs[2]. An organism, highly resistant to penicillin alone and streptomycin alone, is often not affected by a combination of both, when they are combined at their attainable blood levels. In this instance addition of a third antibiotic may yield an inhibitory combination at concentrations of their respective blood levels.

Experiments were carried out with *Staphylococcus aureus* type 80 and Oxford strain, 46422. Each small test-tube contained 0·2 ml. 'Trypticase Soy Broth' (Baltimore Biological Laboratory), and was inoculated with 10^5 organisms. Presence or absence of growth was recorded after 18 h incubation at 37° C. In each experiment, three antibiotics were titrated separately, and simultaneously in different combinations. Results are summarized in Table 1.

Table 1. Mean Inhibiting Doses of Single Antibiotics*

Strain	No. of experiments	Single inhibitive dose			Combined inhibitive doses	Synergism
		Pen.	Strep.	Tet.	Pen. + Strep. + Tet.	
80	30	125	992	25	8 + 8·87 + 4	4 29295
46422	20	22·5	6,125	66 67	5 88 + 11·76 + 2 94	3·25372

Pen., penicillin; Strep., streptomycin; Tet., tetracyn.
* Units (Pen.) or μg (Strep. and Tet.) per ml.

We have endeavoured to determine the smallest amount of streptomycin needed (because of its toxicity) in combination with penicillin and tetracyn (tetracycline hydrochloride) for *Staphylococcus* 80. Penicillin and tetracyn were combined at fixed concentrations, 20 per cent below their attainable blood levels, with different concentrations of streptomycin. From thirty experiments the growth inhibitive combination was found to consist of: 8 u./ml. penicillin, 8·87 μg/ml. streptomycin and 4 μg/ml. tetracyn. For strain 46422 dilutions of the blood-level concentrations of the three antibiotics were

taken. The inhibitive combination proved to be: 5·88 U./ml. penicillin, 11·76 μg/ml. streptomycin and 2·94 μg/ml. tetracyn. All three antibiotics were effective in combination at 58·8 per cent of their respective maximum blood level concentration.

In line with former notation[2] it is assumed that a result of one indicates additive action, smaller than one antagonism, and greater than one synergism. In a mixture of three drugs, the dose of drug 'one', in combination, $D_{A_{1\ \mathrm{comb.}}}$, is usually smaller than its inhibitive dose, D_{A_1}, when acting alone. Thus the expression:

$$\left(1 - \frac{D_{A_{1\ \mathrm{comb.}}}}{D_{A_1}}\right)$$

is a measure of the space which is occupied by drugs 'two' and 'three'. As there are many possible partitions of this space between the two complementing drugs, a simpler approach was followed.

The full inhibitive dose forms the basis of all calculations. The combined effect of penicillin, streptomycin and tetracyn on *Staphylococcus* 80 should serve as an illustration. This organism is inhibited by 125 U./ml. penicillin, when acting alone, or by 8 U./ml. penicillin, when acting in combination with streptomycin and tetracyn. Thus streptomycin plus tetracyn reduce the need for penicillin to 8/125 = 0·064 of the inhibitive dose; likewise, the share of streptomycin in this combination is 8·87/992 = 0·00894 of the inhibitive dose, and the share of tetracyn is 4/25 = 0·16 of the inhibitive dose. All three drugs in combination exert a full inhibition by only 0·064 + 0·00894 + 0·16 = 0·23294 of the inhibitive dose. The reciprocal of this figure, 1/0·23294 = 4·29295, indicates the efficacy of the combination.

In general, let $D_{A_1}, D_{A_2}, D_{A_3}, \ldots D_{A_n}$ be the inhibitive doses of drugs, when acting singly, and $D_{A_{1\ \mathrm{comb}}}, D_{A_{2\ \mathrm{comb.}}}, D_{A_{3\ \mathrm{comb}}}, \ldots D_{A_{n\ \mathrm{comb}}}$, when acting in combination. The action of drugs in combination is estimated from:

$$\frac{1}{\frac{D_{A_{1\ \mathrm{comb}}}}{D_{A_1}} + \frac{D_{A_{2\ \mathrm{comb}}}}{D_{A_2}} + \frac{D_{A_{3\ \mathrm{comb}}}}{D_{A_3}} + \ldots \frac{D_{A_{n\ \mathrm{comb,}}}}{D_{A_n}}}$$

The foregoing expression leads to a conservative estimate, as it is most influenced by the drug, which is reduced least in a combination; in the example given, by tetracyn. The expression also makes it possible to estimate the efficacy of a combination with any number of participating drugs.

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Pharmacology of Akinesia—Investigations on 3,4-Dimethoxyphenylethylamine

AKINESIA in Parkinson's disease is a complex phenomenon which resembles catalepsy and includes a reduction in motor activity as well as a certain degree of catatonia (maintenance of a fixed passively imposed position). Akinesia as a clinical sign may be connected with changes in the metabolism of dopamine (DA), a substance involved in the pathogenesis of Parkinson's disease[1]. Experimental investigations in animals strengthen the evidence for a relationship between catecholamines and control of motility. Fisher and Lopez Amalfara[2] found that epinephrine (E) and norepinephrine (NE) caused a motor depression, while Ernst[3] produced a striking akineto-rigid syndrome in cats with injections of two possible dopamine metabolites: 3,4-dimethoxyphenylethylamine (DIMPEA) and 4-methoxyphenylethylamine (4-MEPEA), both structurally related to mescaline (3,4,5-trimethoxyphenylethylamine). These investigations gained further significance with the identification of DIMPEA in the urine of schizophrenic patients by Friedhoff and Van Winkle[4] and others since[5]. This same compound was found by Barbeau *et al.* in high concentration in the urine of clinically akinetic parkinsonian patients[6].

In order to study the pharmacology of akinesia and its production by compounds related to dopamine, objective and quantitative tests were chosen for their ability to measure, in experimental animals, the elements of 'fixity' and of spontaneous activity. 'Fixity' was evaluated with the 'traction test': a mouse holds a horizontal bar with the forepaws, while the rest of the body is suspended by the tail. When the tail is released by the experimenter, the lower part of the body springs forward; normal animals grasp the bar with the hindlimbs within 5 sec, while paralysed, cataconic or excited animals take a longer time, or fall. Total motor activity was measured in groups of five mice with an open field actophotometer (Woodard Research Corp.) equipped with six converging light beams.

Pilot studies with intraperitoneal injections of DIMPEA (K. and K. Laboratories) in doses ranging from 2 to 200 mg/kg body-weight produced akinesia in six rats, twelve mice, eight rhesus monkeys and four rabbits. This reaction was recorded by cinematography and resembled Ernst's observations in the cat[3]. Our animals slowed down for approximately 5–10 min after the injection and hesitated to initiate a change in position, yet reacted normally to stimuli. The speed of the actual movements was reduced as well. At doses of less than 100 mg/kg the animals were never sleepy. Catatonic activity in mice, as measured with the traction test in 15 groups of twenty animals, was evident at doses approaching 80 mg/kg. This phenomenon started 15 min and lasted 60–150 min after an intraperitoneal injection. At higher doses the animals were excited, occasionally trembled and most fell off the bar before completing the test. At doses above 200 mg/kg many animals convulsed and died.

Table 1. EFFECT OF DIMPEA ON MOTILITY
(Counts/10 min)

Group	N (groups of 5 mice)	$\bar{x}$	S	$S\bar{x}$	't' Drug/placebo	P
Placebo	12	841·9	260·1	75·0	—	—
1 mg/kg	12	879 3	273·2	78 8	0·60	N.S.
10 mg/kg	12	742·0	299·2	86 4	1·32	N.S.
50 mg/kg	12	399·5	213·4	61·6	4 27	<0·01
100 mg/kg	12	289·4	220 0	62·3	6·17	<0 01

Further experiments were designed to evaluate the effect of DIMPEA on motor activity. Twelve groups of five mice were randomly injected with each of five dosage schedules. Immediately after the injection the animals were placed in the actophotometer and, 5 min later, their activity was measured for a period of 10 min. Spontaneous activity was significantly reduced ($P < 0·01$) following injections of 50 and 100 mg/kg of DIMPEA (Table 1). The decrease in activity preceded the catatonic effect. The animals were slower within 5 min but could perform the traction test up to about 15 min after the injection.

Similar investigations were carried out at the standard dose of 100 mg/kg with a number of metabolites of dopamine (Table 2). For each drug, twelve groups of five

randomly selected mice were used. Dopamine decreased the motor activity of mice at 100 and 25 mg/kg. The metabolites behaved in various ways: 3-methoxytyramine was inactive when compared with a saline placebo, while 4-MEPEA caused a depression of motor activity quantitatively similar to that caused by DIMPEA. 3,4-Dihydroxyphenylacetic acid (DIMPAC) (the presumed oxidation metabolite of DIMPEA) is inactive as measured by this test. 3,4-Dihydroxyphenylalanine (DOPA), a precursor of dopamine, also produced a significant slowing of the animals. At doses above 200 mg/kg (not shown in Table 2) the animals receiving DOPA were excited, aggressive, but moved only in slow steps. Although DOPA given alone markedly reduced the activity of mice in the actophotometer, it also effectively prevented the cataleptic and akinetic effects of DIMPEA when given 30 min previously. This phenomenon was better observed in monkeys. The animals given DIMPEA after DOPA pre-treatment could easily initiate a movement (contrary to what is observed with DIMPEA alone), yet the movements were still slow in execution.

could not perform the traction test properly, mainly because they were so excited that they fell off the bar. These animals were not akinetic. After similar time intervals, the animals injected with DIMPEA or 4-MEPEA were slow and markedly akinetic in the traction test.

Chronic injections of DIMPEA at doses of more than 10 mg/kg/day over periods extending to 6 months in rats, rabbits and monkeys elicited a persistent akinetic syndrome. Histopathological study of the six chronically injected rhesus monkeys did not reveal lesions in the liver or brain, particularly the substantia nigra, striatum and globus pallidus. A diffuse discoloration of the brain was seen with the Klüver stains. Specific colour reactions for β-phenylethylamines[7] demonstrated a diffuse penetration of such a substance into the brain presumably across the blood–brain barrier. Mescaline, which is structurally related to DIMPEA, is known to cross the blood–brain barrier in a similar diffuse manner[8]. Thus, DIMPEA and 4-MEPEA appear to cross the blood–brain barrier in greater quantity than either E, NE or

Table 2. Activity of Dopamine Derivatives
(Counts/10 min, mean ± *S.E.*)

Experiment	*A*–1	*A*–2	*B*	*C*–1	*C*–2	*D*	*E*	*F*
Placebo	549 ± 67	549 ± 67	658 ± 40	637 ± 55	637 ± 55	690 ± 45	790 ± 19	608 ± 54
DA 100 mg/kg			356 ± 43					
DA 25 mg/kg						426 ± 45		
HVA 100 mg/kg	705 ± 68							
DOPAC 100 mg/kg		632 ± 51						
3-MEPEA 100 mg/kg				549 ± 55				
4-MEPEA 100 mg/kg							264 ± 23	
DIMPEA 100 mg/kg								269 ± 46
DIMPAC 100 mg/kg					502 ± 67			
Student's '*t*'; drug/placebo	2·6	0·8	4·7	1·0	1·4	5·4	5·7	6·4
'*P*'	< 0·05	N.S.	< 0·01	N.S.	N.S.	< 0·01	< 0·01	< 0·01

N equals twelve groups of five mice per dose

Similar experiments could not be carried out with E and NE at equal doses because of their toxicity; but we confirmed that an intraperitoneal injection of 0·3 mg/kg of body-weight of these catecholamines produced a slowing of motor activity lasting 10–20 min, as previously reported. However, long-term observations in the actophotometer (Fig. 1) revealed that the action of these compounds was biphasic. After the initial depression of movements, there was a recrudescence of activity. The E- and DA-injected animals were more active after 30 min and 1 h than the placebo-injected controls. The NE-injected animals were very active at 30 min after the initial slowness, but resembled the control group at 1 h. In contrast, the animals receiving DIMPEA or 4-MEPEA remained constantly slower than the controls. At 30 min, 1, 1·5 and 3 h the animals injected with E, NE or DA

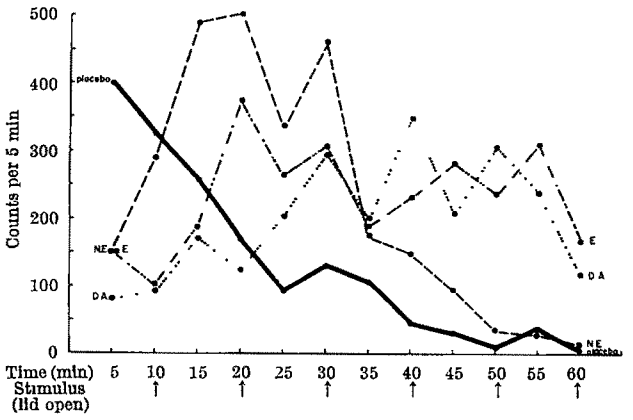


Fig. 1. Long-term actophotometer observation with catecholamines. ——, Placebo, – – – –, norepinephrine, —·—·—, epinephrine; . . . . . , dopamine

DA. The knowledge of this difference is important for the understanding of akinesia.

Indeed, akinesia, best defined as a difficulty or incapability of initiating movements, should not be equated with the so-called 'psychomotor depression' previously described with the catecholamines[2]. The actual effect of parenterally injected catecholamines on motility, as indicated by long-term observation in the actophotometer, is excitation. This is consistent with their selective penetration through the blood–brain barrier and their stimulating effect on the ergotropic systems in the hypothalamus and brain-stem reticular formation. The decrease of motor activity in the immediate post-injection period is not accompanied, in our experiments with the traction test, with 'fixity'. Probably it is related to a peripheral (non-brain) mechanism, perhaps a state of rigidity. In contrast, injections of DIMPEA or 4-MEPEA are always accompanied by both fixity and decreased motility.

If the phenomenon produced by DIMPEA and 4-MEPEA can be equated to akinesia, then akinesia would possess both a central and a peripheral component. In this light it is interesting to note that Smythies and Sykes[9] have recently shown that DIMPEA inhibits the conditioned avoidance response in rats. Motor activity measurements are thus not sufficient as a quantitative evaluation of akinesia or catatonia and, in further investigations, more attention should be directed to tests able to delineate the phenomenon of 'fixity' with its central components. It is our opinion that DIMPEA and 4-MEPEA are related to the symptom of akinesia in Parkinson's disease and that this action probably takes place through competition with the normal metabolism of dopamine in the central nervous system.

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HAEMATOLOGY

A Naturally Occurring Foetal Haemoglobin Hybrid

Recent evidence[1] has shown that abnormal haemoglobins can result from the mutation of genes controlling the synthesis of α-, β- and δ-chains in the adult as well as γ-chains in the foetus. According to this concept, it should be possible for an individual to inherit simultaneously a gene for an α- as well as a β-chain abnormality. Such a person could possess eight haemoglobins: two completely normal (Hb-A and A_2), the abnormal α-chain with a normal β-chain ($\alpha_2{}^x B_2$), the normal α-chain with the abnormal β-chain ($\alpha_2 B^x{}_2$), both abnormal chains ($\alpha_2{}^x B^x{}_2$), the abnormal α- with the normal δ-chain ($\alpha_2{}^x \delta^2$), and finally the abnormal α- with a normal γ-chain ($\alpha_2{}^x \gamma_2$). One of us[2] has already discussed the occurrence of minor haemoglobins of cord blood and the relationship of Hb-H and Bart's to each other and to Hb-F.

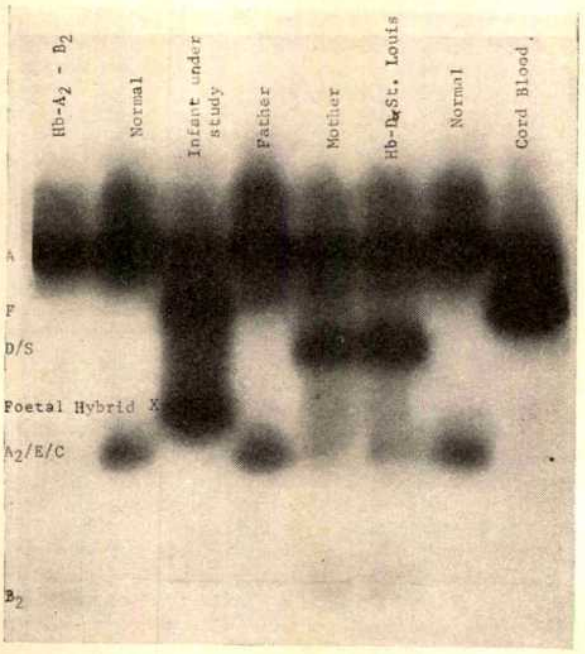


Fig. 1. Starch-gel electrophoresis of selected haemoglobin samples at *p*H 8·1 (*tris*-EDTA-borate buffer); benzidine stain

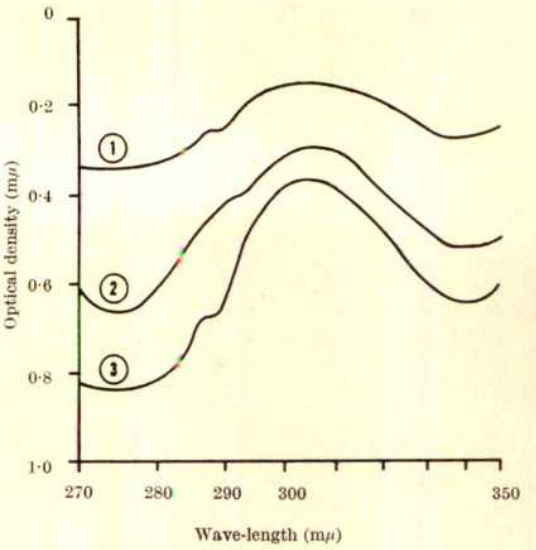


Fig. 2. Ultra-violet spectrum of eluates from DEAE cellulose chromatography. Slit width 0·1 mm, 1 cm cuvette. (1) Pure Hb-F; (2) pure Hb-A; (3) isolated slow foetal fraction *X* under discussion

We have investigated, using routine starch-gel electrophoresis, 1,373 haemoglobin samples from new-born Negro babies. Fifty-two (5 per cent) had Hb-Bart's, forty-three (3·1 per cent) had Hb-S trait, twenty-four (1·7 per cent) had Hb-C trait, while one each had Hb-Gower II, Hb-H and a slow-moving component resembling Hb-Alexandra. Less than fifty cord bloods were obtained on Caucasian babies and no abnormalities were seen. Because of the presence on starch-gel electrophoresis of a slower moving anodal component, in addition to the usual pattern F + A (Fig. 1), the following observations were made.

Comparison with known haemoglobin variants on starch gel at *p*H 8·0–8·6 (ref. 3) disclosed that this component was slower than Hb-S and only slightly faster than C, E or A_2. At *p*H 6·7 (phosphate buffer 0·1 M) this fraction moved rather like S. Hb-O (ref. 4) Alexandra[5] and the foetal component of Dα St. Louis[6] are the only variants known to occupy a position intermediate between C/A_2 and S at an alkaline *p*H. On DEAE cellulose chromatography[3], this slow-moving component occupied a position between Hb-A and S. By agar electrophoresis[6], only two spots corresponding to the positions of F and A were obtained, while a concentrate of the isolated fraction revealed only one spot at the position; such a position serves to distinguish it from Hb-D, which does not separate from Hb-A.

Foetal haemoglobin, as determined by alkali denaturation of the total haemoglobin haemolysate, was 61 per cent, approximately equal to the sum of the slow-moving fraction under investigation (17 per cent) plus the isolated pure Hb-F (42 per cent). The ultra-violet spectrum of the fraction under investigation showed the same tryptophan band at 289μ as Hb-F (Fig. 2). Quantitation of the amino-acid fractions of an acid hydrolysate by means of a five-column Technicon 'Autoanalyser' revealed an isoleucine : methionine ratio of 2 : 1.

The babies presented no clinical or haematological abnormalities. Of the parents, the father was normal, while the mother possessed a slow-moving haemoglobin shown to be Hb-D and, by further investigation, to be identical to Hb-Dα St. Louis (Fig. 1).

Hybridization[7] of the slow-moving component in the mother with isolated Hb-F resulted in the formation of a component similar to the slow-moving foetal fraction

under discussion which was thus the result of the combination of abnormal α- with normal γ-chains.

Our findings suggest that this foetal component is similar to that described by Fessas[5] as Hb-Alexandra, where only one normal parent was available for study, and with a component described by Vella *et al.*[8] in Malaya. In the latter case, the haemoglobins of both parents were said to be identical on starch-block electrophoresis although, in our experience, Hb-A_2 and B_2 may remain undetected if present in quantities less than 1 per cent. Recently, Minnich *et al.*[9] reported a foetal form of Hb-Dα St. Louis which resembles the component under discussion. However, the degree of separation of the components by starch-block electrophoresis was incomplete as shown in their illustrations. It seems probable that Hb-Alexandra, the foetal form of Hb-Dα St. Louis and our component are identical, but until all three components can be investigated in the same laboratory, a dogmatic assertion is not possible.

The tentative name Hb-Fα D St. Louis is suggested for this foetal haemoglobin in accordance with the nomenclature of abnormal haemoglobins.

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IMMUNOLOGY

Formation of Isohaemagglutinins in the Marmoset, *Tamarinus nigricollis*

The small South American primates of the Hapalidae family, the marmosets, are unique in that twin births are an almost consistent occurrence[1]. This twinning phenomenon further is unusual in that all twins appear to be fraternal, and placental vascular anastomoses result in haematopoietic chimerism of the young. Blood chimerism in adult male and female marmosets has been demonstrated by sex-chromosome analyses of cells in metaphase and also by examination of peripheral blood leucocytes for the drumstick-bearing neutrophils[2,3]. Sex-chromosome analysis of lymph node, spleen and bone marrow cells has also revealed chimerism: thus all haematopoietic tissues are involved[3]. Erythrocyte chimerism, although presumed to exist, has not yet been demonstrated owing to the unavailability of blood-grouping reagents in this species. Although previous investigations have indicated the presence of human A- and B-like antigens on the marmoset red cells, differences among marmosets of these human blood group substances are not apparent[4–6]. Tests for naturally occurring isohaemagglutinins in these animals have thus far been negative. The natural blood chimerism assumed to exist in all marmosets because of the twinning phenomenon would appear to preclude or make exceedingly difficult successful red cell isoimmunizations since both donor and recipient could conceivably have two (or more) major genetically distinct blood-group antigens. In a random-bred colony without any breeding records, therefore, the prior knowledge of chimerism in any one animal (as determined by sex-chromosome analysis) could be of considerable value. Thus, if on the basis of sex-chromosome analysis one could find an animal that is definitely a heterosexual chimera, yet in only a small proportion (10–20 per cent), its red cells would be more desirable for immunization purposes in making specific reagents than those of an animal with a relatively high degree of chimerism, or one in which the degree of chimerism is not known. This procedure would have merit only if the degree of chimerism as determined by sex-chromosome analysis of dividing white cells is also representative of the chimerism of the erythropoietic system, an assumption that must be made until evidence shows otherwise. In an analysis of the dividing white cells of the peripheral blood of twenty-six marmosets found to be heterosexual chimeras, two male animals had 12 per cent (No. 21-11-5) and 18 per cent (No. 21-1-1) chimerism of female cells, the lowest observed in our colony. These were then used as red-cell donors for isoimmunizations.

The immunization procedure was as follows: the antigen inoculum for each day consisted of 0·1 ml. unwashed packed red blood cells suspended in 0·1 ml. complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan). Six injections were given on alternate days over a period of 10 days. The first, second and fourth were given intramuscularly in the thigh, the third and fifth subcutaneously in the inguinal area, and the last intraperitoneally. Previous attempts at immunization with Freund's adjuvant injected into the footpad of the hind limbs resulted in deep ulcerations approximately 14–21 days after injection. These invariably led to death of the animals. Slight ulcerations were also observed after intramuscular injections. In the present series, one animal (26-3-2) was removed from the experiment after the third injection for use in our breeding programme and another (23-5-1) was substituted in its place. This animal (26-3-2), however, later (1 month later) developed a severe ulceration on one thigh at the site of the intramuscular injection and was killed to prevent further suffering.

The isoagglutinin responses obtained in five animals at varying intervals after the last injection are indicated in Table 1. Each serum was tested *in vitro* in saline with the specific cells used for immunization and also with the cells of a second marmoset. An excellent agglutinin response was obtained with marmoset 26-5-5 after immunization with red cells from marmoset 21-1-1. This serum also reacted strongly with cells of 21-11-5, although later bleedings showed a faster decline in antibody reactive with these cells than with the immunizing cells. Marmoset 27-4-4 became ill (from an unknown cause) 16 days after the last injection and was killed as death appeared certain. His serum contained agglutinating antibody to the specific immunizing cells only. Marmoset 26-3-2, which was removed from the experiment after only three injections of red cells, also formed specific agglutinins. Of interest was the response of marmoset 21-7-2, which, although immunized with cells of 21-1-1, failed to develop agglutinins reactive with these cells, but instead formed agglutinins reactive with cells of 21-11-5. This development would be difficult to explain except for the blood chimerism of these animals. It is possible that the 18 per cent chimeric portion of red cells of marmoset 21-1-1 has antigenic determinants common with the 88 per cent chimeric portion of the red cells of 21-11-5, and immunization of marmoset 21-7-2 may have been effected through the minor population of red cells present in donor 21-1-1. Consequently, in the *in vitro* test, the antibody formed is

Table 1. Formation of Isohaemagglutinins in Marmosets following Immunization with Marmoset Red Blood Cells

Red cell donor	Immunized recipient	Immune serum obtained on day	Reaction *in vitro* with red cells of:	Agglutinin reaction at serum dilution (log_2)*								
				1	2	3	4	5	6	7	8	9
21-1-1	26-5-5	22	21-1-1	4+	4+	4+	4±	3+	3±	2+	1±	–
		40		4+	4+	3+	3+	2+	1+	1±	–	
		70		4+	4+	3+	2+	1+	–			
		22	21-11-5	4+	4+	4+	4±	3±	3±	2+	±	
		40		4+	3+	3+	2+	1+	–			
		70		3+	3+	2+	1+	–				
	21-7-2	22	21-1-1	–	–	–	–					
		40		–	–	–	–					
		70		–	–	–	–					
		22	21-11-5	1+	–	–						
		40		3+	3+	3±	1+	–				
		70		3+	3+	2+	1+	–				
21-11-5	27-4-4†	16	21-11-5	4+	4±	4±	3+	1+	–			
			21-1-1	–	–	–	–					
	26-3-2‡	40	21-11-5	3+	3±	3±	2±	1+	–			
		50		3+	3+	3+	2+	1+	–			
		40	21-1-1	–	–	–	–					
		50		–	–	–	–					
	23-5-1	22	21-11-5	2+	1+	–						
		50		3+	2+	2+	1+	–				
		70		2+	1+	1±	–					
		22	21-1-1	–	–	–	–					
		50		–	–	–	–					
		70		–	–	–	–					

* Reactions performed at room temperature after serial dilution of serum in saline. –, Indicates no macroscopic agglutination; 4+, indicates complete agglutination of all cells.
† Animal became ill and was killed by cardiac puncture.
‡ Animal received only three injections of antigen and was killed by cardiac puncture on day 50 after development of severe ulceration at site of intramuscular injection.

evident when tested with cells of marmoset 21-11-5, since the percentage of cells having the specific antigens available for reaction would be greater. The smaller proportion of the specific antigenic cells of 21-1-1 may not have been sufficient to detect any *in vitro* agglutinating activity of the weakly reactive antibody. That there is some common antigenicity between red cells of marmosets 21-1-1 and 21-11-5 was most evident when the reaction of serum 26-5-5 was tested *in vitro* with these cells. A clearer understanding of this interpretation should be forthcoming when the blood-group relationships of the immunized recipients and cell donors become established using reagents that are being developed in this laboratory.

My investigations show that it is feasible to develop isohaemagglutinin reagents in the marmoset, even though blood chimerism exists. The rationale for the experimental immunization procedure, that is, the use of animals known to have a low degree of such chimerism, can now be extended to testing of such immune reagents *in vitro* with red cells of animals also assumed to have a low red-cell chimerism. My present efforts are directed toward such panel testing of cells from our colony of marmosets with the hope of detecting suitable antigenic differences to permit further isoimmunizations. The delineation of blood groups in these animals will subsequently permit the evaluation of the erythrocyte chimerism as well as that of the white cells. Certainly, the haematopoietic chimerism of the marmoset makes it a most interesting laboratory primate for studies in haematology, immunology and radiation effects[7].

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Immunoelectrophoretic Examination of Avian Serum Esterases

It has been recognized for some time that immunochemical properties provide a useful criterion for differentiating enzymes. When immunoelectrophoretic analysis was used in investigations of esterases in sera of birds it showed that greater variations occurred in their properties than has been revealed by simple agar electrophoresis[1–3].

Two species were more extensively investigated, namely, duck and quail, and other Gallinaceae were used for comparison; sera of individual birds were analysed after storage at either −12° C or +4° C for from 1 day to 1 year (as the esterases in the plasma were the same as in the sera, the analyses were in some cases performed as early as 1 h after bleeding).

The routine method used, based on that of Uriel[4], involved photographically recording lines of precipitation, usually after 48 h, washing in saline for 6 days, photographing again, and then drying; the esterases were then revealed by a suitable substrate and the result was recorded photographically; for the detection of proteins, the plate could be later stained with amido black. A duplicate plate was stained with Sudan black to detect the lipids present.

Using such techniques the avian sera disclosed several esterases which differed in electrophoretic localization, antigenic specificity, biochemical characteristics, behaviour during storage, and intraspecific variability.

The total number of esterase-active components detectable in a serum depends on the immunoserum (large differences have been found in the number and levels of antibodies produced between identically immunized rabbits) and on the substrate used to reveal enzyme activity (some antigen–antibody complexes preferentially hydrolyse the β-naphthyl acetate or butyrate, others the indoxyl acetate, while in simple agar electrophoresis these substrates appear to be hydrolysed to the same extent).

On the whole, six active components were observed: two β_1-globulins, one α_2- and one α_1-globulins; the remaining two esterases could not be assigned a definite rate of migration, as the localization of one of them varied from sample to sample between α_2- and α_0-regions, and the other from α_1 to a very rapid ρ (Fig. 1).

All these esterases were antigenically independent and this was demonstrated by the crossing of precipitation or activity lines when their electrophoretic localization was sufficiently close (Fig. 2). One of the β_1, the α_2–α_0 and the α_1–ρ esterases are lipoproteins (Fig. 3).

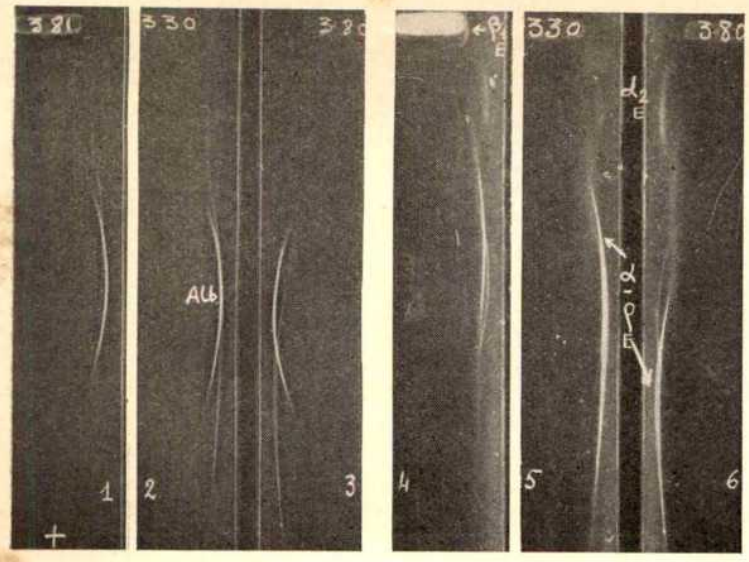


Fig. 1. Precipitation lines (1, 2, 3) and corresponding esterase staining (4, 5, 6) for quail serum. 1 and 4, Fresh sample; 2 and 5, serum stored for 8 days; 3 and 6, serum stored for 1 month

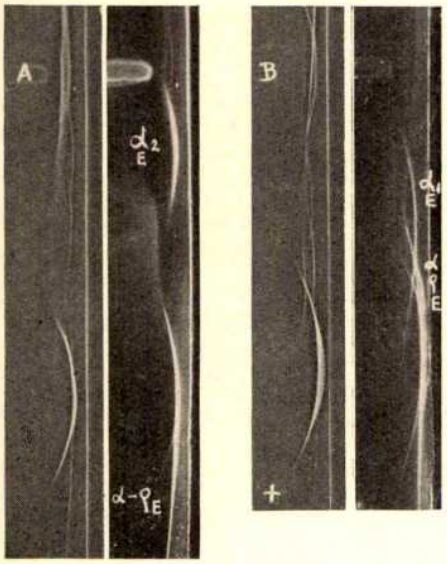


Fig. 2. Precipitation lines and corresponding esterase staining for duck serum. Serum *A* contains the α_2-esterase (note the visible precipitation line); serum *B* shows the α_1-esterase (no corresponding line can be seen) crossing the α–ρ esterase

Changes in the migration rate of the ρ lipoprotein in duck sera have already been reported[1,3,5] to be approximately proportional to the length of time it is kept in storage. The immunoelectrophoretic results confirm these observations and reveal, for fresh samples, an arc in the α_1-position (Fig. 1); in older samples the line is progressively displaced towards the anode and, in sera stored for 1 month, it is definitely faster than albumin (Fig. 1). The line is then long and straight, which indicates that the molecular charges are very heterogeneous; however, the enzymatic and lipidic properties of these samples do not appear to be significantly altered (Fig. 3).

Effects analogous to those autolytic modifications were obtained with various agents: (1) digestion of serum by porcine pancreatic lipase; (2) intravenous injection of heparin to the animal 15 min before bleeding; (3) incubation with sodium taurocholate. The biochemical alterations induced by these agents are not, however, identical, and the acceleration of the migration rate of ρ esterase by lipase or heparin is significantly enhanced by further incubation with taurocholate.

Previous results disclosed differences in the number of esterases present in sera of birds of the same species[2]. The interpretation given to these electrophoretic findings might, however, be incorrect due to the aforementioned differences in the rate of migration of the α–ρ esterase, which in fresh serum samples may mask the 'true' α-esterase or suggest its presence when it is actually absent. For this reason, use of the antigenic characters makes possible a more reliable confirmation of the presence or absence of a given component.

Taking into account the state of the serum analysed, the quality of the immunoserum and the kind of substrate used, the following esterases were found in all sera examined: (1) the non-lipo-β_1; (2) the α_2–α_0 lipo-esterase; (3) the α–ρ lipo-esterase. The three other components seem to be individual features, either as genetically determined factors or as expressions of a physiological or pathological alteration.

The esterase–antibody complexes are generally detected as precipitation lines exhibiting hydrolytic activity towards suitable substrates. With some antisera, however, the precipitation line of α–ρ does not react with the usual substrates[6], or, if it does, only very weakly; this might be due to the presence of neutralizing antibodies. On the other hand, the α_1-esterase is exclusively revealed by incubation with the substrate: its coloured arc could not be attributed to any of the visible precipitation lines nor could it be stained with amido black[5,6] (Fig. 4). One possible explanation of this finding is that the total amount of protein contained in the antigen–antibody complex is too low to form a visible precipitate or even to take up the strain. Attempts to increase this amount or to modify the antigen–antibody proportions have so far been unsuccessful.

The avian serum esterases are, finally, characterized by species-specific antigenic properties similar to other serum proteins: for example, using the anti-quail antiserum, the α–ρ esterase was detected in sera of turkey, chicken, pheasant, guinea-fowl; a weaker reaction was observed with duck and goose sera.

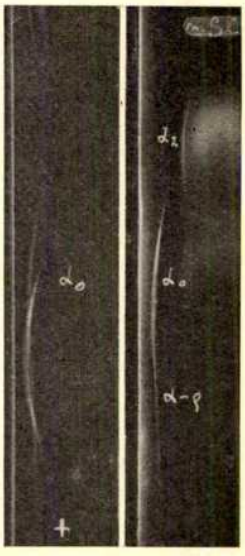


Fig. 3. Precipitation lines and lipid staining for quail serum

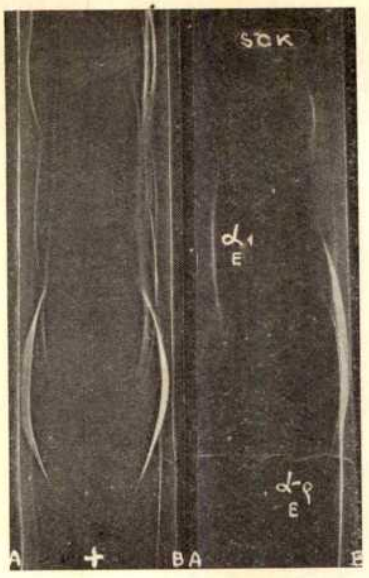


Fig. 4. Comparison of precipitation lines and esterase activity revealed by two immunosera: with *A* the α–ϱ line does not react with the substrate, the α_1 esterase gives a strong reaction; with *B* the α–ϱ line is active but the α_1-esterase is scarcely visible

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Fluorescent Antibodies in the Fluid of the Conjunctival Sac of Trachoma Patients

THE indirect fluorescent antibody technique was used to investigate the possible presence of antibodies in the lacrimal fluid or, to be more exact, the fluid of the conjunctival sac of 21 patients with active trachoma (Stage II–III), nearly all of them children, 9–14 years old. In most instances, the small quantity of fluid normally present in the conjunctival sac, or produced by slight mechanical irritation rather than freely flowing tears, was examined. The fluids, obtained with the aid of sterile pipettes used for white blood-cell count, were diluted 1 : 10 in phosphate buffered saline *p*H 7·4. Trachoma elementary body antigens were prepared and examinations were carried out by techniques previously described[1–3]. Commercial fluorescein isothiocyanate labelled anti-human γ-globulin (Microbiological Associates, Bethesda) and rhodamine were used for the purpose of staining. Fluorescence with trachoma antigen was observed with the conjunctival fluids of ten from the twenty-one cases of trachoma. The conjunctival fluids from twenty-two trachoma-free children gave negative results.

In the positive samples, elementary bodies stood out clearly with a minimum of non-specific background staining.

A number of experiments were carried out to examine if specific antibodies were involved in the reactions. Conjunctival fluids which gave a positive reaction with elementary bodies of trachoma strains failed to react with elementary bodies of psittacosis (6*BC* strain) prepared by the same technique. Heating positive conjunctival fluid for 30 min at 56° C did not abolish fluorescence. After digestion of positive conjunctival fluid with trypsin solution (twice recrystallized, 1 mg/ml. in saline phosphate buffer *p*H 7·4 kept for 150 min at 37° C) fluorescence with trachoma antigen was no longer observed.

The high degree of specificity, thermostability and sensitivity to trypsin observed with positive conjunctival fluids suggests that true antibodies were responsible for the fluorescence reactions.

In sixteen trachoma patients, antibody determinations by the indirect fluorescent method were simultaneously performed in conjunctival fluids and blood serum. Table 1 presents the results for nine positive cases and demonstrates that in some instances antibody titres were higher in the conjunctival fluids than in the serum. Blood and conjunctival fluids were obtained from these patients repeatedly during a 6-week period. Similar antibody titres were observed on each occasion. It was also found that similar antibody titres were present in the conjunctival fluids of both eyes from the same patient.

The relationship of antibodies in the conjunctival fluid to those in the serum remains to be clarified. Flemming and Allison reported that no serum proteins were present in the tears of four healthy individuals[4]. Normally, antibodies in the serum do not seem to pass into the conjunctival fluid. No fluorescent antibodies were observed in the conjunctival fluid of two rabbits hyperimmunized against trachoma and possessing serum antibodies in titres greater than 1 : 10,000, determined by the indirect method. Inflammatory processes may lead, however, to an increased permeability of the capillary walls. Thus, antibodies may appear and possibly become concentrated extravasally at the infected site. The higher titre of antibodies in the conjunctival fluids than in the blood, observed in a number of our cases, may be due to such a mechanism. The possibility of locally produced antibodies must also be considered. Large numbers of lymphocytes and plasma cells are present in the trachoma-infected conjunctiva. These cells, as well as local lymph nodes, might furnish the necessary apparatus for antibody production. It seems less probable that antibodies are produced in the lacrimal glands.

Table 1. FLUORESCENT ANTIBODIES IN CONJUNCTIVAL FLUIDS AND SERA OF TRACHOMA PATIENTS (INDIRECT TEST)

Patient No.	Conjunctival fluid	Serum
1	80	20
2	20	< 5
3	40	5
4	120	40
5	10	40
6	10	< 5
7	10	30
8	30	160
9	20	40

The practical importance of these generally low-titred antibodies in conjunctival fluids of trachoma patients remains to be demonstrated. The chronic character of the disease points to a low efficiency of the immune response. It is also known, however, that the isolation of trachoma agents from the conjunctiva of patients and the demonstration of Halberstaedter-Prowazek inclusion bodies succeed only in the early stages of the disease. The gradual elimination of the agent from the infected tissues may be due to low-titre antibodies present in the conjunctival fluids.

Preliminary results indicate that it is possible to raise the antibody titre in the serum and the conjunctival fluids of some trachoma patients by a single injection of an inactive trachoma vaccine. The effect of optically applied antigen is under investigation. It will also be of interest to examine whether antibodies can be produced in the conjunctival fluids of normal individuals and whether passage of serum antibodies into the conjunctival sac can be elicited by a local inflammatory reaction.

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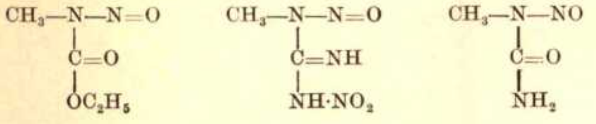
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PATHOLOGY

Carcinogenic Activity of *N*-Methyl-*N*-nitroso-*N'*-nitroguanidine

N-Methyl-*N*-nitrosourethane (MNU) (I) and its ethyl homologue have been found to be effective carcinogens, which can induce stomach, oesophageal, intestinal and lung tumours with one or a few doses[1–6]. As urethane itself is carcinogenic it seemed of interest to test the related *N*-alkyl-*N*-nitroso-*N'*-nitroguanidines, in order to assess the role of the ethoxycarbonyl-group in the carcinogenic action.



(I) (II) (III)

The commercially available crystalline compounds, *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNG) (II), *N*-ethyl-*N*-nitroso-*N'*-nitroguanidine (ENG), and *N*-methyl-*N'*-nitroguanidine (MG) were tested in rats by intragastric administration. For this purpose, the compounds, which are very sparingly soluble in water or in ethanol, were used as suspensions in 30 per cent aqueous ethanol, and were given by stomach tube to young male and female white rats, weighing 50–100 g. The number of doses varied between 3–5; each represented about 50–100 mg/kg body-weight. The doses were given at several months' intervals and amounted to 10–50 mg per rat *in toto*. The animals tolerated the treatment well; their growth rate did not significantly differ from that of control rats. All the animals were maintained under uniform conditions, and were given food (MRC diet 41*B*) and water *ad libitum*.

So far, four male and four female rats killed between 12 and 24 months after the first dose of MNG had tumours of the fore-stomach. The tumours were mostly multiple; when still small they appeared to have multicentric origin. Some formed narrow, elongated, tongue-like projections into the stomach lumen, others formed nodules containing keratin pearls in the centre. The larger tumours formed co-fluent masses of erupting keratinizing nodules covering the whole squamous part of the stomach and spreading among the muscle bundles of the stomach wall and also under the mucosa of the glandular part. One rat had numerous metastatic nodules in the peritoneal cavity (Figs. 1 and 2). Among these rats no tumours of the glandular stomach were seen, though abnormalities and hyperplasia of the glandular mucosa were present in some. Among the rats killed after similar time and having been given similar doses of *N*-ethyl-*N*-nitroso-*N'*-nitroguanidine and of *N*-methyl-*N'*-nitroguanidine no stomach tumours have as yet been seen.

The finding that *N*-methyl-*N*-nitroso-*N'*-nitroguanidine is an effective carcinogen for the rat fore-stomach provides additional evidence that the carcinogenic activity must be connected with the *N*-alkyl-*N*-nitroso- groups of this class of compound (Magee and Schoental[5]). The ethoxycarbonyl- (in the case of the urethanes, I), the amido (in the case of the very carcinogenic urea compound, III (ref. 6)) and the carbimino-amine group (in the case of guanidine, II) appear to affect the rate of interaction of the respective compounds with cell constituents. *N*-Methyl-*N*-nitroso-*N'*-nitroguanidine (II) is an effective mutagen[7,8], resembling also in this respect *N*-methyl-*N*-nitrosourethane (I) (ref. 5). *In vitro*, MNG (like MNU[9]) interacts with sulphydryl compounds at neutral *p*H, and nitrogen is evolved in the process (Schoental, unpublished results). The relatively low toxicity and lack of necrotizing action of MNG may be related to its low solubility and possibly to a slower rate of its interaction with tissues. Although nitrogen of the *N*-nitroso-group is evolved as gas in this process, it still remains to be shown whether the diazoalkanes or the 'active alkylenes' are the effective agents in the carcinogenic action of the *N*-alkyl-*N*-nitroso compounds.

The induction of squamous carcinomata in the fore-stomach of rats by intragastric administration of MNG provides additional evidence that the carcinogenic action

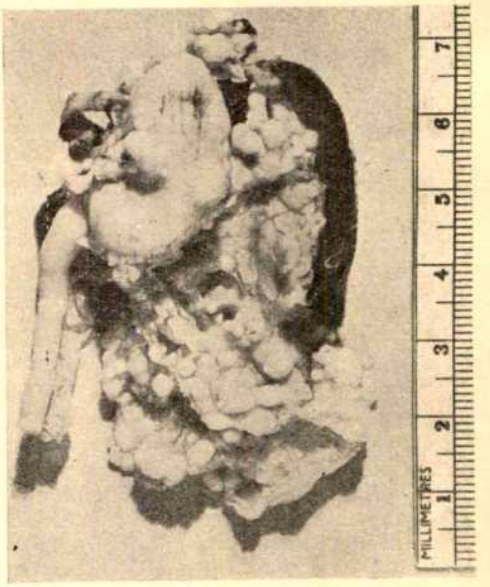


Fig. 1. Stomach of a female rat, killed 15½ months after the first of three doses of MNG (30 mg/rat *in toto*) with numerous metastatic nodules

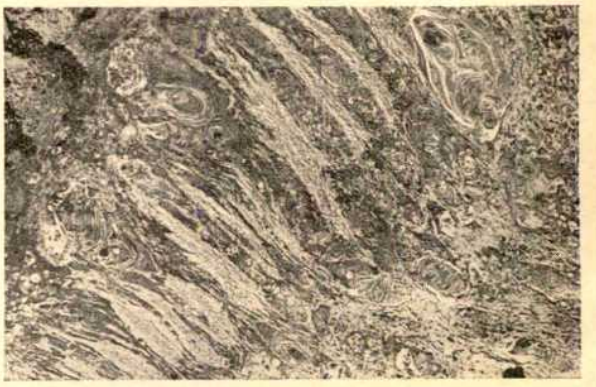


Fig. 2. Squamous carcinoma of stomach found in a female rat, killed 15½ months after the first of three doses of MNG (26 mg/rat *in toto*). (Haematoxylin and eosin, × *c.* 22)

of the *N*-alkyl-*N*-nitroso compounds is related to their alkylnitroso groups.

I thank Mr. I. Caldwell for his technical assistance.

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Lactate Dehydrogenase Isoenzymes in Malignant Tissues

PREVIOUS reports from this laboratory have shown that the electrophoretically fast lactate dehydrogenase isoenzymes, LD_1 and LD_2, of human and animal tissues exhibit high activity with 3·3 mM 2-oxobutyrate as substrate relative to that with 0·7 mM pyruvate[1,2].

Values for the ratio, $\text{activity}_{\text{2-oxobutyrate}}/\text{activity}_{\text{pyruvate}}$, approach unity in tissues such as heart muscle in which LD_1 and LD_2 predominate and fall to about 0·3 in skeletal muscle in which LD_4 and LD_5 are the principal isoenzymes. By comparison with those of normal tissues, the proportions of the slow isoenzymes LD_4 and LD_5 in certain malignant tissues have been shown by DEAE-'Sephadex' chromatography[3] and by electrophoresis on agar-gel[4] and cellulose acetate[5] to be increased. We therefore considered it of interest to determine whether such a shift in the isoenzyme pattern occurs in tumours generally and if so whether it is reflected by a change in the $\text{activity}_{\text{2-oxobutyrate}}/\text{activity}_{\text{pyruvate}}$ ratio.

Portions of tumours and of contiguous normal tissues removed at operation or at autopsy were extracted immediately or after storage at −15° overnight. The tissues were homogenized in a Potter–Elvehjem homogenizer in 10 volumes of 0·067 M Sørensen phosphate buffer at *p*H 7·4, and the homogenates were centrifuged at 5,000*g* for 10 min at 0°–5° C. Dehydrogenase activities in the supernatant fractions were then determined spectrophotometrically in a Unicam *SP*500 or a Unicam *SP*800 spectrophotometer at 25° with 0·7 mM pyruvate[6] or 3·3 mM 2-oxobutyrate[1] as substrate and 0·12 mM reduced nicotinamide-adenine dinucleotide as coenzyme.

Lactate dehydrogenase isoenzymes were separated by electrophoresis on either cellulose acetate[7] or in acrylamide-gel[8]. The isoenzymes were located by the MTT tetrazolium staining technique[9], and a quantitative assessment of the relative amounts was made by scanning cellulose acetate electropherograms with a 'Chromoscan' (Joyce, Loebl and Co., Ltd.).

The isoenzyme pattern observed in a lymph node tumour shows a marked shift towards the slow isoenzymes relative to that of a normal lymph node extract (Fig. 1). There is admittedly some doubt concerning the purity of the control specimen, which shows greatest activity at LD_3, but

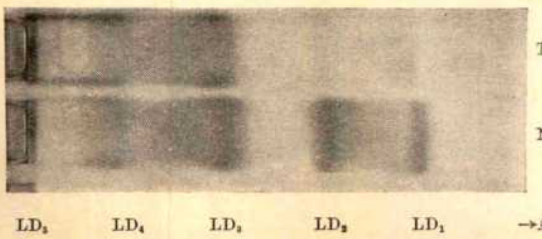


Fig. 1. Lactate dehydrogenase isoenzyme patterns of normal and neoplastic lymph node extracts separated by acrylamide gel electrophoresis

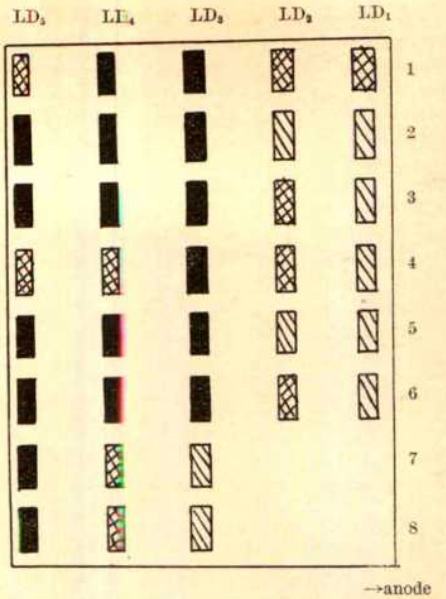


Fig. 2. Diagram of lactate dehydrogenase isoenzyme patterns of normal and neoplastic human tissues and rat tissues separated by acrylamide gel electrophoresis. 1, Normal colon; 2, neoplastic colon; 3, normal colon + neoplastic colon; 4, normal rectum; 5 and 6, rectal tumours; 7, fresh rat tumour; 8, rat tumour 10 days old

the tumour which appeared to be free from contaminating tissues showed most activity in LD_3, LD_4 and LD_5. Similar observations were made with tumours of other tissues, including breast, rectum, colon, stomach, testis and lung. Some of the isoenzyme patterns obtained are illustrated in Fig. 2.

Although visual impressions of the changes in isoenzyme distribution were supported by scanning electropherograms run on cellulose acetate foil, it must be borne in mind that several possible sources of error are inherent in the use of staining techniques. Since isoenzymes differ in their substrate affinities, certain components may function under optimal conditions while others may not be fully saturated or may even be inhibited by excess substrate. Moreover, the more active fractions may utilize all the substrate in their immediate environment and consequently tend to be somewhat under-estimated relative to the weaker fractions.

In an attempt to overcome these objections, $\text{activity}_{\text{2-oxobutyrate}}/\text{activity}_{\text{pyruvate}}$ ratios were determined and the results are given in Table 1. In every case in which it was possible to make a comparison, the tumour showed an activity ratio lower than that of the corresponding normal tissue. The differences are more pronounced when the tumours originate in tissues which normally contain a predominance of fast isoenzymes with relatively high activity ratios, but the changes are apparent even in those tissues normally characterized by moderately low activity ratios.

These quantitative investigations confirm that the shift in the isoenzyme patterns in malignant disease detected by other techniques[2–5] applies to neoplasms generally,

Table 1. LACTATE DEHYDROGENASE ACTIVITIES OF NORMAL AND MALIGNANT HUMAN TISSUES

Organ	Normal			Malignant		
	Enzyme activity (25° C) (I.U./mg N)					
	HBD*	LD†	HBD/LD	HBD	LD†	HBD/LD
Rectum	0·027	0·051	0·53	0·038	0·79	0·48
Rectum	0·058	0·117	0·50	0·072	0·182	0·40
Rectum	0·031	0·064	0·48	0·029	0·065	0·44
Colon	0·026	0·043	0·61	0·034	0·058	0·58
Colon	0·024	0·038	0·63	0·029	0·051	0·57
Colon	0·036	0·053	0·67	0·039	0·071	0·56
Breast	0·019	0·024	0·79	0·014	0·029	0·48
Breast	0·013	0·016	0·81	0·016	0·039	0·42
Stomach	0·035	0·090	0·39	0·045	0·125	0·36
Lungs	0·028	0·057	0·49	0·040	0·098	0·41
Lymph node	0·051	0·078	0·65	0·025	0·048	0·52

*Dehydrogenase activity determined with 3·3 mM 2-oxobutyrate as substrate.
†Dehydrogenase activity determined with 0·7 mM pyruvate as substrate.

but it was necessary to exclude the possibility that the change might be due to the presence of necrotic cells in the tumours. We therefore compared the lactate dehydrogenase isoenzyme patterns and activity ratios of experimental tumours (Walker 256) in the rat 4–5 days after implantation with those observed in a second series of rats 10 days after implantation, by which time the tumours had become necrotic. Similar isoenzyme patterns and similar (low) activity ratios were found in both fresh and necrotic tumours. It seems unlikely, therefore, that the presence of necrotic cells plays an important part in causing the shift in the isoenzyme pattern.

We thank Dr. D. H. Mackenzie and Dr. K. Lewin for the human tissues and Dr. D. Burston for the rat tumours used in this work. The investigation was carried out during the tenure of a World Health Organization fellowship awarded to one of us (H. P.-L.).

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Collagenolytic Activity in Gingivae of Man

Recent investigations by Gross *et al.*[1–6] indicate that substantial quantities of collagen are lost from the tail fin of tadpoles during metamorphosis. They have also noted that the back skin, gill and gut, but not muscle, notochord, kidney, gonads, heart, liver and spleen from tadpoles are able to lyse collagen gels when cultured *in vitro*. This collagenolytic activity can be accounted for at least in part by an enzyme active against native collagen which is produced by these tissues[1–6]. Collagenolytic activity has also been reported in the uterus and healing wounds[1].

Since considerable remodelling of periodontal tissues normally occurs, and since large amounts of collagen are lost during the course of periodontal diseases, we have investigated the possibility that these tissues also secrete substances active against native collagen.

More than one hundred specimens of gingivae were obtained from various periodontists who had removed the tissues for the treatment of periodontal diseases. Gingivectomy specimens were placed directly into Tyrode's solution to which had been added 100 u. penicillin/ml., 100 μg streptomycin/ml., and 100 u. 'Mycostatin'/ml. Tissues were shaken briefly, left standing at room temperature for about 30 min, and then placed in another vessel containing Tyrode's solution with antibiotics for a few minutes in order to disperse and remove micro-organisms. Tissues were then removed from the solution, diced into 1–2 mm cubes, and incubated in the Tyrode's solution plus antibiotics either on the surface of a collagen gel or without.

In order to test the ability of the tissues to lyse collagen gels, collagen was prepared from salt and acetic acid extracts of rat skins[7]. In general, collagen gels were prepared according to the method of Gross and Lapiere[2], and Lapiere and Gross[4]. Satisfactory gels were obtained with 0·25 per cent collagen in 0·4 M phosphate buffer. The collagen solutions were poured into 35 or 55 mm plastic Petri dishes to a depth of about 3 mm and incubated at 37° C until firm. The collagen gel was equilibrated with Tyrode's solution with antibiotics for 30 min and drained. Diced tissues were placed on the gel and incubated at 37° for 30 min in a moist chamber to ensure their sticking to the surface of the gel. 1 ml. (55 mm Petri dish) or 0·5 ml. (35 ml. Petri dish) Tyrode's solution with antibiotics was added to the surface of the collagen gels. The cover was sealed with silicone grease to prevent loss of carbon dioxide and resulting *p*H change during the incubation period of 72–96 h.

After 24 h, clearing around gingival tissues (indicating collagenolysis) was generally evident, and clear zones increased with time (Fig. 1). Clearing was not observed around small pieces of rat pancreas, heart, intestine and kidney incubated under comparable conditions for as long as 1 week. Substitution of Eagle's medium with added antibiotic for Tyrode's solution did not appear to affect the extent of lysis[8]. Application of crystalline trypsin to the gels did not produce visible clearing. Collagen gels were readily lysed in the region of 3-mm rings of filter paper soaked in purified collagenase from *Clostridium histolyticum* (0·5 mg/ml. 0·01 M *tris* buffer, *p*H 7·4, containing 10^{-3} calcium ions) and incubated overnight at 37° C.

Collagenolysis could be observed best in stained preparations. Van Gieson's stain and the following procedure are favoured. A few millilitres of 10 per cent formalin are placed on the tissues and the collagen gel for a few hours. The formalin is poured off, and the gels with tissues *in situ* are stained with Van Gieson's mixture (5 ml. 1 per cent acid fuchsin added to 95 ml. saturated picric acid) for a few minutes. The excess stain is rinsed off. Collagen lysis is evident as clear zones. Alternatively, the gels with attached tissues may be stained for a few minutes with an aniline blue solution (0·05 per cent aniline blue in aqueous 0·5 per cent hydrochloric acid) followed by rinsing.

Various observations indicated that cellular activity was required for collagenolytic activity; namely, (*a*) tissues frozen and thawed, and incubation under conditions which otherwise would result in clearing of the gels failed to manifest activity; (*b*) clearing of the gels was not observed in gingival cultures to which puromycin (25 μg/ml.) had been added. Similar observations have been made with tadpole tissues[1–6].

The possibility that bacteria were present in the cultured gingival tissues was examined by placing specimens exhibiting collagenolytic activity into heart-brain infusion and thioglycollate broth. No growth was observed after one week. These and previous investigations carried out by one of us (W. G.) indicate that bacterial contamination does not constitute a problem with gingival cultures prepared in this manner.

In other experiments, excised gingivae were treated with Tyrode's solution with antibiotics, diced into approximately

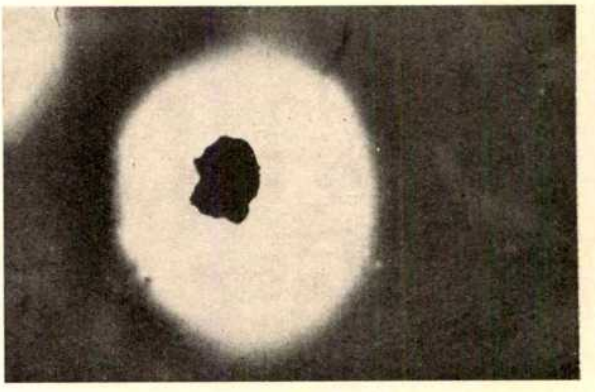


Fig. 1. 72-h gingival culture on a collagen gel afterwards fixed and stained with aniline blue. Note clear zone around incubated tissue indicating collagenolytic activity. Similar tissues frozen and thawed before culture, as well as tissues incubated with puromycin failed to manifest collagenolytic activity

2-mm cubes and cultured for 1 week in 2 ml. Tyrode's solution with antibiotics in sealed 55-mm Petri dishes at 37° C. Tissues were then separated from the culture media by centrifugation at 18,000*g*. The ability of the supernatant to reduce the viscosity of collagen solutions was studied in Ubbelohde viscometers at 20° C in 0·1 M *tris* buffer at *p*H 7·4. Calcium chloride was added to maintain the collagen in solution. Under these conditions, the viscosity of 0·25 per cent collagen solutions was reduced 14–35 per cent as compared with control solutions. This activity was precipitable with 50 per cent ammonium sulphate and largely inactivated by boiling.

These experiments indicate that diseased gingival tissues produce a heat-labile collagenolytic factor when cultured *in vitro*. Inasmuch as large amounts of collagen are lost during the course of periodontal diseases, the contingency that this factor, possibly an enzyme, is concerned with the process is intriguing.

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ANATOMY

Double Central Canal in Spinal Cord of Rat

WHILE studying the morphology of thiamine pyrophosphatase (TPPase)-positive Golgi complex in the central nervous system, it was observed that two central canals (Figs. 1 and 2) were present in the mid-thoracic region of the rat spinal cord.

Frozen cryostat sections, 10μ thick, were post-fixed in 10 per cent formalin. The central canal was in the normal position (Fig. 1). The second canal was observed a short distance in front of the grey commissure in the midline in the anterior white commissure (Figs. 1 and 2). This second canal was elongated anteroposterially similarly to the central canal at the same level (Fig. 2), and the canals were of equal length (anteroposteriorly).

When we observed some of the sections of the spinal cord above this level, it appeared at first that the central canal was greatly lengthened in an anteroposterior direction. Then we observed the separation of this lengthened canal into two parts within the grey commissure itself. Observations of additional sections in the same region revealed that the anterior segment of the separated canal gradually extended farther and farther from the central canal and was finally to be seen in the anterior white commissure. Thus the second anterior central canal originally observed in the anterior white commissure was derived from the central canal and was directly continuous with it.

The caudal part of the spinal cord was discarded before this double canal was seen and, for that reason, we do not know how it terminated—whether blindly or by rejoining the central canal. There are numerous reports of the occurrence in human beings of a double spinal cord with a central canal in each division[1], usually in association with various anomalies in the vertebral column, and with signs and symptoms of nervous system involvement. But there is no report of a double spinal canal in a normal spinal cord. The animal described in this report did not have a double spinal cord and the vertebral column was normal; there were no signs and symptoms of nervous system involvement. It would be of interest to know if similar double central canals are found in other species of animals, including man, and how commonly they occur.

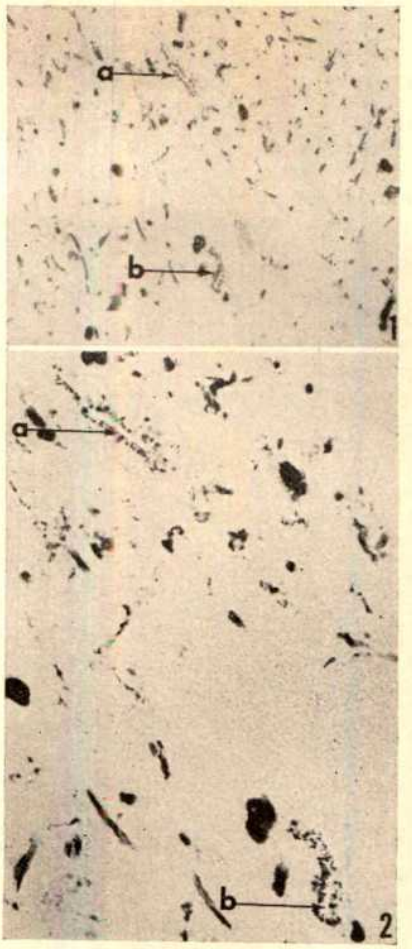


Fig. 1. Low-power photomicrograph of the spinal cord of the rat showing two central canals. One central canal (*a*) is found in the centre of the grey matter as observed in normal spinal cords. The second central canal (*b*) is found in the anterior white commissure. Note that both these canals are placed in the midline. (×*c*. 100)

Fig. 2. Higher magnification of Fig. 1 showing double central canal (*a*, *b*) more clearly. Both figures are thiamine pyrophosphatase (TPPase) preparations. Note the TPPase-positive Golgi material found at the apical borders of the ependymal cells surrounding the lumen (×*c*. 250)

It is interesting that the ependymal lining of both these central canals were cytologically similar. They had discrete granular, vesicular, and comma-shaped TPPase-positive Golgi complex, supranuclear in position. Further details on the TPPase-positive Golgi complex of the ependymal lining as compared with the choroid plexus and ciliary process will be published elsewhere[2–4].

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RADIOBIOLOGY

Factors influencing the Staining of Histones in Irradiated Rat Thymocytes

THE most striking cytological event which accompanies the death of thymic lymphocytes is the complete loss of nuclear structure (pycnosis), as seen in fixed and stained preparations or in unstained cells with phase contrast optics[1–3]. Irradiation strongly accelerates this phenomenon and structurally homogeneous nuclei begin to accumulate in thymocyte cultures between 0·5 and 1·0 h after irradiation with doses between 500 and 1,500 r. According to Whitfield *et al.*[1,2], the loss of nuclear structure is accompanied by marked changes in the localization and extractability of histones with hot trichloroacetic acid (TCA). The histones, which are normally localized in the condensed chromatin aggregates of the healthy interphase nucleus, come to be evenly distributed throughout the damaged nucleus while some escape into the cytoplasm. The histones which do remain in the nucleus become abnormally extractable with hot TCA.

Other workers[4,5] have not observed the presence of histones in the cytoplasms of either irradiated or unirradiated cells containing pycnotic nuclei. In the present communication we will show that this discrepancy is mainly due to differences in the methods of mounting stained cells for microscopic examination.

The detailed method of preparing suspensions of thymocytes from the thymus glands of 27-day-old Sprague–Dawley rats has already been described[1]. The suspensions were irradiated at 37° C with 1,500 r. of 300-kV X-rays from a Seifert 'Isovolt' X-ray machine operating at 10 m.amp. The dose was delivered at a rate of 135 r. per min. The details of the irradiation procedure have been given previously[1]. After irradiation, the cultures (contained in 25 × 150 mm tubes) were rotated about their long axes at 40 r.p.m. in an incubator at 37° C.

The media used were medium 199 (Burroughs Wellcome) and a serum–salts medium. The serum-salts medium was similar to that used by Whitfield and Rixon[3] and consisted of 50 per cent foetal calf serum and 50 per cent Krebs–Ringer solution. The *p*H of cultures in both media was maintained at 7·2 by flushing their gas phases with a mixture of 10 per cent carbon dioxide and 90 per cent air.

The optimal fixation procedure for thymocytes consisted of placing a few drops of suspension on a slide, mixing them with a drop of foetal calf serum and then drying the resulting film in a current of air heated to 37° C. The dried films were then fixed in 10 per cent neutral formalin for at least 30 min. The histones were stained by first extracting the nucleic acids from the cells with hot (100° C) 5 per cent TCA and staining in a 0·1 per cent aqueous solution of fast green FCF at *p*H 8·2 for 1 min (ref. 6). The stained preparations were then either dehydrated with alcohol and xylol and mounted in Canada balsam or they were directly mounted in the water mounting medium 'Aquamount' (made by Edward Gurr, London). Cytoplasmic histones were specifically demonstrated by incubating formalin-fixed cells for 1 h at 37° C in distilled water at *p*H 6·0 containing 300 μg of crystalline ribonuclease (Nutritional Biochemicals Corp., Cleveland, Ohio) per ml. This was followed by staining in 0·1 per cent fast green (*p*H 8·2) for 1 min and mounting in 'Aquamount'.

The reason for using hot TCA in the fast green technique is to remove the nucleic acids to which the histones are bound. Once the nucleic acids are removed, the reactive groups of the histones can then be stained with alkaline fast green. The enzyme ribonuclease should act in the same way to unmask any histones which escape from the nucleus and become bound to the cytoplasmic RNA. We have found this to be true; extraction of the formalin-fixed damaged cells with RN-Aase followed by staining with alkaline fast green and mounting in 'Aquamount' produced the same green-stained crescentic forms as did TCA-fast green procedure (Fig. 1*B*). This observation strongly supported our previous conclusion that a considerable fraction of the histones leave the radiation-damaged nucleus, enter the cytoplasm and there combine with RNA[1,2].

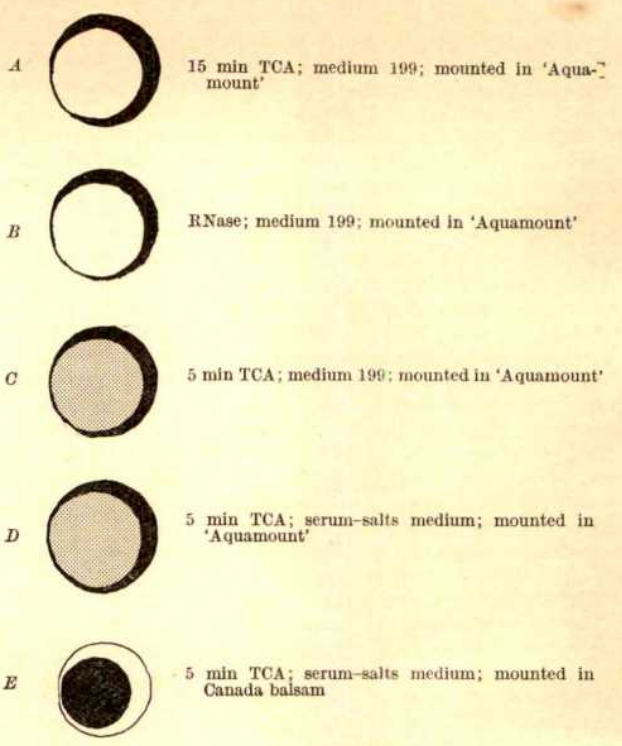


Fig. 1. Diagrammatic representations of the localization of histones in the X-irradiated rat thymocyte which has lost its nuclear structure. The effect of different mounting procedures on the cell size and histone localization can be seen. The histones were stained with alkaline fast-green (ref. 6)

When irradiated thymocytes cultivated in medium 199 were fixed in neutral formalin, extracted for 15 min with TCA and stained with fast green (at *p*H 8·2), the damaged cells with structurally homogeneous (pycnotic) nuclei always appeared as heavily stained green crescents. The homogeneous nuclei of these cells were totally unstained and the stained crescents were found to be the crescentic cytoplasmic masses which surrounded the eccentrically located nuclei[1] (Fig. 1*A*). These observations were invariably obtained with stained preparations which were mounted in 'Aquamount'. If the same stained preparations were then dehydrated with alcohol and xylol and re-mounted in Canada balsam, the cytoplasmic stain was totally extracted and the cells were shrunken. Examination of stained preparations before and after mounting in 'Aquamount' showed that this procedure caused no changes whatsoever in the histone localization of apparent cell size.

The histones in cells (both normal and damaged) cultivated in the serum–salts medium used by Rixon[5] and Whitfield and Rixon[3] were much more easily extracted by hot TCA than those in cells cultivated in medium 199. Extraction of such cells (again both normal and damaged) with TCA for 15 min rendered them totally unstainable. It was necessary to reduce the extraction time with TCA from 15 to 5 min in order to stain the cells. When such cells (extracted with TCA for 5 min) were stained and mounted in 'Aquamount', the homogeneous nuclei of the damaged cells were lightly but diffusely stained with fast green while their cytoplasms were intensely stained (Fig. 1*D*). This picture was identical to that obtained with cells cultivated in medium 199, extracted with TCA

for 5 min and mounted in 'Aquamount' (Fig. 1*C*). When these stained preparations were then dehydrated and remounted in Canada balsam, the cell diameter was reduced, the cytoplasmic stain had been totally extracted and the shrunken nucleus was intensely stained (Fig. 1*E*). This picture of the damaged cell agreed exactly with the one described by Rixon[5] and with the structure of pycnotic follicle cells of the guinea-pig described by Alfert[4].

We conclude from these observations that the optimal procedure for investigating the histone changes in radiation-damaged cells with the fast-green technique of Alfert and Geschwind[6] requires that the stained cells be mounted in a water-mounting medium such as 'Aquamount' and that dehydration with alcohol and xylol be avoided. Dehydration causes severe shrinkage of the cell and it extracts the stain from the cytoplasm. The water-soluble mounting medium, 'Aquamount', does not change the cell size or the degree of staining of any part of the cell. The shrinkage of the nucleus by dehydration is largely responsible for the impression that pycnotic nuclei are severely condensed and hence heavily stained; in reality they are not severely condensed.

Dehydration, after staining with alkaline fast-green, of other types of mammalian cells also extracts the stain from cytoplasmic structures containing basic proteins. Preliminary observations on the localization of basic proteins in fixed and stained normal *L* strain mouse fibroblasts have shown that the cytoplasmic basic proteins located in perinuclear fibrillar structures can be strongly stained with alkaline fast-green provided the stained cells are mounted in 'Aquamount'. However, if the stained cells be dehydrated with alcohol and xylol and mounted in Canada balsam, only the nuclei are stained while their cytoplasms are completely unstained.

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BIOLOGY

'Erythroblastosis Foetalis' in the Primate, *Tamarinus nigricollis*

Foetal and neonatal haemolytic anaemia, erythroblastosis foetalis, occurs naturally in man and is a major cause of human intrauterine and *post partum* death. However, it occurs spontaneously in few other species; in the equines especially after cross-breeding between horses and donkeys, and in some pigs after anti-viral immunizations[1]. It has been experimentally produced in dogs and rabbits by isoimmunization with paternal erythrocytes[1]. To our knowledge it has not previously been reported as occurring naturally or experimentally in primates other than man. The present report offers both histopathological and serological evidence suggesting isoimmunization during pregnancy in the primate, *Tamarinus nigricollis*, a New World monkey of the marmoset family.

Although this marmoset has been successfully bred in our laboratory, many pregnancies have terminated in spontaneous abortion, stillbirths and premature deliveries. These misfortunes had been attributed entirely to unknown inadequacies of the laboratory environment until after a recent delivery of a stillborn foetus and its twin that lived for 16 days. Pathological examination suggested that a disease similar to erythroblastosis foetalis was present in both. Similar histological evidence of the disease was also obtained in twins that died near term *in utero* after the traumatic death of their mother. Unlike the disease in man[2], foetal hydrops and icterus neonatorum were not evident but severe hepatic haemosiderosis was present, as shown by positive Prussian blue staining of the hepatic parenchyma (Fig. 1*A*). Although small foci of extramedullary erythropoiesis are not uncommon in late embryonic and foetal life, these animals showed many enormous erythroblastic islands in distended hepatic sinuses (Fig. 1*B*) and dilated blood vessels of the placental trabeculae (Fig. 1*C*). The placentae of single-born young that have survived do not show these foci (Fig. 1*D*). The young with the disease show smaller erythroblastic foci in the adrenal glands, myocardium and lungs. The bone marrow did not show generalized erythropoietic hyperplasia, but focal areas were seen. The fact that the pulmonary capillaries of the marmoset are not as rich in erythroblasts as are those of the human with erythroblastosis may be due to the more effective filtration of the blood by the double placental circulation shared by these chimeric twins[3]. As in the human disease few other organs show any effects, there being typically only parenchymal hepatic atrophy, and lymphoid degeneration and atrophy in the spleen, thymus and lymph nodes.

Independent serological investigations in both deliveries served to substantiate the pathological interpretation of haemolytic disease in these animals. Thus, in the first case, following the death of the second twin, the serum of the mother was tested for antibody to the paternal cells. As shown in Table 1, both saline and Coombs reactive antibodies were found, the latter having a $\log_2$ titre of 9. In the second case, the cells of both foetuses showed a Coombs positive reaction on direct test, one showing a definite 1 + agglutination, the other a 2 + reaction. The maternal serum also yielded a positive Coombs reaction when tested with the paternal cells. This agglutination, however, as shown in Table 1, was only weakly positive (1 +) over a series of serum dilutions. In view of the blood chimerism known to exist in these

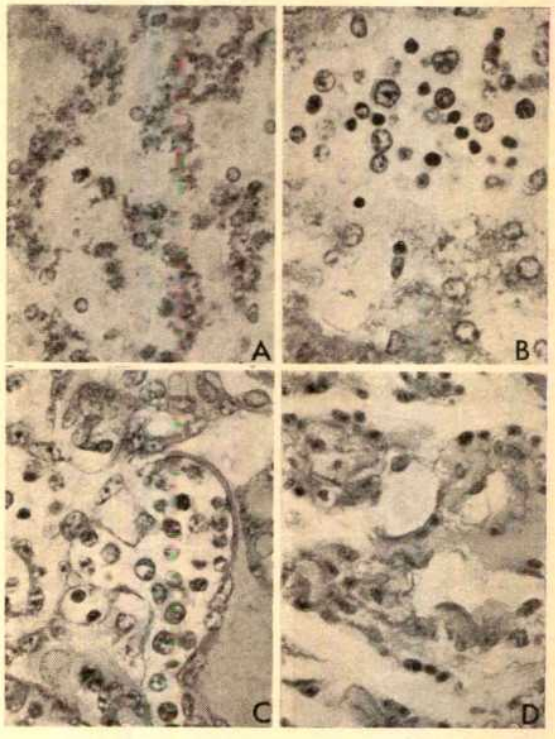


Fig. 1. Photomicrographs showing: *A*, haemosiderin granules revealed by the Prussian blue reaction in hepatic parenchyma in a marmoset which died 16 days *post partum*; *B*, an intralobular hepatic sinusoid distended with erythroblasts in various stages of maturation in a marmoset which died *in utero* at term; *C*, a placental vessel occluded by an erythroblastic focus in the same animal; and *D*, the appearance of normal placental vessels in a surviving single birth (× 450)

Table 1. Detection of Maternal Antibodies to Paternal Cells in Two Cases of Haemolytic Disease of the New-born

	Serum dilution ($\log_2$)								
Agglutinin type:	2	3	4	5	6	7	8	9	10
Case 1									
Saline	3+	3+	3+	1+	–	–	–		
Incomplete*	4+	3+	3+	3+	2+	2+	2±	1+	–
Case 2									
Saline	–	–	–						
Incomplete	1+	1+	1+	1+	1±	±	–	–	–

* Detected by Coombs test with rabbit-anti-marmoset immune serum.

animals, this finding may be interpreted as being due to the existence of two genetically different populations of red cells in the father, only one of which is reacting with the maternal antibody. This interpretation may also apply to the reactions observed with the direct Coombs test of the foetal cells noted here.

The present studies also indicate the existence of strong antigenic blood group differences in the marmoset, confirming an earlier report by Gengozian on successful isoimmunizations in these animals[4]. Whether the blood group antigen(s) responsible for the haemolytic disease of the new-born is similar or related to the Rh–Hr system of humans[5] is not known, although previous attempts to detect such factors on marmoset red cells have failed. Only the presence of human A- and B-like antigens on their cells has been established thus far[6–8]. In any event, the delineation of isoimmunization during pregnancy in these animals indicates antigenic determinants on their red cells capable of reacting in a manner analogous to the Rh_0 factor of humans. As in humans, the transmission of maternal antibody to the foetus occurs *in utero*, although a significant effect may be exerted through the colostrum following birth. This role of colostrum is suggested by the death of the infant 16 days *post partum*, where pathological findings suggest that the severe haemolytic process in this animal continued after birth (Fig. 1*A*).

The natural occurrence of haemolytic disease of the new-born in this species is of particular interest in view of the nature of twinning among these primates. Thus, the marmosets are unique in that (*a*) fraternal twin births are an almost consistent occurrence[3,9], and (*b*) placental vascular anastomoses result in foetal haematopoietic chimerism[10,11]. The trabecular rather than villous structure of these placentae also affords many areas of intimate contact of maternal and foetal tissues that could potentiate isoimmunization of the mother. This anatomical structure combined with consistent fraternal twinning and blood chimerism would seem to improve the chances of this disease occurring naturally. This primate, therefore, would appear to offer many experimental approaches to the study of the prevention and cure of this disease. Also, one obvious way to promote a successful breeding programme would be the selection of suitable male–female pairings for mating, and we are presently attempting this.

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Specificity of Male Sex Attractants in some Australian Scorpion Flies

Species of the Australian genus *Harpobittacus* (Bittacidae, Mecoptera) have a complex mating habit not recorded elsewhere in the Insecta (Bornemissza[1]). It involves the release of a sex pheromone by two intertergal vesicles on the abdomen of the males which lures females to its vicinity. Mating follows, during which the male presents to the female a nuptial meal consisting of a previously captured insect[2]. Of the six known species of *Harpobittacus*, the mating behaviour of four has been investigated and found to be essentially identical.

Species of *Harpobittacus* (in contrast with those of the world-wide *Bittacus* (see Setty[3])) are allopatric in distribution. Both they and their sub-species are readily distinguishable although, rather surprisingly, difficulty arises with the females of *H. similis* E.-P., which is restricted to an area within 100 miles of Perth, Western Australia, and of *H. australis* (Klug), occurring only in the eastern States. Riek[4] considers, on morphological grounds, that these two taxa may be only sub-specifically distinct. In view of the probability that the western shield has been isolated from the eastern part of this continent since the Middle Cretaceous[5], it was of interest to determine whether the two populations had become reproductively isolated also. The investigation was broadened by the inclusion in the tests of a coastal species from New South Wales, *H. tillyardi* E.-P.

Two types of laboratory experiments were conducted: (*a*) For each sex of each species, small numbers were housed in rectangular gauze cages. Pairs of such cages were arranged so that the airflow from an electric fan passed first through one containing males, and then through one containing females; the reaction of the latter to the male pheromone was recorded. (*b*) Different sex pair combinations of the three species were placed in transparent cylindrical containers closed with nylon mesh, and their behaviour noted.

In all cases, caged females responded to the pheromone emanating only from the males of the same species by congregating on the side of the cage nearest to them.

Caged pairs of the same species mated normally, but when pairs consisting of different species were confined attempts at mating rarely occurred. Mating attempts (which never resulted in impregnation of the female) occurred only when the female present was *H. australis*, and then only in one of fifteen replicates of male *H. similis* and in one of ten replicates of male *H. tillyardi*.

In the field, free-flying *H. australis* females responded to caged males of the same species 10 metres distant, but ignored cages containing *H. tillyardi*.

These results indicate a high order of specificity of the sex pheromones (although to the human sense all three have a uniform butyric-musty odour), and suggest that *H. australis* and *H. similis* are valid species, exhibiting both geographical and reproductive isolation.

Despite the lack of overt reaction by unmated females to the pheromones of other species, the males appear to be highly sensitive to them. When caged pairs were exposed to the pheromone of a second species, production of pheromone and even mating by the former ceased abruptly. Due to the allopatry of *Harpobittacus* spp., interference under field conditions could not arise.

The males of *Bittacus*, according to illustrations by Setty[3] and Tjeder[6], have apparently similar intertergal vesicles. It is reasonable to assume that these vesicles produce sex pheromones similar to those of *Harpobittacus*. It would therefore be of considerable interest to investigate the phenomena of sex attraction and mating in dense sympatric populations of *Bittacus* spp., such as occur in North America (Setty[3]).

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Temperature Optima for Algal Development in Yellowstone and Iceland Hot Springs

In analysing the influence of specific environmental factors on growth, it is desirable to study habitats where only a single factor varies. Hot springs provide suitable habitats where temperature is the only variable, so that the relation of temperature to biological development can be measured directly.

Yellowstone National Park has probably the largest number of thermal features for a comparable area of any region in the world. The numerous springs and hot pools have been well characterized in the classic monograph of Allen and Day[1]. In general, the hot springs are concentrated in basins, each often containing numerous thermal features differing in size, temperature, chemical characteristics and water flow. Although some springs are highly variable, others are remarkably constant in thermal, chemical and hydrological properties. Indeed, we have found the thermal characteristics of some springs studied by Allen and Day in the 'twenties to be unchanged in 1965, and there is good reason to believe that they have essentially remained constant for many centuries. Thermal activity at Yellowstone is thought to pre-date the last glacial age[1].

The Yellowstone springs vary in chemical composition, and three types have been recognized: alkaline springs, acid springs, and travertine-depositing springs which are high in calcium carbonate. These three types of springs also differ considerably in biological characteristics. We have concentrated our work primarily on the alkaline springs because these are the most numerous, exhibit the greatest biological development, and because they yield the largest amounts of water. Most alkaline springs of any antiquity have built up large cones composed primarily of siliceous sinter: these deposits are laterally flat and have a remarkably gentle and uniform longitudinal physical descent from the source. Source water overflows and descends across the sinter cone in one or a few narrow channels or in broad sheets of water, cooling as it flows, and in a favourable situation one can find a constant and uniform thermal gradient from a temperature in the high 80's or low 90's down to 25°–30° C. Along this thermal gradient, blue-green algae proliferate, and much of the attractive colour of Yellowstone springs is due to these organisms (bacteria are also present, but, by microscopic observation, it is estimated that they comprise less than 5 per cent of the total biomass). Thermal activity usually prevents terrestrial vegetation near the springs so that there is no shading, and organisms along the thermal gradient are equally exposed to light. It is not difficult to find pools where the water of the overflow is derived from a single source, so that the initial chemical composition of the water flowing over all the organisms along the thermal gradient is identical.

Although we have made casual observations in many regions of the Park, the quantitative investigations reported here were performed on Firehole Pool and two unnamed springs located along the Firehole Lake Loop Road in the Lower Geyser Basin. The extent of algal development in any spring is strongly influenced by the amount and rate of water flow, and since the springs differed in this factor, they also differed in algal content, but all exhibited similar thermal gradients. To eliminate flow rate as a variable all samples from a single outflow were taken as nearly as possible from areas which had the same amount and rate of water flow. Temperature was measured either with a conventional mercury thermometer or with a Yellow Springs Instrument Co. model 42 thermistor. Duplicate samples of the algal mat and underlying siliceous sinter were taken with a brass coring device 2·27 cm² in area. Preliminary experiments showed that essentially all the chlorophyll-containing material was above the sinter, even where very loose sinter was encountered; therefore a core depth of 1–2 cm, which included 0·5 cm or more of underlying sinter, was routinely used. One sample at each temperature was placed in a 15 ml. polypropylene tube and within an hour was covered with 3 ml. of acetone. Within 12 h these cores were homogenized in the tubes with a 'Teflon' homogenizer. The tubes were centrifuged at 5,000 r.p.m. for 5 min and the pellets treated once or twice more with acetone until all the chlorophyll was extracted and the siliceous sinter (which was an excellent abrasive) had been converted to a fine powder. All the extracts from a core were combined, the volume determined, and the chlorophyll content estimated by reading the optical density at 665 mμ with a Bausch and Lomb 'Spectronic 20' colorimeter. (In all cases, it was ascertained by reading at other wave-lengths that the peak absorption was at 665 mμ.) Chlorophyll *A* was estimated from the optical density readings with the relationships given by Odum *et al.*[2].

The pellet from the acetone extraction was then treated with 5 ml. of 0·5 N perchloric acid at 90° C for 20 min, centrifuged, and the supernatant retained for RNA assay using the orcinol[3] method. DNA could not be assayed because of some material interfering with the diphenylamine colour reaction. The pellet was suspended in 5 ml. of 1 N sodium hydroxide and heated at 90° C for 20 min. After centrifugation, this supernatant was assayed for protein by the method of Lowry *et al.*[4] using crystalline lysozyme as a standard. Control samples of sinter, taken from water at 93° C and free of living organisms (by microscopic observation), were treated in the foregoing manner and these gave essentially no colorimetric readings, showing that the assays of experimental samples had biological meaning. The other sample at each temperature was preserved in 4 per cent formalin and assayed several weeks later in the laboratory, using the procedures already outlined. In general, there was good agreement between the values for the duplicate samples, and only the data for the samples extracted directly into acetone are given.

Fig. 1 shows the relationship between temperature of sample and biochemical content per unit area for one of the springs. In general, there was a good correlation among the contents of chlorophyll, nucleic acid and protein for each sample. Each curve exhibits a characteristic sharp optimum at a temperature of 54° C. A sharp temperature optimum in another spring (Firehole Pool) was found at 51° C, whereas in the small unnamed spring just east of Pink Cone Geyser, the optimum was 56° C. The three springs differed considerably in the maximum amount of algal development, probably due to differences in flow rate, but in each case the temperature optimum was quite similar. Microscopic investigations showed that the number and composition of species changed as one moved down the gradient, as has also been observed by others[5], but these changes are of no consequence in our investigations, since we assume that the organisms found at a given temperature are those best adapted to that temperature.

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The fact that the optimum temperature for algal development in these alkaline springs is 51°–56° C can be readily verified by mere visual inspection in a variety of springs where quantitative sampling is not feasible, since the marked algal growth in this temperature range leads to the appearance of the brilliant colours so characteristic of these alkaline springs. (The bulk of the colour seen is not chlorophyll, however, but an orange pigment which has an absorption peak about 450 mμ.) Indeed, even the casual tourist, armed with a thermometer, can verify the optimum shown by our quantitative data.

Hot springs are found in large numbers in only two other areas, Iceland and New Zealand. We have made preliminary investigations in Iceland which essentially confirm the Yellowstone results. The geology of hot springs in Iceland has been described in detail by Barth[6]. Hot springs are much more scattered in Iceland than in Yellowstone and are in general less accessible, so that it has not been possible to find many springs which provide complete thermal gradients. We have performed quantitative studies on only one spring in the Geyser group, and the results are shown in Fig. 2. In this spring sharp optimal temperatures for chlorophyll, protein and RNA were found at 48° C. These optima are slightly lower than those found in Yellowstone, but the maximum temperature for growth is also lower, about 60° C. The *p*H of this spring, 9·1, is higher than those of the Yellowstone springs, and this may account for the lower maximum and optimum temperatures. We have also investigated an acid spring at Yellowstone (Roaring Mountain, *p*H 2·2) which visually shows an optimum temperature of about 45° C; however, the algal development in this spring is so sparse that we were not able to perform quantitative assays. Clearly, the temperature optimum will differ in various environments and may be influenced by such other factors as light, nutrient supply, hydrostatic pressure or *p*H.

Probably the most noteworthy aspect of these results is the fact that in all cases the optimum temperature is so high. The factors limiting photosynthetic efficiency have been discussed by Daniels[7], and he points out that in terrestrial environment the concentration of carbon dioxide is usually the limiting factor, rather than light. If the concentration of carbon dioxide is raised so that it is not limiting, then "the bottleneck in increased photosynthesis is the rate of using the photochemical products in the later thermal reactions"[7]. In the alkaline hot springs, where neither light nor carbon dioxide would be expected to be limiting, it seems likely that temperature

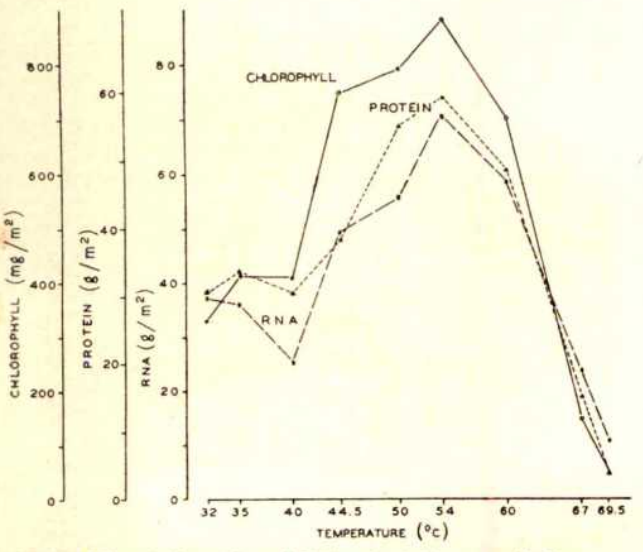


Fig. 1. Chlorophyll, protein, and RNA content of core samples from a large spring in Yellowstone National Park, located on a hillside east of and across the road from White Dome Geyser. Source temperature 72° C, *p*H 8·4

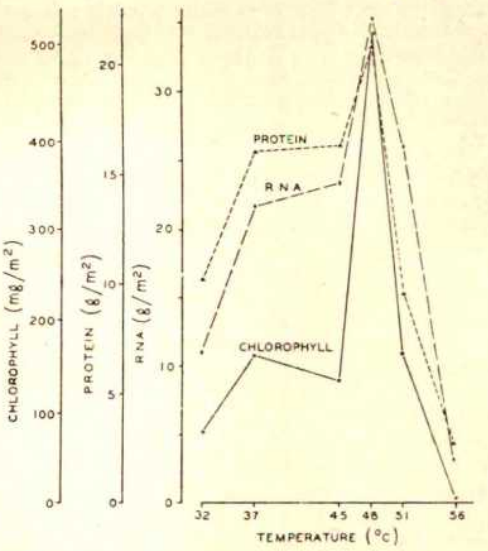


Fig. 2. Chlorophyll, protein, and RNA content of core samples from an Icelandic spring in the Geyser group, near Sisjothandi (Barth's No. 51). Source temperature 92·3° C, *p*H 9·1

is the limiting factor for photosynthetic growth. Only when the temperature reaches values so high that it is probably affecting protein stability or the stability of enzyme-substrate complexes is a further decrease in biological development seen.

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Autoradiography as a Tool in Microbial Ecology

The importance of micro-organisms as agents of biochemical transformations in natural environments is well established. But micro-organisms are small and thus their environments are also small, so that micro-organisms can be investigated directly in Nature only by the use of the microscope. Although the microscope can be used to see where various organisms are situated in a micro-environment and how organisms are disposed with relation to one another, one cannot, however, study microscopically many of the characteristics of an organism which are of greatest ecological interest: that is to say, nutritional requirements, responses to environmental changes, production of metabolites and growth rate. The usual approach

for the study of these latter phenomena has been to isolate micro-organisms in pure culture and attempt to infer from the behaviour of the pure culture the behaviour of the parent organism in Nature. However, it is clear that the behaviour of the pure culture reveals the potentiality of the organism in Nature, but does not show what the organism was actually doing, since the organism has undoubtedly adapted to the cultural conditions used. Further, even if the laboratory conditions precisely mimic the natural conditions, the laboratory environment cannot reproduce the natural environment, since the natural environment contains the additional elements of competition and co-operation with other organisms.

The technique of microscopic autoradiography presented here rectifies in part some of these difficulties, and enables the investigator to obtain some idea directly in Nature of the growth and function of micro-organisms. The technique makes use of the fact that if a micro-organism has assimilated into its protoplasm a radioactive substance with which it is supplied, the presence of this radioactive substance within the microbial cell can be ascertained. The main limitation of this technique is that it must be possible to recognize microscopically in some ecologically relevant way the organisms of interest. After the organisms have assimilated the radioactive material, they are fixed in some way which does not make them morphologically unrecognizable, and they are then affixed to microscope slides. In certain cases, histological procedures may first be needed to make the preparation suitable for microscopy. For example, a plant part or soil sample might be embedded and sectioned. The prepared slides are then dipped in liquid photographic emulsion. (We have used Eastman Kodak '*NTB*-2' nuclear track emulsion, but others may be more desirable for certain purposes, and Prescott[1] should be consulted for details.) After a suitable exposure, which depends on the amount and specific radioactivity of the substance supplied, the extent of radioactivity of the organisms, the nature of the isotope, the thickness of the specimen, etc., the slides are developed and examined phase microscopically. (We have found that a very convenient lens for this examination is the Carl Zeiss Water-Immersion lens, normal aperture 0·75, which can be used directly in a drop of water without a cover slip.) Both radioactive and non-radioactive micro-organisms can be identified, the former being distinguished by the silver grains which appear adjacent to or on top of them. To obtain the best morphological definition, it is essential that the number of silver grains per unit area of organism is not so great as to obscure completely the cell. With a micro-organism of sufficient size, it is often possible to see that one portion of the organism is radioactive, whereas an adjacent portion is not, indicating a functional differentiation within a cell or filament. If fixatives are used, it is probable that radioactivity not present in macromolecules is lost, but in the usual situation the material lost is probably a minor proportion of the total, and can be ignored. Techniques are available for visualizing the radioactivity which is present in a cell in soluble form[2] if required.

This technique can be used in a wide variety of ways to answer a wide variety of questions, and this communication briefly mentions several of them.

For example, in the usual oceanographic or limnological investigation of primary productivity, the photosynthetic fixation of carbon dioxide by aquatic algae is studied by supplying $^{14}CO_2$ to the mixed natural population, and after a given time period the whole population is removed from the water and its radioactivity determined. In this way it is not possible to determine which organisms in the mixed population were actively photosynthesizing at the time of examination. But this question could be answered if a sample of this incubation mixture were examined autoradiographically.

The assimilation by micro-organisms of any other compound available in radioactive form, such as a nutrient or a pesticide, could also be investigated. It should be emphasized that the radioactive compound will be diluted with non-radioactive molecules of the same substance which might be present in the environment, so that it is essential to know the actual specific radioactivity of the compound in the environment before any quantitative significance can be attached to the result. The rate of incorporation of the radioactive material can be estimated by preparing a series of parallel samples incubated for various times. We have used this technique to measure the assimilation of ^{14}C-glutamate by bacteria epiphytic on marine algae, and a representative photograph is shown in Fig. 1. Carbon-14 β-rays have too much energy for high-resolution work, and consequently the morphology of an organism is masked, but the localization of regions of microbial colonization is made easier. For example, the dark area at the axillary region represents a micro-colony of bacteria, while the linear band of silver grains seen in the adjacent area derives from a filament of *Leucothrix mucor*. This picture illustrates another feature frequently observed: the heaviest colonization of marine algae is frequently in axillary regions, presumably where diffusion is restricted and organic matter can accumulate.

It is frequently of interest to know whether a micro-organism is actively multiplying (as opposed to merely assimilating substrate) in a particular environment at a given time. Indeed, it can be argued that it is those environments where an organism can multiply which are of most interest, since in many other environments the organism may be found in viable form, but may not be able to reproduce. We have approached this question with the use of tritiated thymidine. It is well known that cells undergoing division duplicate their DNA, whereas non-growing cells do not. Therefore, cells which are multiplying will incorporate the DNA precursor tritiated thymidine, and will become radioactive, whereas non-dividing cells will not become radioactive. We have used this technique to study the growth of filamentous bacteria of the species *Leucothrix mucor* growing epiphytically under natural conditions on marine seaweeds. We find that in a given area of an algal frond, certain *L. mucor* filaments incorporate tritiated thymidine very rapidly, but other filaments in the same region are completely non-radioactive, even though both radioactive and non-radioactive filaments appear morphologically similar. In many cases, we find that even within a single *L. mucor*

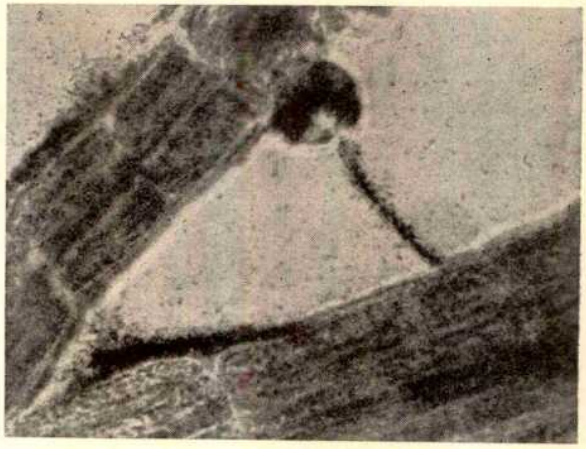


Fig. 1. Autoradiograph of red alga incubated with ^{14}C-glutamate. Incubation, May 1, 1965, 6.00–7.00 p.m., Long Island Sound, Woodmont, Connecticut. Red alga fronds taken directly from Nature, placed in a vial with 1 ml. sea water containing 1 μc./ml. ^{14}C-glutamate (27 mc./mmole), incubated *in situ* (temperature 9° C). Sample fixed overnight in 4 per cent formaldehyde, then washed in water, and affixed to slides with Ulrich's adhesive. Kodak '*NTB*-2' liquid emulsion. Exposure time, 7 days

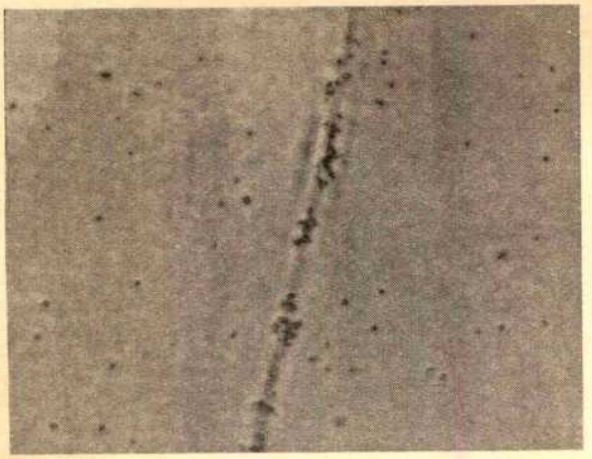


Fig. 2. Autoradiograph of *Leucothrix mucor* filament epiphytic on red alga incubated with ^{3}H-thymidine. Incubation, May 2, 1965, 3.00–4.00 p.m., Narragansett Bay, Quahaug Rock. Red alga, *Polysiphonia lanosa*, taken directly from Nature, placed in a vial with 1 ml. sea water containing 1 μc./ml. ^{3}H-thymidine (6·7 c./mmole), incubated *in situ*, temperature 12° C. Sample fixed 3 days in 4 per cent formaldehyde, then treated as in Fig. 1

filament, growth is localized, and Fig. 2 shows an example of a filament where growth is occurring in four or five localized bands. Thus we can determine by this technique the proportion of filaments, or of cells within filaments, which are growing. This same technique could be used with any other organism which is able to incorporate exogenous thymidine into its DNA.

We believe that the present technique has extremely wide application in microbial ecology, and can be adapted to a variety of problems where it is required to know something about the physiological or biochemical aspects of the organism–environment relationship. Although we have used it in the aquatic habitat, there is no reason why it could not also be applied to soil, the animal body, or the plant root. The effect of environmental changes, seasonal cycles, alterations of temperature, *p*H, and other factors on the biochemical functions of micro-organisms can now be studied for the first time directly in the natural situations which are the primary interest of the investigator. No longer need the microbial ecologist be satisfied with merely looking and counting. This technique provides a means for directly studying in Nature the behaviour of micro-organisms with only minimal disturbance to the system by the investigator.

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MICROBIOLOGY

Effect of Growth Rate on the Lipase Activity of a Rumen Bacterium

In 1961 Hobson and Mann[1] reported the isolation of lipolytic bacteria from the sheep rumen. The predominant lipolytic bacteria were anaerobic, Gram-negative, vibrio-shaped organisms which were not named. Since this time other strains of these bacteria have been isolated and one (strain 5*S*) is being used in a detailed investigation of the lipolytic enzymes. Lipase activity was found to be associated with growing cells in batch culture and so the enzyme activity of the cells grown in continuous culture was tested so that the optimum activity might be obtained for further investigations. The bacterium was grown anaerobically in a medium as previously described[2] and in a chemostat essentially similar to that described by Hobson[3], but of larger vessel volume and fitted with a *p*H control system. In the results described here fructose (concentration 29 μmol/ml.) was the limiting nutrient, and the culture *p*H was controlled at 6·1–6·2, but similar results were found when glycerol was the limiting nutrient. The organism had previously been shown to hydrolyse glycerol esters of short-chain and long-chain fatty acids (tributyrin, triolein and linseed oil) but a more rapid enzyme assay was obtained using the method of Nachlas and Seligman[4] in which hydrolysis of the naphthyl esters of acetic, lauric and stearic acids is followed. The results are taken from a continuous culture run of 1,100 h, and when the culture had stabilized at each growth rate a sample (10 ml.) was taken from the culture vessel and centrifuged to compact the cells. The supernatant was pipetted off and the cells resuspended in 1·0 ml. of Veronal buffer, *p*H 6·8, containing 0·02 per cent (w/v) sodium sulphide.

A suitable volume, 0·2 ml., of the cell suspension or 0·5 ml. of the culture supernatant was then added to a test-tube containing the substrate and buffer in the proportions specified by Nachlas and Seligman[4] and the tube incubated at 38° under a stream of nitrogen which served both to keep the cells suspended and to keep the system anaerobic. The naphthol released was determined by a colorimetric method[4] after incubation for 45 min or 30 min for the laurate and acetate and 90 min for the stearate. The percentage hydrolysis of the substrate was then corrected in each case to that which would be produced by a culture of standard turbidity.

The results for stearate and acetate substrates are shown in Fig. 1. The dilution rates shown were not taken in ascending order but were taken at random to obviate any continuous change in constitution of the cells caused by growing them at a steadily increasing growth rate. The results for laurate were essentially the same as those for stearate, and a determination at a growth rate of 0·03 h^{-1} showed practically no enzyme activity. From the relative hydrolysis produced by cells and supernatant the organism would seem to produce two enzymes, an esterase associated mainly with the cells and a lipase which is mainly secreted into the culture medium.

The activities in the supernatants relative to the cells are higher than shown on the graph, as the cells equivalent to 2 ml. of culture were used in the activity digests, whereas only 0·5 ml. of culture supernatant was used. What is unusual about these results is the pronounced dependence of enzyme activity on growth rate. Superimposed on the graphs of enzyme activity is a graph of culture turbidity at the dilution rates corresponding to various steady states, from which the maximum growth rate of the bacteria in this medium can be seen. The culture turbidity has been shown to be proportional to

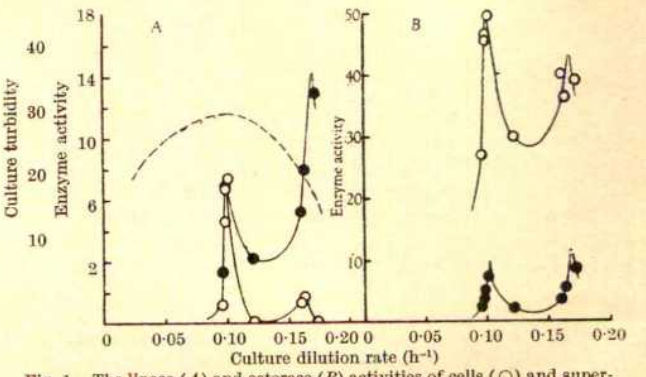


Fig. 1. The lipase (*A*) and esterase (*B*) activities of cells (○) and supernatants (●) from a continuous culture growing at different rates. Substrates, naphthyl stearate (*A*), naphthyl acetate (*B*). Superimposed on *A* is a graph of the steady-state cell concentrations at different growth rates

the concentration of cells as mg dry wt/ml. (ref. 2). There is a peak of enzyme activity at about the optimum growth rate when energy of fermentation of the substrate is being used optimally for cell formation and not for maintenance requirements, and this might be expected, but there seems to be no explanation of the second peak of enzyme activity, unless two separate enzyme systems are being formed. This will be further investigated.

The optimum growth rates for lipase and esterase activities correspond, although that the lipase and esterase activities are due to two different enzymes is suggested by the different distribution of activities shown here: the apparently different *p*H optima for activity, and the fact that when the cells are grown at a constant dilution rate of 0·1 h^{-1} the specific activity of the lipase seems to be much more dependent on the *p*H at which the culture is growing than does the esterase activity.

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VIROLOGY

Early Diffuse Chromosome Alterations in Monkey Kidney Cells infected *in vitro* with Herpes Simplex Virus

Herpes simplex virus induces chromosomal alterations in cultures of Chinese hamster cells[1–4] and human cells[4]. We have observed numerous mitoses, with chromatid and chromosome gaps as well as breaks, in primary cultures of monkey cells inoculated with herpes simplex virus; with a virus–cell ratio of about 1 : 8, 25 per cent of mitoses showed such lesions 19 h after the viral inoculation; this percentage rose to 60 per cent at 27 h. Besides mitoses with one or few gaps and breaks, diffuse metaphase chromosomal alterations were observed, but only after 26 h, and then in only a very small number of cells[5].

In order to investigate more accurately the time of appearance of the chromosomal lesions, cells were infected with a high multiplicity of virus. Primary cell cultures of *Papio papio* kidney cells were made on 6 × 32 mm coverslips in Leighton tubes in a medium consisting of 47·5 parts Hanks's solution, 47·5 parts Parker's medium, and 5 parts heat inactivated calf serum. After 5 days, the average number of cells was $2{\cdot}5 \times 10^5$ per tube.

Herpes simplex virus (*HFYS*$_{21}$ strain, American Type Culture Collection) propagated in our laboratory in *KB* cells was then added; each tube received 0·3 ml. of virus suspension containing $10^{6{\cdot}5}$ *T.C.I.D.* 50, giving a virus–cell ratio of about 10 : 1.

Control tubes received the same dose of virus previously inactivated with ultra-violet irradiation. Other controls received 0·3 ml. of supernatant from uninfected *KB* cell cultures.

Microscopic observations were made at hourly intervals to estimate the gross cytopathic effect. Two replicate tubes from each of the three groups were sampled at intervals from 30 min to 4 h after inoculation, in order to examine the mitoses.

Colchicine was added to most tubes (see Tables 1 and 2), and the cultures were incubated for an additional period of 2 h. The coverslips were then removed, immersed in a hypotonic solution, fixed in Carnoy's solution, air dried, and stained with Unna-blue. Mitoses were observed using oil immersion and photographed. In order to detect early alterations and to recognize any artefact due to colchicine itself, a few tubes were incubated with colchicine for 30 min, 1 h or 1·5 h or received no colchicine at all.

The infected tubes showed no cytopathic effect until the tenth hour. At that time, the cytopathic effect appeared and increased rapidly, and the whole cell layer was destroyed by the twenty-fourth hour. In contrast, controls appeared intact at 24 h.

Tables 1 and 2 show the number and percentage of abnormal mitoses observed at early times of inoculation. In the infected tubes, the lesions were of two types: (*a*) distinct gaps and breaks, usually in one chromatid more often than in both; (*b*) diffuse lesions of many or most chromosomes. These diffuse lesions are similar to those reported by several authors[4,6,7], some of which appeared to be due to a partial despiralization of the chromosome.

The distribution of the cells in cultures was not uniform along the length of the coverslips, as the Leighton tubes were incubated in a tilted position, and it was observed that the percentage of damaged metaphases of both types was much higher where the cells were more sparse. The results given in Tables 1 and 2 are those relating to regions with a low density of cells. Gaps and breaks slowly became more numerous in the infected tubes whereas the number of diffuse lesions appeared to be very high as early as the second hour, since only one such figure was seen in the controls. Comparison of the results with those from coverslips not treated with colchicine showed that no artefact is due to the alkaloid.

In tubes not exposed to colchicine, a possible colchicine-like action of the infectious virus was noted. The chromosomes of 50 per cent of metaphases appeared well spread and condensed, whereas the figure for cells exposed to virus previously inactivated by ultra-violet light was 30 per cent and for those receiving *KB* cell culture supernatant only 25 per cent. Anaphase abnormalities and formation of micronuclei were also observed with infectious virus.

The chromosomal lesions described here are due to herpes simplex virus infection: they were not observed in controls when herpes simplex virus was previously irradiated with ultra-violet light, or when supernatant from *KB* cells was added alone. It has already been shown[5] that few or no lesions are seen if the virus is inactivated by heat or neutralized by a specific antiserum. If these results are compared with those previously reported[5] it appears that the chromosomal injuries occur much sooner with a higher virus dose and the figures for diffuse damage are surprisingly higher.

Table 1. Abnormal Mitoses after Inoculation with Infectious Virus

Time of incubation with infectious virus (h)			No. of mitoses examined	Abnormal mitoses			
Total time	Before addition of colchicine	After addition of colchicine		Few gaps and/or breaks	%	Diffuse alterations	%
1	0·5	0·5	186	8	4·3	0	0
1·5	0·5	1	112	3	2·7	0	0
2	0·5	1·5	147	8	5·5	28	18·3
2	1	1	164	12	7·3	34	20·8
3	1	2	140	6	4·3	41	29·3
3	2	1	183	20	10·9	46	24·3
4	2	2	112	9	8	35	31·2
4	3	1	147	25	16·9	45	24·5
0·5	0·5		100	1	1	0	0
1	1	No	109	4	3·6	0	0
1·5	1·5	colchicine	104	3	2·9	0	0
2	2	added	160	16	10	21	13·1
3	3		132	19	14·4	26	19·7
4	4		117	9	7·7	41	28·3

Table 2. Abnormal Mitoses in Control Tubes

Control series	Time of incubation (h)			No. of mitoses examined	Abnormal mitosis		
	Total time	Before addition of colchicine	After addition of colchicine		Few gaps and/or breaks	%	Diffuse alterations
Ultra-violet-inactivated virus	1	0·5	0·5	112	5	4·4	0
	2	1	1	90	2	2·2	0
	3	1	2	126	7	5·2	0
	4	2	2	166	3	1·8	1
Ultra-violet-inactivated virus	1	1	No	73	4	5·4	0
	3	3	colchicine	110	2	1·8	0
	4	4	added	128	6	4·7	0
Uninfected *KB* culture supernatant	1	0·5	0·5	114	7	6·1	0
	2	1	1	108	3	2·9	0
	4	2	2	105	4	3·9	0

The early appearance of such gross lesions is noteworthy; they were obvious at 2 h, that is, when about 80 per cent of the virus had been absorbed to the cells[11]. According to Roizman, these first 2 h correspond to the stage at which herpes simplex virus induces the preliminary synthesis necessary for the initiation of viral DNA duplication[8].

These diffuse chromosomal lesions could be noted 1·5 h before formation of the earliest type-*A* intra-nuclear inclusion bodies in interphase cells[9], 2 h before the onset of virus DNA synthesis[8], and 4 h before new particles could be detected inside the nucleus by electron microscopy[10].

The occurrence of early diffuse chromosome fragmentation may help to explain the apparent inhibitory effect of herpes simplex virus on mitoses[11]. Experiments are now in progress to determine whether other strains of herpes simplex virus induce the same chromosomal lesions.

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Inhibition of Adenovirus 12 Oncogenicity in Hamsters by Simultaneous Inoculation with Adenovirus 5

Trentin *et al.*[1] demonstrated that hamsters inoculated at birth with adenovirus 12 develop a high incidence of malignant tumours, and that viral multiplication does not seem to occur under these conditions. On the other hand, adenovirus 5 is apparently not oncogenic for hamsters[2], but it has been reported to induce a lethal infection in new-born animals including lesions of liver cells similar to those observed in cultured human epithelial cells in which the virus is multiplying[3]. Further investigations[4] have recently shown that the pathogenicity of adenovirus 5 for new-born hamsters depends on the virus doses inoculated, no pathological symptoms being observed below 10^8 *T.C.I.D.* 50.

This communication presents results indicating that the oncogenicity of adenovirus 12 for hamsters can be reduced by simultaneous administration to new-born animals of both adenovirus 5 and 12.

Adenovirus 12 (strain Huie) and adenovirus 5 (strain Adenoid 75), supplied by the American Type Culture Collection, were inoculated on *KB* cells grown as monolayers in Roux bottles in a casein hydrolysate medium supplemented with 5 per cent calf serum and antibiotics (penicillin, streptomycin, kanamycin).

When all the cells exhibited typical cytopathic effects, they were freeze-thawed three times in dry ice–alcohol, and the cell-supernatant mixtures were centrifuged at 2,000 r.p.m. for 5 min to remove cellular debris. Supernatants, considered as virus stocks, were placed in 2-ml. vials stored at −20° C, and used for all the experiments described here.

Virus assays were carried out in *KB*-cell tubes. The final reading was made on the tenth day following virus inoculation, and *T.C.I.D.* 50 was calculated by the method of Kärber.

The titres of the virus stocks were, respectively, $10^{2.5}$ *T.C.I.D.* 50 per 0·1 ml. for adenovirus 12 and $10^{6.5}$ *T.C.I.D.* 50 per 0·1 ml. for adenovirus 5.

Litters of 1–3-day-old, male and female, non-inbred golden hamsters (*Cricetus auratus*) from a closed colony established in our Institute were randomly distributed prior to inoculation.

All animals were inoculated by the subcutaneous route in the dorsal region with an unvarying volume of 0·3 ml. The following series were established: (*a*) animals inoculated with adenovirus 12 (0·2 ml.) adjusted to a volume of 0·3 ml. with Hanks' solution; (*b*) animals inoculated with adenovirus 12 (0·2 ml.) mixed immediately before inoculation with adenovirus 5 (0·1 ml.), mixing of both viruses being carried out at room temperature in a vial by means of pipetting; (*c*) animals inoculated with adenovirus 12 (0·2 ml.) mixed under the same conditions as above, with 0·1 ml. of uninoculated *KB* cell extract.

The effects of diluting or inactivating adenovirus 5 prior to mixing with adenovirus 12 were investigated in a complementary experiment. Adenovirus 5 was diluted 10^{-3} and 10^{-6} in Hanks' solution, or inactivated by heating to 100° C for 15 min.

The hamsters were examined twice a week. Tumours were detected through palpation and confirmed at autopsy and by histological investigations. A preliminary experiment was undertaken with the view of detecting an eventual pathogenicity for new-born hamsters of adenovirus 5 *T.C.I.D.* 50 used in our experiments. No clinical manifestations of disease were found in fifteen hamsters observed for over 1 year.

Table 1 shows a summary of the results of three independent experiments which were performed under identical conditions, in which adenovirus 5 was mixed undiluted with adenovirus 12. These results indicate that significant decrease of the percentage of adenovirus-12 induced tumours in hamsters can be achieved by simultaneous injection of adenovirus 5 into new-born animals.

Table 2 shows the results of experiments in which adenovirus 5 was diluted or inactivated prior to mixing with adenovirus 12.

Table 1. Inhibition of Adenovirus 12 Oncogenicity in Hamsters by Simultaneous Inoculation with Adenovirus 5

Experiment	Composition of inoculum	No. of viral infective doses inoculated (*T.C.I.D.* 50)	Observation period (days)	Tumorous hamsters / Injected hamsters *	Tumorous hamsters (%)	Statistical evaluation †
1	Adeno 12 (0·2 ml.) + Hanks' (0·1 ml.)	632		19/23	82·6	
	Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632 $3·16 \times 10^6$	360	4/12	33·3	$P = 0·01$
2	Adeno 12 (0·2 ml.) + Hanks' (0·1 ml.)	632		7/8	87·5	
	Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632 $3·16 \times 10^6$	240	1/6	16·6	$P = 0·03$
3	Adeno 12 (0·2 ml.) + Hanks' (0·1 ml.)	632		9/16	56·2	
	Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632 $3·16 \times 10^6$	210	0/13	0	$P = 0·005$
4 (controls)	Adeno 12 (0·2 ml.) + Hanks' (0·1 ml.)	632		7/8	87·5	
	Adeno 12 (0·2 ml.) + Uninoculated *KB* cell extract (0·1 ml.)	632	240	7/8	87·5	

* Numerator indicates No. of hamsters which developed tumours and denominator No. of hamsters surviving at weaning (3 weeks).
† Corrected χ^2 tests.

Table 2. Effect of Inactivating or Diluting Adenovirus 5 on the Inhibition of Adenovirus 12 Oncogenicity

Composition of inoculum	No. of viral infective doses inoculated (*T.C.I.D.* 50)	Observation period (days)	Tumorous hamsters / Injected hamsters *	Tumorous hamsters (%)	Statistical evaluation †
Adeno 12 (0·2 ml.) + Hanks' (0·1 ml.)	632	210	7/8	87·5	—
Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632	210	7/8	87·5	$P > 0·05$
	0 (virus inactivated)				
Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632	210	8/11	72·7	$P > 0·05$
	3·1				
Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632	210	4/10	40	$P > 0·05$
	3,160				
Adeno 12 (0·2 ml.) + Adeno 5 (0·1 ml.)	632	210	1/6	16·6	$P = 0·03$
	3,160,000				

* Numerator indicates No. of hamsters which developed tumours and denominator No. of hamsters surviving at weaning (3 weeks).
† Each series of animals inoculated with adenovirus 12 and various adenovirus 5 doses was compared with the series of animals inoculated with adenovirus 12 and Hanks' fluid by means of corrected χ^2 tests.

It can be seen that: (*a*) High doses of adenovirus 5 (3×10^6 *T.C.I.D.* 50) are apparently required to afford protection from tumour development. (*b*) Lower doses of adenovirus 5 are not effective in preventing the induction of tumours. (*c*) Protection from tumour growth cannot be obtained by inoculation of heat-inactivated adenovirus 5.

Experiments are now being carried out in our laboratory to determine whether the protection afforded to hamsters is linked with adenovirus 5 virions or with a soluble factor present in adenovirus 5 cultures. If the former hypothesis is correct, several explanations can be invoked, such as a direct interference phenomenon or an immunological mechanism.

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VETERINARY SCIENCE

A New Sterilizing and Hereditary Defect (the 'Dag Defect') located in the Bull Sperm Tail

Hitherto, cases of sterility in the bull, where the abnormal sperm population shows a dominant sperm defect mainly or exclusively located in the spermatozoan tail, have only rarely been reported.

Hancock and Rollinson[1] found in the Guernsey breed the so-called 'decapitated sperm defect', a congenital defect showing 95–100 per cent of the spermatozoan heads detached from the still motile spermatozoan tails. According to Jones[2], this defect is hereditary within the Guernsey breed.

Williams[3] found in man what he called a 'centriolar-mitochondrial defect' in less than 0·5 per cent of his cases of sterility, often involving the entire sperm population. The defect is characterized by a prolongation or thickening of the mitochondrial sheath and is often combined with a lack of the main piece of the tail. Savage and Isa[4] and Coubrough and Barker[5] have found similar cases of spermatozoan tail defects in bulls of different breeds, but so far there is no report of any of these cases being of congenital nature.

A characteristic sterilizing abnormality, the 'corkscrew sperm defect' located in the middle piece, was demonstrated in bulls of the RDM breed[6]. A more detailed investigation of this defect showed an irregular distribution of the mitochondrial sheath in smaller or bigger lumps along the middle piece[7]. This also provides an explanation for the high incidence of persistent proximal droplets in many of the affected sperms. The defect has since been demonstrated also in the Jersey, Frisian and Aberdeen Angus breeds, and usually the bulls in question have shown a normal spermiogram and normal fertility up to the age of 5–10 years (unpublished observations).

Swanson and Boyd[8] have reported in detail their investigations of three bulls of the Jersey breed which showed a high percentage (50 per cent or more) of coiled, looped or bent spermatozoan tails. Their illustrations, and moreover the fact that spermatozoan material taken from cauda epididymidis *post mortem* showed practically normal tails, seem to indicate that in these cases the prevailing tail defect was the well-known secondary sperm defect: *bent tail*[9]. Usually the bent tail defect takes the form of a single bending of the tail at the distal end of the middle piece, with or without the presence of the distal cytoplasmic droplet within the loop.

Also in a recent case of akinesia of bull sperm reported by Gustafsson[10] the dominating type of sperm abnormality seems to have been the simple bent tail, and electron-microscopical investigations did not show any pathological changes in the ultrastructure of the sperm.

The case histories reported here refer to two 'full-brother' bulls of the Jersey breed, both sub-fertile and both showing the same abnormal spermiogram characterized by a high percentage of defective spermatozoan tails.

A semen sample from bull *A* ('Dag', 11 months old) was received from an artificial insemination (A.I) centre for routine brucellosis control in October 1963. It was the first time that the bull had been used, and the sample (3 ml.) was normal in most respects (concentration about 1 million spermatozoa/mm^3) except that motility was very low and the spermiogram showed a very peculiar picture. There were very few defects among the spermatozoan heads, but about 40 per cent of the spermatozoan tails were strongly coiled, folded together or split up into fibres. Such a high rate has not been seen before (Fig. 1). After retesting several times during the next two months, when similar results were obtained, the A.I. centre was informed that the bull would probably show a lowered fertility and therefore could not be recommended for A.I. Consequently the bull was sold to a private breeder for natural service within his own herd. During the spring of 1964 it was used for 30 cows and heifers (2–3 matings each) with very little success (only one cow conceived). Finally, the bull was taken over by the laboratory for final investigation.

As members of the Jersey Committee of the A.I. centre were still very interested in the sire–dam combination of the bull 'Dag', his full-brother (case *B*), who was born during November 1963, was purchased for the centre. When this younger brother was about 11 months old, the first semen samples were collected and examined, with the astonishing result that they showed almost the same quality semen as that of bull *A*: *Sperm concentration*, about 0·7 million spermatozoa/mm^3 or slightly lower; *motility*, low (10–15 per cent) with weak sperm movements; *morphology*, 40–50 per cent strongly coiled, folded or broken sperm tails (Fig. 3). A total of eight ejaculates (including II and III ejaculates) from bull *B* were examined, all with the same result. The semen was used for insemination (dilution 1 : 10) of sixteen cows and heifers, with rather limited success (only three conceptions were recorded). The bull was slaughtered when 13 months old.

Bull *A* was kept at the experimental farm of the laboratory until it was 2¼ years old. A total of eleven ejaculates were examined from this bull during the observation period, and no improvements in the semen quality were observed.

Post-mortem examination of the genital organs of the two bulls gave the following results: testis + epididymis normal in shape and size; weight: bull *A*, 370 + 380 g, and bull *B*, 340 + 330 g. Histology of testis was normal; no signs of degeneration or inflammation could be demonstrated in the spermiogenic epithelium.

Sperm concentrates from ampulla ductus deferentis and cauda epididymidis were collected. In bull *A*, a haemocytometer count of the cream-like fluid from ampulla ductus deferentis showed a concentration of 9–10 million spermatozoa/mm³. Slides were prepared from these concentrates and stained in the same way as the ejaculates by the eosin–nigrosin and indian ink methods. In both bulls the differential counts of the ampulla ductus deferentis and the cauda epididymidis material gave similar results to those already recorded for the ejaculates, namely, 40–50 per cent of all the sperm tails were strongly coiled, folded or broken (Figs. 2 and 4). In some not too crowded areas of 5μ sections from the cauda epididymidis, it was also possible to demonstrate the typical coiled or folded sperm tails.

It would be of the greatest interest to trace a number of bulls closely related to bulls *A* and *B*, and to investigate their spermiograms. The sire of the two bulls is still in use at a Danish A.I. centre. That animal has been examined once a year during the period 1959–64, and has always showed a normal semen picture. No period of infertility has been observed. Half-brothers to bull *A* and *B* are also in use at different A.I. centres (two on the sire-side and two on the dam-side) and all four are normal so far as semen and fertility are concerned.

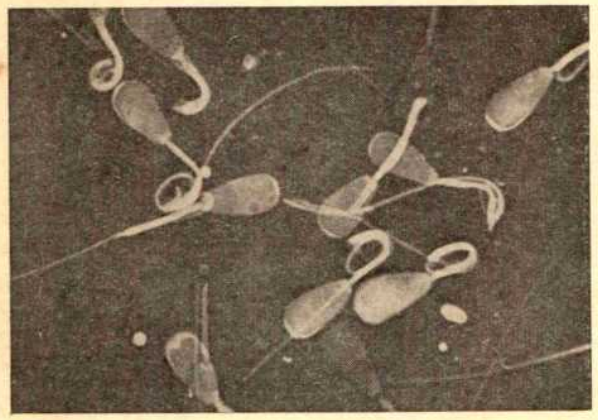


Fig. 1

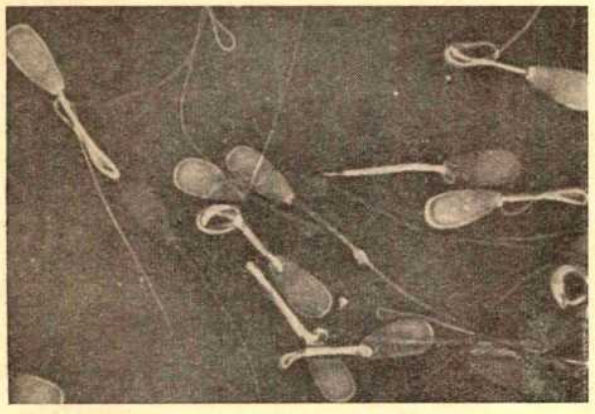


Fig. 2

Figs. 1 and 2. Photographs showing the typical spermatozoan tail defect in smears from bull *A*. Fig. 1 has been prepared from the first ejaculate examined of the bull. Fig. 2 shows the morphology of the sperm-concentrate from ampulla ductus deferentis at slaughter 16 months later. (Eosin-nigrosin stain, × 1,000)

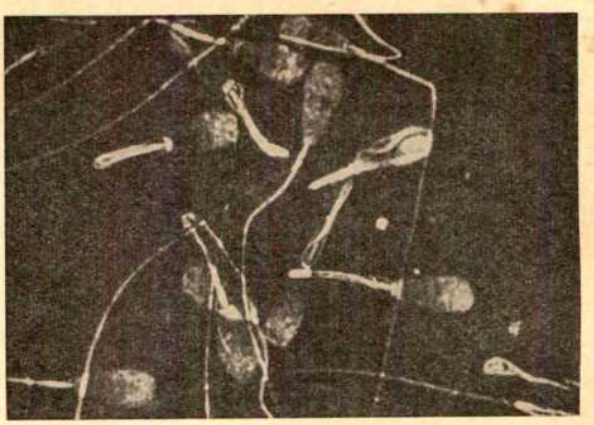


Fig. 3

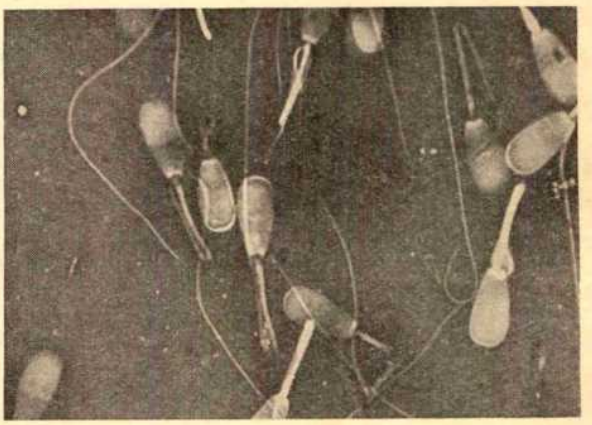


Fig. 4

Figs. 3 and 4. Photographs showing the typical spermatozoan tail defect in smears from bull *B*. Fig. 3 shows the defects in an indian ink preparation from ejaculated semen. Fig. 4 demonstrates an eosin-nigrosin smear from the contents of cauda epididymidis at slaughter, 2 months later (×, 1,000)

The two cases reported are especially remarkable because they are found within the Jersey breed where so far congenital and hereditary defects have only rarely been found. It would appear that a fundamental defect in the sperm tail structure has occurred as the result of the specific sire–dam combination used, and a closer investigation by means of the electron microscope would probably provide a more detailed explanation.

These findings seem to indicate that 'strongly coiled and folded tails' should always be included in the group of 'primary sperm abnormalities'[9] in the morphological sperm examination for fertility in the bull.

Thanks are due to Dr. J. Müller, head of department, for his assistance in preparing the photo-micrographs.

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[1] Hancock, J. L., and Rollinson, D. H. L., *Vet. Rec.*, **61**, 742 (1949).
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[9] Blom, E., *On the Evaluation of Bull Semen*, Diss. (Copenhagen, 1950).
[10] Gustafsson, B., *Nord. Vet.-Med.*, **17**, 65 (1965).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 14

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 5.15 p.m.—Prof. Theodor Bücher (University of Munich): "Dynamics of Liver Metabolism". (Further lecture on February 17.)*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. James D. Ebert (Carnegie Institution of Washington). "Interacting Systems in Development: I. Beyond the Ribosome".*

Tuesday, February 15

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Barley".

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Dr. Elliott Jaques: "The Nature of Decision-Making".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. R. May: "Colonial Morphology, Antigens and Pathogenicity of Haemophilus Influenzae". (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. James D. Ebert (Carnegie Institution of Washington): "Interacting Systems in Development: II. Biochemistry and Physiology of the Developing Heart". (Further lecture on February 17.)*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Dr. Vassos Karageorghis: "Recent Excavations in the Archaic Necropolis of Salamis".*

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Chemistry Lecture Theatre, Queen Elizabeth College, Campden Hill Road, London, W.8), at 6.30 p.m.—Scientific Films.

Wednesday, February 16

SOCIETY OF CHEMICAL INDUSTRY (joint meeting of the Fine Chemicals Group and the Microbiological Group, at Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), from 3 p.m. to 6.45 p.m.—Meeting on "The Cephalosporin Story: Biosynthesis—Chemistry—Pharmaceutical Aspects". Lecturers: Dr. G. G. F. Newton, Dr. A. G. Long and Dr. P. W. Muggleton.

UNIVERSITY OF LONDON (at the Institute of Dental Surgery, Eastman Dental Hospital, Gray's Inn Road, London, W.C.1), at 5.30 p.m.—Dr. J. A. Fraser Roberts. "The Genetic Element in the Causation of Disease". (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)*

INSTITUTE OF INFORMATION SCIENTISTS (at the Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. J. McDaniel: "Recent Developments in Mechanical Translation".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the Physics Block, Imperial College of Science and Technology, London, S.W.7), at 6.30 p.m.—Dr. M. F. Ansell: "Recent Advances in the Chemistry of Unsaturated Acids".

Thursday, February 17

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (joint meeting with the Road and Building Materials Group, at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "Aspects of the Surface Chemistry and Practical Application of Bitumen Emulsions".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. A. G. Atkins and Dr. D. Tabor, F.R.S.: "Strength Properties of Solids at Very High Temperatures"; Mr. J. W. Steeds: "Dislocation Arrangements in Copper Single Crystals as a Function of Strain".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Symposium on "Intertidal Studies at Scolt Head, Norfolk".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. J. Dieudonné: "The New Algebraic Geometry".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Mechanized Permanent Way Maintenance", introduced by Mr. F. Fawcett and Mr. A. C. Edrich.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. F. Soothill: "Quantitative Disturbances of Plasma Proteins in Disease". (Eleventh of fifteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation.)*

SOCIETY FOR ANALYTICAL CHEMISTRY, PARTICLE SIZE ANALYSIS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Inaugural Meeting, followed by Scientific Papers.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with West Ham College of Technology Chemical Society, at the West Ham College of Technology, Romford Road, London, E.15), at 7.30 p.m.—Mr. W. G. Busbridge. "The Use of Radioisotopes".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. G. Webbe and Dr. P. Jordan: "Recent Advances in the Knowledge of Schistosomiasis in East Africa".

UNIVERSITY OF LONDON (at Wye College, near Ashford, Kent), at 8.15 p.m.—Prof. E. W. Russell: "The Application of Agricultural Science to Tropical Agriculture".*

Thursday, February 17—Friday, February 18

ASSOCIATION OF APPLIED BIOLOGISTS, AND THE BRITISH MYCOLOGICAL SOCIETY (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 11 a.m. daily—Symposium on "Physiology of Parasitism".

Friday, February 18

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, Institute House, 32 Welbeck Street, London, W.1), at 2 p.m.—Radiobiology Meeting.

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Captain A. Wepster: "Navigation in Main Western European Rivers".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. E. J. Denton, F.R.S.: "The 'Mirrors' of Fishes".

Saturday, February 19

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Frank W. Lane: "The Elements Rage—Storm, Flood and other Forms of Violence in Nature".*

Monday, February 21

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Lt. Col. J. A. Simson, M.C., and Mr. C. J. Sweeney, O.B.E.: "The Ordnance Survey and Land Registration".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. C. Wood: "Developments in the Control of Ectoparasites of Livestock".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. F. Bourlière (University of Paris): "The Ecology of Herbivores in the African Savanna and Forest". (Further lecture on February 23.)*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

GEOLOGISTS (4) (40 or under, with a degree, or equivalent with a pass in geology at final examination) IN THE GEOLOGICAL SURVEY OF THE DEPARTMENT OF INDUSTRY AND COMMERCE—The Secretary, Civil Service Commission, 45 Upper O'Connell Street, Dublin, 1, Republic of Ireland (February 17).

ASSISTANT LECTURER OR LECTURER IN THE FIELD OF STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (February 19).

CHEMIST (with a degree or equivalent qualification in chemistry and preferably a knowledge of chromatographic techniques) to assist in developing and applying analytical techniques for insecticide residues associated with studies on the behaviour of insecticides in soils and plants—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (February 19).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER (with a university degree or H.N.C. and preferably a special knowledge of digital electronics and data handling systems) IN THE DEPARTMENT OF PHYSICS OF THE FACULTY OF SCIENCE, to take part in the experimental programme in nuclear physics associated with the Heavy Ion Linear Accelerator and other accelerators—The Registrar, The University, Manchester, 13, quoting Ref. 17/66 (February 23).

LECTURER (preferably with a veterinary qualification and interested in chemical pathology) IN THE DEPARTMENT OF PATHOLOGY—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (February 25).

ELECTRON MICROSCOPIST (with experience in the use of the electron microscope in biological research, and preferably with a degree or equivalent qualification) IN THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND PHYSIOLOGY, to take charge of the electron microscope serving these two departments and, for the present, certain others—The Secretary, The University, Aberdeen (February 26).

LECTURER or ASSISTANT LECTURER IN STRUCTURES IN THE DEPARTMENT OF ENGINEERING IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester 13, quoting Ref. 19/66/Na (February 26).

READER IN MICROBIOLOGY IN THE DEPARTMENT OF BOTANY; and a LECTURER (preferably with a special interest in enzymology) IN BIOCHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (February 26).

UNIVERSITY LECTURERS (2) IN THE DEPARTMENT OF METALLURGY—The Secretary of Faculties, University Registry, Oxford (February 26).

CHAIR OF ARCHAEOLOGY—The Secretary, The University, Southampton (February 28).

ENTOMOLOGIST (under 40) on the scientific staff of the Royal Horticultural Society at Wisley, for duties which are principally advisory and instructional, but may include investigations on insect pests affecting horticultural plants—The Secretary, Royal Horticultural Society, Vincent Square, London, S.W.1, marking envelope "Confidential" (February 28).

JUNIOR LECTURER/LECTURER IN CHEMISTRY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, February 28).

LECTURER or ASSISTANT LECTURER (preferably with a special interest in operational research and/or statistics) IN THE ELECTRONIC COMPUTING LABORATORY—The Registrar, The University, Leeds, 2 (February 28).

LECTURER TO SPECIALIZE IN ANIMAL NUTRITION IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY—The Registrar (Room 39, O.R.B.), The University, Reading (February 28).

LECTURER (with special qualifications in the teaching of biology, and if possible teaching experience in other subjects) IN EDUCATION—The Registrar, King's College (University of London), Strand, London, W.C.2 (February 28).

READER IN INORGANIC CHEMISTRY; and a SENIOR LECTURER/LECTURER IN ANALYTICAL CHEMISTRY—The Staff Officer, The College of Advanced Technology, Gosta Green, Birmingham, 4, quoting Ref 451/2 (February 28).

SENIOR RESEARCH OFFICER (with a higher degree in biochemistry or chemistry and several years' experience in research) IN MOLECULAR BIOLOGY to be engaged full-time in research in the field of protein chemistry—The

Director of Research, Dr. P. Edman, St. Vincent's School of Medical Research, Victoria Parade, Fitzroy, N.6, Victoria, Australia (February 28).

Temporary Lecturer or Temporary Assistant Lecturer in Mathematics—The Registrar, The University, Keele, Staffs (February 28).

Veterinary Officer (member of the Royal College of Veterinary Surgeons and preferably with experience of exotic as well as domestic animals), to be in charge of a well-equipped animal hospital, and to collaborate with Curators over all matters of animal welfare and husbandry—The Secretary, The Zoological Society of London, Regent's Park, London, N.W.1 (February 28).

Assistant Lecturer in the Department of Medical Physics—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (March 1).

Imperial Chemical Industries Research Fellows (normally below the age of 30 with the degree of Ph.D. or with equivalent research experience) in Chemistry, Physics, Metallurgy, Engineering, Geology, Agricultural Science or related subjects—The Registrar of the University of Wales, University Registry, Cathays Park, Cardiff (March 1).

Lecturer or Assistant (Assistant Lecturer) in Electron Microscopy in the Department of Genetics—Secretary of the University Court, The University, Glasgow (March 1).

Secretary (with a good professional qualification in physics and experience in administration) of The Institute of Physics and The Physical Society—Sir James Taylor, M.B.E., F.Inst.P., Honorary Treasurer of The Institute of Physics and The Physical Society, 47 Belgrave Square, London, S.W.1, marking envelope "Personal" (March 1).

Whitley Professor of Biochemistry—The Registrar, University Registry, Oxford (March 3).

Senior Lecturer or Lecturer in Mathematics at the University of the West Indies, St. Augustine, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 4).

Assistant Lecturer or Lecturer in the Department of Zoology—The Registrar, The University, Leeds, 2 (March 5).

Post-Doctoral Research Fellow in the Department of Chemistry, University of Ibadan, Nigeria, to carry out studies on the comparative physical chemistry of haemoglobins—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 7).

Research Fellow in the Department of Statistics of the Research School of Social Sciences, Institute of Advanced Studies, Australian National University, to work in the field of mathematical statistics and its applications to other sciences—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 11).

Research Assistant (graduate, or equivalent, in physics, engineering or textile technology) to work on the development of woven, knitted and other fabrics in the Department of Textile Technology—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester, 1 (March 15).

Research Assistant (graduate or person of equivalent status) in the School of Chemistry to be responsible for the maintenance of a mass spectrometer, type MS9, and for recording and interpreting spectra—The Registrar, University of Bristol, Senate House, Bristol 2 (March 15).

Senior Lecturer (with a higher degree in pharmacology, physiology, medicine or pharmacy) in the Department of Pharmacology—The Clerk to the Council, School of Pharmacy (University of London), 29/39 Brunswick Square, London, W.C.1 (March 25).

Assistant or Associate Professor in the Department of Physics—J. M. Robson, Chairman, Physics Department, University of Ottawa, Ottawa 2, Canada.

Biologist (with a good honours degree and preferably some research experience) to join a small team carrying out research in the fields of protein chemistry; and a Physical Chemist or a Physicist (graduate) to take part in research and development work connected with the production and properties of powders—The Director of Research, The Research Association of British Flour-Millers, Cereals Research Station, Old London Road, St. Albans, Herts.

Chair of Geography—The Registrar, University Senate House, Bristol, 2.

Head (suitably qualified and experienced to supervise teaching and ancillary staff and 150 students following a full-time pharmacy course) of the New Zealand School of Pharmacy—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1; or The Director of Education, Private Bag, Wellington, New Zealand.

Master to teach Mathematics up to advanced and scholarship levels—The Rector, The Edinburgh Academy, Henderson Row, Edinburgh, 3.

Master to teach Physics at all levels up to University Scholarship—The Headmaster, Mill Hill School, London, N.W.7.

Research Assistant for a research programme entitled "An Investigation of Plant Indicators of Mineralization" being conducted by Prof. Monica M. Cole, Head of the Department of Geography at Bedford College—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1.

Senior Research Officer with primary duties in field research, but also some lecturing, etc.—The Director, Edward Grey Institute of Field Ornithology, University of Oxford, Oxford.

Visiting Professor (with a degree from a university in Great Britain, member of a professional institution and relevant industrial experience) of Ferrous Production Metallurgy at Banaras Hindu University, Varanasi, India—The Recruitment Division, The British Council, 65 Davies Street, London, W.1, quoting Ref. D20/65/UNI/9.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 17, No. 6: Fulgoroidea from Southern Chile (Hemiptera). By R. G. Fennah. Pp. 231–272. 17s. Geology. Vol. 10, No. 9: Dechenellid Trilobites from the British Middle Devonian By Dr. Edwin Brian Selwood Pp. 317–333 + plate 1. 11s. Zoology. Supplement 1: Marine Molluscs in the Cuming Collection, British Museum (Natural History), described by William Harper Pease. By E. Alison Kay. Pp 96+14 plates. 75s. (London: British Museum (Natural History), 1965.) [812

The National Central Library. 49th Annual Report of the Executive Committee for the year ending 31 March 1965. Pp. 24. (London: The National Central Library, 1965.) [812

Commonwealth Mycological Institute. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, Set 7, Nos. 61–70. Pp. 20. (Kew, Surrey: Commonwealth Mycological Institute, 1965.) 5s. [812

Department of Education and Science. Building Bulletin No. 27: Boarding Schools for Maladjusted Children. Pp. v+16. (London: H.M. Stationery Office, 1965.) 4s. net. [812

Ministry of Aviation; Ministry of Defence (Army Department); Ministry of Defence (Navy Department); Inter Service Metallurgical Research Council. Fibre Reinforcement of Metals: a Critical Review. By A. Kelly. Pp. v+26. (London: H.M. Stationery Office, 1965.) 4s. net. [812

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1092, Vol. 258 (2 December 1965): The Effect of a Sudden Change of Shape of the Bottom of a Slightly Compressible Ocean. By C. C. L. Sells. Pp. 495–528. 12s. 6d.; 1.85 dollars. No. 1093, Vol. 258 (2 December 1965): Flow Properties of Mineral Oils in Elastohydrodynamic Lubrication. By A. Sydon. Pp. 529–564. 13s. 6d.; 2 dollars. No. 1094, Vol. 258 (2 December 1965): The *PVT* Properties of Water. 1: Liquid Water in the Temperature Range 0 to 150° C and at Pressures up to 1 kb. By G. S. Kell and E. Whalley. Pp. 565–617. 19s.; 2.85 dollars. (London: The Royal Society, 1965.) [812

Other Countries

Skogshogskolan, Stockholm. Studia Forestalia Suecica. Nr. 28: Om Variation och Stickprovstorlek vid Uppmätning an Frofall, Observation av Kottsättning och Taxering av Plantuppslag. (Variation and Size of the Random Samples when Measuring Seed Fall, Cone Setting, and Stocking) Av Stig Hagner. Pp. 18. 3 kr. Nr. 29: Samband Mellan Personvariabler och Prestation vid Huggningsarbete. (The Relationship Between Individual Characteristics of the Worker and Output of Work in Logging Operations.) Av Jan-Erik Hansson. Pp. 90. 15 kr. Nr. 30: Distribution of the Costs of Joint Forest Roads According to Crosswise and Lengthwise Road Function. (Kostnadsfördelning för Enskilda Skogsbilvägar, Grundad på Vägens Längs- och Tvärsfunktioner.) By Ulf Sundberg. Pp. 22. 5 kr. (Stockholm: Svenska Skogsvardsforeningen, Box 16316, 1965.) [812

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Interim Reports, Series A. Publication No. 76: List of Publications Resulting from the Work of Australian National Antarctic Research Expeditions (to December 1964). Pp 48. (Melbourne Antarctic Division, Department of External Affairs, 1965.) [812

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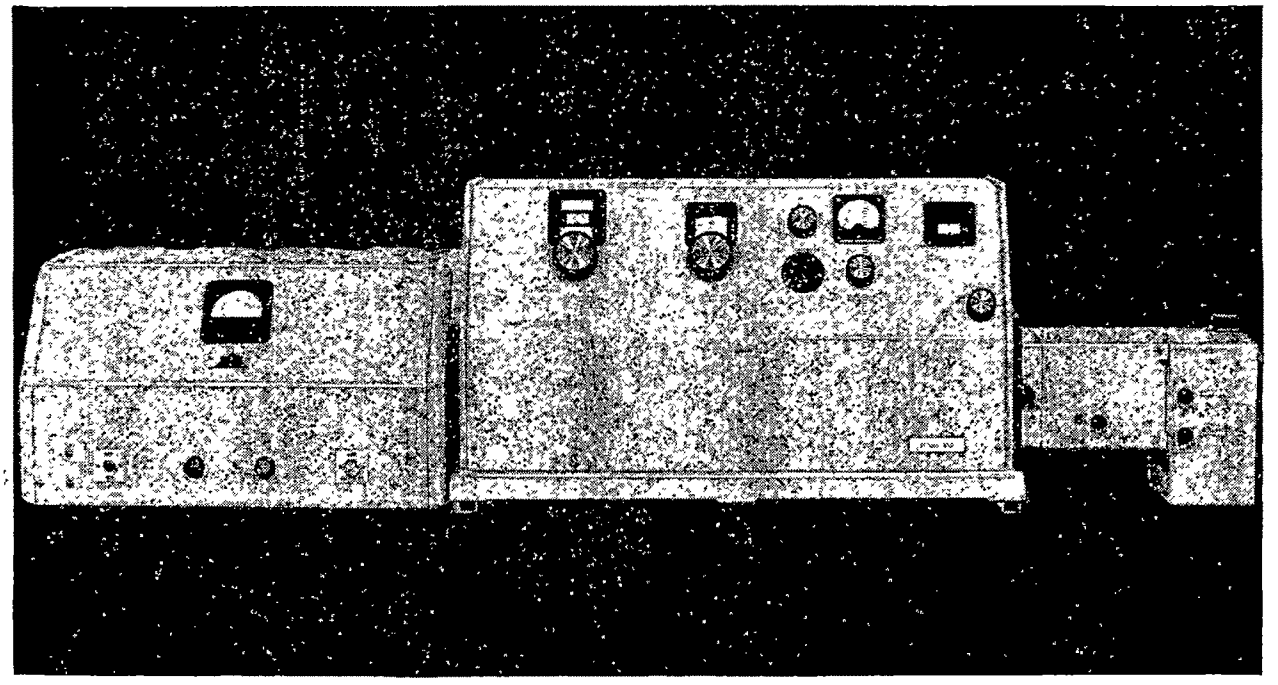
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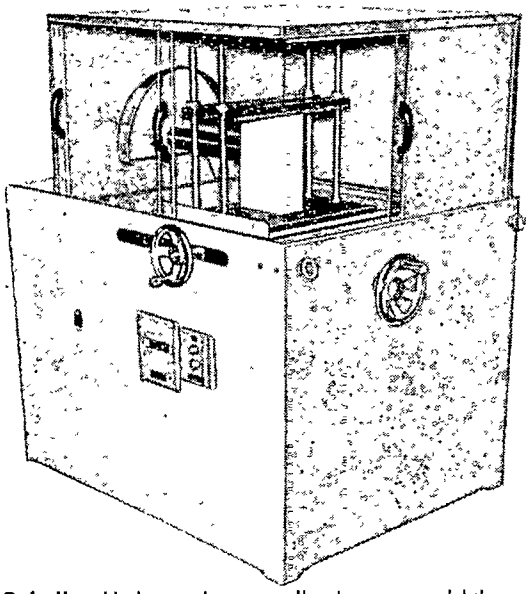
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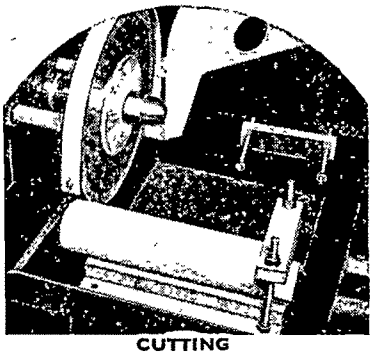
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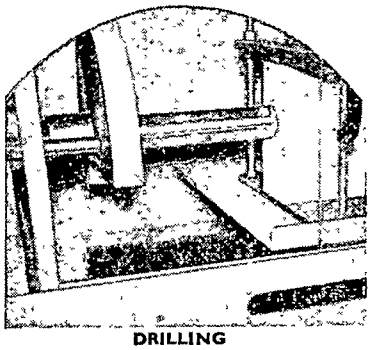
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THE IMPACT OF SOCIETY ON SCIENCE

IN his presidential report to the Carnegie Institution of Washington for 1964–65*, Dr. Caryl P. Haskins once again sustains the sweep and balance, the insight and judgment, which have made these annual presidential surveys as welcome and as stimulating in Europe as they are in the United States.

Dr. Haskins begins by announcing his primary concern with the future of science in the United States, the nature of its growth, its place in society, the quality of its evolution and its rank among the deepest values of the American people. Any examination of such questions, with the penetration and perspective that have characterized Dr. Haskins's reports, inevitably is of considerable interest and validity far beyond the national scene with which it is primarily concerned: his address is once again of deep interest to all concerned with science, whether with the evolution of science itself over the next two decades or with the relations of science to modern society.

Dr. Haskins's report might well be described—and with some justification—as an essay on the impact of society on science. He adopts a historical approach, treating the past history of science as a prologue and using as illustrations the work of Galileo Galilei and Mendel. The great disciplines, for which the anniversaries of Galileo's birth and Mendel's paper commemorate major turning points, are once again so transforming our visions, both of our outer universe and of our inner worlds, as to constitute in our time what may be reckoned as a contemporary scientific revolution fully comparable with earlier ones that are now classic. No later scientific revolution, he observes, has stood apart from the source-stream of that dramatic Greek period when science may literally have been born. In substance as in philosophy, each later burst of scientific growth has been deeply nourished by its heritage of earlier discovery and concept, transmitted in a continuous, if often tortuous, flow.

He holds, moreover, that, however fragmented the course of science may appear when viewed in any of its detailed reaches, it has, in its deeper and more general features, preserved a striking unity over the centuries. He is not, in truth, greatly concerned with the structure of scientific revolutions, and it will be apparent that he is far asunder from Prof. R. S. Kuhn and that his treatment is simpler. He stands rather with Dr. S. Toulmin, noting that while the evolution of the substance of science is—of its very nature—free and untrammelled and not to be confined within the bounds of detailed forecast, the methods of probing Nature have varied widely among different branches of science, sometimes even being in opposition. He shows that, even within a single discipline, the philosophy of approach may change drastically as the field evolves.

Dr. Haskins notes, moreover, a further point which is of special importance at the present time, and this he develops as a main theme. Each burst of scientific growth has been closely linked with the technology of its time, sensitive to the technical needs and demands of its society, profoundly influenced by them, and profoundly affecting them in turn. It is in the context of these profound interactions that he considers the relations between science and technology and the implications and the dangers attendant on the passage of the patronage of science to Government agencies and ultimately to the general public which supports those agencies. Both the magnitude and the proportion of the support which science draws from Government sources are often almost indistinguishable from support for technology. In effect, this support is ultimately apportioned according to the public will and is likely to increase still further.

Already, however, science in the United States, as in Britain, is materially endowed at a level which is unique in the history of man, and at a level also that already seriously taxes our human resources. That is the real reason why widespread understanding of the nature of science, of its distinction from technology, and of the requirements for healthy scientific growth is of increasing and critical importance. Only to a minor extent is support for science to-day drawn from the kind of patrons to whom it had become adjusted during its long formative centuries, and who in turn had become adjusted, in a considerable degree, to its demands and needs.

Dr. Haskins points out that in the past the great successes of science have depended not only on the abilities of scientists themselves but also on the understanding, patience and perceptiveness of supporting patrons. The demand for such understanding is now made not on the few but on the many, on an infinitely greater scale and on a plane far more difficult and more powerful. Meeting this demand is ultimately a problem of education and of communication, of marshalling our developing resources in those two fields to meet our pressing obligations effectively and urgently. Dr. Haskins's two vignettes are well chosen to show just how, in this way, the future of science as a dynamic cultural force in society will be determined.

In the first place, these vignettes well illustrate the pace of change and the limits of our ability to predict the implications of scientific advance and of the impact of science on society. We are scarcely aware, for example, of the potentialities of many of the new tools developed in the past decade. By far the most easily projected visions of the future are those concerning technology and scientific application, and these, precisely because the consequences of advances in science itself, are less predictable. Danger arises in the failure to distinguish between science and technology. There are also, indeed, wide differences in the contours of advance and strategies of attack between different branches of science. Dr. Haskins observes that in many important respects the development of strategy, and indeed of detailed philosophy, has been almost opposite in the physical and in the biological sciences. Concepts and approaches that have proved unsuitable in the past in one area have on occasion been highly evocative in another.

Furthermore, strategies of investigation and foci of interest have often shifted radically during the evolution of a single science. He quotes Sir Peter Medawar's view that the sheer factual content of specific scientific fields is likely to diminish with their growth, rather than pile up over the years to yield even greater and more un-

* Carnegie Institution of Washington. *Report of the President*, 1964–1965. Pp. 73. (Washington, D.C.: Carnegie Institution of Washington, 1965.)

assimilable masses. The great frames of investigation, like astronomy and the biological sciences, however, have continued to build steadily over the centuries. To-day they are frontiers as vital as ever and it is almost inconceivable that they will not maintain that vitality into the foreseeable future.

Dr. Haskins notes one aspect of the critical periods of fundamental change wrought by science which has characterized them since that period of Greek thought in which the scientific mode originated: their remarkable brevity. He suggests that one of the more important lessons of the past is that the eras of major change characteristically ran swift and deep and were incredibly dynamic. Furthermore, periods of intense change have tended, on the whole, to alternate with quieter periods of consolidation. An important factor here is possibly the real need, following a time of violent change, to pause and consolidate, to re-weave the torn fabric of universal ideas. If this factor was and is really significant, Dr. Haskins suggests that, as the pace and variety of scientific advance increase, we may well look for important shifts of pattern. Major sweeps in our ideas about the nature of the universe may not coincide precisely with periods of major growth in our ideas about, for example, the nature of life. We may come to live almost continually, not only with technical change itself but also with a plethora of revolutionary ideas.

That might almost be our present situation and it carries psychological hazards of which we should be sensitively aware. In any event, mankind is undoubtedly in the midst of an era of change in total outlook fully comparable with that of the classic scientific revolutions. Our technological society, however, shows little awareness of the real implications in its efforts to develop institutions to meet our changing needs. The extraordinary degree to which both the substantive content and the character of development of the earlier revolutions reflected and responded to the predominant and technical environments and the social and technical demands of their times is seldom considered. Nor is it always remembered that science seems to have flourished most, and made its richest contributions, in an atmosphere that combined the elements of a maximum freedom of enquiry with those of a lively—though often incoherent—stimulus of social demand and need.

It is in this context that Dr. Haskins considers the nature of the predominant claims made on the contemporary scientific revolution and of the stimuli given to it by our own environment in the years ahead. Both for science and technology the intricate social milieu of our day is, in many respects, a world society, but Dr. Haskins suggests that it is already apparent that in the future the environment created by humanity itself, the environment of human social forms, of individual human needs, must rank high in our reckoning and may equal or surpass more traditional concerns in its relevance and import. This predominant feature of our world must clearly have a profound influence in defining new sectors to command scientific attention, and could be a dominant factor in the internal growth of science.

The development of science in its relations to the society it serves, and of which it forms an integral and decisive part, is another matter, and Dr. Haskins suggests that this influence of society on science is made trebly important by the dynamic growth of our civilization and by its changing concerns and character. He sees science, indeed, as one of the cultural elements of our age, but he also recognizes that the effects on society of, for example, automation raise large questions of social and intellectual satisfaction for individuals. Dr. Haskins sees that science will not make its full contribution to the growth and welfare of society unless the nature and purpose of science are far more widely and deeply understood. There is a genuine social peril in the popular notion of the fragmentation of science, when the distinction between method and goal is not understood and the picture of fragmentation is extended to the modes of the search for knowledge.

Dr. Haskins notes the consequences for science itself if the idea that the whole search for truth leads not to a single coherent vision but to fragmentation. He suggests that we may come to think of scientific truth as being above all else a measure of the coherence of systems of thought and of their capacity to communicate among themselves; as a property primarily of interchangeability both among observers and among scientific propositions relating to differing situations in Nature. If the primary task of science remains the search for truth in such a definition, we may well conclude that the highest aim of scientific enquiry is to make knowledge communicable in all possible situations and that the measure of the validity of such knowledge may be the degree of its coherence among such situations. In striving towards such an objective, the fear of losing overriding purpose loses its reality in a scientific content.

There remains, however, a considerable hazard for the relation of science to our changing society, to which Dr. Haskins now directs attention. If the world remains free from major military conflict, what is likely to happen to the public understanding and evaluation of the scientific outlook in a materially opulent society, which provides generously for the individual wealth and leisure of its citizens? There is a widespread disposition to equate science and its purposes primarily with the two great technical areas of military defence and protection against bodily ills. The public esteem of science is strongly coloured by these identifications, and Dr. Haskins asks whether public concern for the military aspect of technology may not wane in long years of freedom from the threat of military conflict, and wonders how this will affect popular concern with science itself. Furthermore, the tendency to confuse the objectives of technology in general with those of science, although a natural misconception, is too prevalent. In the United States, technology and science have from the beginning served in the closest partnership, the one being indispensable to the other, and throughout the modern world, wherever technology is strong and well-developed, there is also science. Where one is weak the other cannot be strong. Yet their objectives differ in some important respects and the perception of these distinctions is fundamental to a genuine understanding of science.

At this point, Dr. Haskins looks again at the past, where the primitive roots of science and technology once lay far apart. A well-developed technology could and did flourish, for example, in the India and China of Lao-tse, Confucius and their contemporaries, but a scientific evolution was not possible in that philosophical climate. This difference between science and technology remains to-day, and is epitomized by their widely divergent objectives. At one extreme of the spectrum from science through technology, the essential purpose of science is the broadening of knowledge, the deepening of understanding. At the other extreme of the spectrum, the ultimate merit of technology is the effectiveness with which it can bend the knowledge won by science to real social use.

In this comparison, the attitudes and goals of technology, its procedures and its practical scale of values, may approach the general daily working philosophy of society as a whole, rather than the philosophy of science itself. In fact, the characteristic processes and goals of technology are commonly far more visible to a society than are those of its science. This immediate visibility of the aims of technology is enhanced by the circumstance that technology, unlike science, normally produces conspicuous and useful products, which, devised as they ordinarily are with the aid of the knowledge that science provides, are liable to be popularly attributed to science itself. This gives rise to the danger with which Dr. Haskins is fundamentally concerned in his review; in a society as technologically polarized as our own, public misunderstanding of the nature of science and the requirements for healthy scientific growth could, in the long run, seriously jeopardize the quality of science itself. Under such conditions the approaches and goals of science could be subjected, however gently or subtly, to assessments and demands formulated primarily in those terms.

Dr. Haskins's plea for special vigilance on this score in the years ahead, as both the magnitude and proportion of the support of science from Government sources—often almost indistinguishably bound up with support for technology, and in effect ultimately apportioned according to the public will—increase still further, is substantiated by developments in Britain in the past 2 years. It may as yet be too soon to judge whether the Ministry of Technology is really closer to industry than the Department of Scientific and Industrial Research, but there is little evidence that the creation of a Ministry of Education and Science in place of a Ministry for Science offers any advantage. On the contrary, there is evidence to justify the uneasiness of the universities, if not also of the University Grants Committee, particularly in the absence of effective action to remedy weaknesses in the old arrangements which persist into the new.

It is only too clear that the warning addressed by Dr. Haskins to the United States could apply to many other countries. It is quite possible—some might suggest it is already happening—for the fabric of science to be subtly distorted over the long term by public support unwisely or improperly administered, by the lavish granting of funds for this and the withholding of funds for that, on the judgment of a collective patron insufficiently knowledgeable or politically biased. Equally serious is the real danger that any long persistence of such a situation could corrode the inner structure of science by presenting a strong and lasting temptation to disregard scholarly and objective standards, or at least to lower them. Such a situation could cause science to hold out to its public patron arresting technical prospects and thereby acquire additional means to conduct some of the supporting research, or even to add a whole sector of undirected scientific research that could not otherwise have been undertaken.

Dr. Haskins believes that there are contemporary examples of this situation and that it can threaten the very essence of the scientific way itself. It can favour in practice the effective promoter, whose primary value may be the conventional and tired goal of operation for its own sake, over the investigator whose efforts and attention are more deeply committed to winning new knowledge. Even worse, it can further compromise the future by playing on, and thus deepening, the confusion between scientific and technical processes and goals.

Such circumstances give, in Britain, special force to the recommendation of the Parliamentary and Scientific Committee to the Select Committee on Procedure that a new select committee should be set up to examine the annual reports of the Research Councils, the Atomic Energy Authority and other similar bodies and also such scientific and technological matters as were under consideration in Government Departments as a basis for legislation and formulation of policy. The Select Committee on Procedure, in its fourth report last July, embodied this proposal in a wider one which was not very favourably received for the Government by the Lord President of the Council. Nevertheless, the proposal is important and deserves further consideration. Examination in this way of the annual reports of the Research Councils, for example, could well contribute to informed public discussion on the balance of Britain's research effort. It could equally bring to light departmental shortcomings, and it is clear from recent reports from the Estimates Committee that select committees do not regard with favour a tendency to put administrative or departmental convenience before the public interest. The lukewarm reception of the proposal by the Government should be a further reason for its sympathetic and immediate examination.

Dr. Haskins's warning is thus one to be heeded seriously in Britain. It is a part of the problem of the communication of ideas, of the emerging science of science. No doubt, it will be noted by Prof. Blackett and his colleagues in the Royal Society in considering what part that Society should play in the future in the mechanism of scientific advice and in determining the balance of Britain's scientific effort. But the debate must range wider, and the very reluctance of the Ministry of Technology and of the Department of Education and Science to report on their activities makes public debate the more imperative, both within and outside Parliament. The recent plea of the Chairman of the Parliamentary and Scientific Committee deserves the strongest support for the reasons which are so lucidly displayed in Dr. Haskins's review.

A STRAND OF ACADEMIC HISTORY

300 Jahre Physik und Astronomie an der Kieler Universität

Von Charlotte Schmidt-Schönbeck. Pp. 261. (Kiel: Verlag Ferdinand Hirt, 1965.) 15 D.M.

THE University of Kiel was founded in 1665 as part of the reconstruction after the Thirty Years' War and was initially intended for the training of clergy and state officials. During its first hundred years its expansion was checked by dependence on the resources of an impoverished duchy; thereafter it flourished for a century under Danish rule until 1863, when it passed, with Schleswig-Holstein, to Prussia. Under the German Empire the University enjoyed its heyday and scientific and medical studies came into prominence. It was devastated during the Second World War, but has since made a remarkable recovery as a national institution.

300 Jahre Physik und Astronomie an der Kieler Universitat traces the fortunes of physical and astronomical studies at Kiel through the past three centuries by presenting a series of brief biographical studies of forty-seven of its most distinguished teachers in these fields. These notices are grouped according to the special sciences and the periods covered, and each section is prefaced with an account of the relevant developments in the wider field

of European science. Introductory to the whole book are a brief history of the University and an outline of the rise of the physical sciences from antiquity through the Middle Ages and up to the point at which Kiel began to play its not inconsiderable part in their further advancement.

It is of particular interest to note the points at which revolutions in scientific thought began to be reflected in the courses of instruction given at this little University. On the physical side, Kiel appears to have lagged behind the advance of European science in general. Founded some twenty years before Newton's *Principia* appeared, the University courses had assigned no secure place to the Copernican theory by 1700, and it was not until about 1800 that they embraced Newtonian physics. However, about the middle of the nineteenth century there was a great advance on this state of affairs when Kiel established links with more progressive German Universities; thereafter, teachers of the calibre of Heinrich Hertz, Max Planck and Hans Geiger were attracted. The twentieth century brought financial stringency and political persecution, but now, once again, the omens for this historic academy seem favourable.

During the seventeenth and eighteenth centuries, no sharp distinction was drawn at Kiel between physics and astronomy. In the mid-nineteenth century, however, the two sciences drew apart following the foundation of the Kiel Observatory and the creation of various physical institutes. Thereafter, the progress of these two disciplines receives separate treatment in Dr. Schmidt-Schönbeck's pages. The Observatory was completed in 1876, though equipped at first only with old instruments brought from the disused observatory of Altona. Under its first director, C. A. F. Peters, the resources of the new institution were chiefly applied to the time-honoured problems of positional astronomy—the precise determination and cataloguing of star places and motions—while contributing also, latterly through the labours of Elis Strömgren, to the refinement of planetary and cometary orbits. The publication of the *Astronomische Nachrichten* long remained a responsibility of the Kiel astronomers.

This useful handbook presents an immense amount of information which has hitherto not been available, based on a bibliography occupying thirty-seven pages and illustrated with numerous portraits and pictures of historic instruments and institutions. Though primarily concerned with the fortunes of one provincial 'Hochschule', in one aspect of its work, the book possesses a wider interest, for the developing situation at Kiel was typical of the circumstances encountered by several other small German universities.

A. Armitage

EDUCATIONAL RESEARCH

Innovation and Research in Education

By Michael Young. (Institute of Community Studies.) Pp. viii + 184. (London: Routledge and Kegan Paul, Ltd., 1965.) 35*s*. net.

Student Guidance

By Claude Palmer. (Education Today.) Pp. xvii + 108. (London: Longmans, Green and Co., Ltd., 1965.) 10*s*. net.

Innovation and Research in Education was written by Dr. Young before the report of the Heyworth Committee on Social Studies was published, in order to clear his own mind as to priorities in educational research. Since its publication, however, the Government has announced its decision to establish a Social Science Research Council and Dr. Young has been appointed its chairman. Accordingly, the book derives further interest as an indication of Dr. Young's own attitude towards research in the social sciences in general. It is described by him as an invitation to teachers in schools and in training, as well as to administration, to join in a common endeavour to promote research and innovation in education. He argues that the social sciences will be greatly advanced when the entanglement of the social scientist in society is better understood and that this understanding will be promoted if the social scientist deliberately seeks to become involved in action rather than to keep clear of it. Only by linking innovation and research will the methods of the social sciences be greatly improved. Progress will be made not so much by seeking to copy the natural sciences as by recognizing that the social scientist is closely involved with his material and that the problems he has to examine are proposed by his social environment, by his research community, or by other people. Dr. Young strongly emphasizes the importance of the research worker as a variable in any social research, and he insists that, without recognizing the interaction of the research worker and his material, there can be no growth in precision. Unless research workers are more precise, they will not know the grounds of disagreement, and unless disagreement between workers is reduced there can be no cumulative advance of the kind the social sciences need if they are ever to match the progress of the natural sciences. On this view, the most important initial task is to endeavour, with the help of control groups, to measure the effect of observation on its field and that here the vital question is of method.

Dr. Young believes that education offers the greatest scope for advance along these lines in social science research, and points to the closer co-operation between people with different kinds of training which would be generated by experiments along the lines that he suggests. In successive chapters he thus considers the influence of fashion in research, the problems presented by innovation in the classroom, by families and pupils, and in co-operation between home and school. In his final two chapters he discusses methods of research and the organization of research in education. Appendixes are provided describing the extent of research in Departments and Institutes of Education, the organization of educational research in the U.S.S.R., by Dr. W. D. Wall, and finally S. S. Dunn assesses educational research in the light of Australian experience. Apart from an unhappy opening sentence, the book is well written and Dr. Young argues his case clearly and cogently; education, he maintains, needs innovation more than it needs research, and he points out that all-important social changes are made on inadequate evidence and probably always will be. He considers the possibilities of innovation in examinations as well as in the classroom itself, and in co-operation between home and school. In educational research, he concludes that, in the near future at any rate, progress is most likely to be made by setting up some experimental schools and by backing small-scale research run by teachers and research workers in co-operation. He maintains that the Social Science Research Council, which he takes for granted, should be concerned with rather more fundamental research into education, while the Department of Education should be concerned more with the applied aspects. No organization, however efficient, will itself produce research of first quality; that depends, he reminds us, not on committees but on people with a sense of commitment and with a flair for choosing the most hopeful pathway into a problem. He does not overplay the administrator and his hope is that a more scientific approach to education will do something to make it more human. Dr. Young's criticisms are searching but they are fair and open-minded, and the tone of the book, for all its uncomfortableness, is healthy and refreshing.

Minor support for Dr. Young's argument is forthcoming from Mr. Palmer's book, which gives a detailed account of his experience as student adviser at the Barnet College of Further Education. It goes to the root of many student failures and provides convincing evidence that if students are to do themselves justice in further education some

form of student guidance must be an integral part of our educational system in the foreseeable future; it will remain an important element in our social services for young people so long as adolescence remains a time of increasing strain and difficult decisions. The value of such an appointment is fully demonstrated, on the grounds of its contribution to student welfare, to improving their intellectual efficiency, and thus adding to the amount of the talent available to the nation as a whole—and to minimizing the diversion of ability into anti-social channels. In the long run, it offers a saving in time and emotional strain to other members of the staff which it affects, improving teaching and making education in these colleges more efficient, which, in the terms of results achieved, more than repays the financial outlay. *Student Guidance* provides an example of exactly the kind of innovation desiderated by Dr. Young, but Mr. Palmer also pleads for a great expansion of research in all fields of education with as much freedom as possible for the research workers. Likewise, he emphasizes the importance of some independent sources of finance. Some of Mr. Palmer's observations are provocative, and he reminds us that teachers and administrators and many employers can all be a source of frustration. Emphasizing the importance of the teaching of English—failure to master which he regards as an integral part of the overall failure of the student to cope with his or her environment—he observes that we are wasting the talents of thousands of our bright boys and girls, young men and women, for just this neglect. Here he comes near to echoing Dr. Young's own remark that teachers need to concentrate on their proper function of encouraging the desire to learn. In his open-mindedness, he also comes near to reaffirming Dr. Young's further remark that research on the raising of the school-leaving age and what is involved is unlikely to go far if the investigators are already firmly convinced that the extra year is in itself a good thing, or at the least can do no harm. For such open-mindedness, as is shown by these remarks alone, both books are timely and stimulating.

R. BRIGHTMAN

MOLECULAR GENETICS

The Evolution of Genetics

By Arnold W. Ravin. (Academic Paperbacks.) Pp. x+216. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 22*s*. 9*d*.; 2.95 dollars.

IN one way *The Evolution of Genetics* is an excellent book, in another it is depressing. The trouble arises from its title, which leads one to expect some cogent illustration of the way in which contemporary genetics has evolved, an expectation to which the author adds when he complains in his preface that "the debt of modern genetics to the past is easily overlooked".

Yet with what are we presented? 32 pages on the legacy of classical genetics, 147 pages on modern molecular genetics, and 23 pages on the future of genetics. In these first 32 pages we have as the main legacy of classical genetics the concept of a unit of inheritance—the gene (Mendel); the concept of relation between genes and metabolic blocks (Garrod); the concept that genes are chromosomal (Sutton); linkage (Morgan); proof of chromosome exchange in crossing-over (Stern); the origin of theoretical population genetics (Hardy–Weinberg; Fisher, Haldane, Wright); and proof that the cytoplasm counts too (anon).

In the rest of the book we find that, prior to 1940, Griffith had discovered transformation and Muller and Stadler had discovered the mutagenic effects of radiation. Furthermore, and here we come to the crux, we are told (p. 87) that "The very power of the microbial system led to a breakdown of one of the tacit assumptions of classical genetics. The gene, it had been presumed, was a unit of the genetical material that functioned as a single entity in controlling some phenotypic character and responded as a whole to mutation and to recombination". "It was Seymour Benzer who brought this conclusion emphatically home" using phage.

Opinions of which were the key points that led to the opening up of molecular genetics may vary; but none could omit any of the following. The introduction of micro-organisms to genetics by Dodge and Lindegren and to biochemical genetics by Beadle and Tatum; the demonstration by Avery that the transforming factor is DNA; the production of a genetically intelligible structure for DNA by Watson and Crick; and—the point which the author rightly describes as basic—the discovery that genes defined as segregational units, as recombinational units, as mutational units and as physiological units are not necessarily coextensive. But this discovery did not depend on micro-organisms. Well before Benzer's work began, Fisher, Goldschmidt, Darlington, and Mather, among others, were aware that different definitions of the gene were not synonymous, and the demonstration that physiological units were complexes that could be broken down by recombination was made primarily with *Drosophila* (Lewis, Pontecorvo). To say this is not to minimize Benzer's contribution. It is merely to contradict the central point in this account of the evolution of genetics.

As an account of contemporary molecular genetics, the book is simple, straightforward, well written, and has much to recommend it, and the concluding section is a thoughtful account of the views of the author of the problems geneticists must face in the future. It is a pity the author, in his title and preface, should have suggested he was trying to show how molecular genetics arose, a task which, had it been well done, would have been very well worth doing.

J. M. THODAY

A MOLECULAR BASIS OF EVOLUTION

Evolving Genes and Proteins

Edited by Vernon Bryson and Henry J. Vogel. (A Symposium held at the Institute of Microbiology of Rutgers, The State University, with support from the National Science Foundation.) Pp. xxi+629. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 156*s*.

THIS well-presented though expensive volume makes available a detailed account of the proceedings of a symposium held at Rutgers University in September 1964. An impressive array of scientists delivered papers which are collected into nine parts, eight of which begin with a chairman's introductory remarks and which end with open discussion. There are two parts dealing with the evolution of biochemical pathways and three each with the evolution of proteins and genes.

Dedicated to the late D. M. Bonner, *Evolving Genes and Proteins* starts with a list of participants, a short preface by the editors, and a welcome by J. O. Lampen. At the end of the book there is a complete author index and a sensible subject index.

An opening address was delivered by E. L. Tatum, who attempted to place the present knowledge about the molecular basis of evolution in perspective within the framework of the rapidly developing discipline of molecular biology.

In the first section, N. H. Horowitz surveyed the evolution of biochemical syntheses. He proposed a process of retro-evolution whereby evolution started with an end-product of a biosynthetic pathway and worked backward stepwise towards the beginning of the reaction

chain. Thus the first enzyme should carry a memory of its origin. Next H. J. Vogel showed how a biochemical characteristic such as the lysine pathway could be useful to observe evolutionary relationships among a variety of lower organisms.

Part 2 started with K. Bloch's discussion of the evolutionary patterns observed in an examination of the lipids from a variety of organisms. S. Granick then proposed how the biosynthetic chains involved in respiratory and photosynthetic functions developed to produce haem and chlorophyll. He also described the recent evolution of a haem oxidative enzyme and its controls when animals progressed on to land.

The first section on the evolution of proteins began with a long description of evolutionary divergence and convergence in proteins by E. Zuckerkandl and L. Pauling. They assumed that the most important thing to do in the life sciences is to uncover the molecular mechanisms which underlie the observations of classical biology. Next, J. Buettner-Janusch and R. L. Hill discussed the interplay between molecular and organismal aspects of evolutionary events through an investigation of the haemoglobin of man and his primate relatives. Indeed, the differences and similarities among primate haemoglobins reflect to a great extent present ideas of phylogeny. Finally, in this section, G. Braunitzer and his colleagues surveyed amino-acid composition and sequence data of selected respiratory and viral proteins.

The first two papers in the second section on proteins dealt with the structural and functional aspects of proteins presumed to be biologically ubiquitous. Thus phosphoglucomutase and cytochrome *c* were studied by P. Handler's group and E. Margoliash and E. L. Smith respectively in the hope of identifying a protein which is the expression of a homologous gene, thereby exemplifying the common ancestry of life on this planet. N. O. Kaplan then described methods whereby structural and functional relationships among dehydrogenases from different organisms can be compared. He showed that there has been little or no evolutionary change in the structure of coenzymes and discussed the significance of alterations in enzymes. The final paper of this section by W. J. Rutter analysed the molecular features of functionally related proteins derived from various biological sources to provide evidence for the interpretation of basic evolutionary mechanisms.

P. H. Abelson, in his introduction to the final section on proteins, gave an example in which phylogeny based only on protein structure yielded a pattern clearly inconsistent with the taxonomy of the zoologist. The results of detailed studies in the laboratory of the late D. M. Bonner on the tryptophan synthetase systems from *E. coli* and *N. crassa* supported the proposal that the interaction between catalytic units might be followed by conversion to single polypeptide systems. The next paper described work in W. D. McElroy's laboratory and compared luciferase preparations from various species of firefly. E. Freese and A. Yoshida then reviewed the role of mutations in evolution and suggested that rules concerning the preferred types of spontaneous mutations can be used to deduce the nucleotide sequences of normal and mutant genes from the amino-acid sequences of the corresponding proteins. After a summary by S. E. Luria of the evolution of the lactose utilization gene system in enteric bacteria, the final paper in this section was delivered by S. W. Fox. He described *in vitro* experiments which suggest that proteins evolved before cells and genes.

An evening lecture by T. M. Sonneborn on the degeneracy of the genetic code has been expanded for inclusion in this volume. He proposed that natural selection should operate to minimize nonsense and maximize sense.

Part 7, the first section on the evolution of genes, began with a survey of the synthesis of polydeoxynucleotides by A. Kornberg, who suggested that a reiterative copying process might operate occasionally *in vivo* with reference to the natural evolution of DNA. Next E. K. F. Bautz pointed out that RNA species with no coding function have fixed nucleotide compositions whereas those with coding properties can exhibit extensive variations as reflexions of the DNA's characteristic of particular organisms. Using data from analytical studies of *m*RNA made on *T4* DNA as template, he was able to make the much anticipated but important observation that *in vitro* replication and *in vivo* transcription have the same polarity. Finally, in this section, J. Marmur's group described defective bacteriophage particles found in *B. subtilis* and suggested that such particles might represent an evolutionary stage close to temperate phages.

A. Rich started the following section on genes by surveying the details available about the mechanism of protein synthesis. With reference to the evolutionary history of the protein synthesizing mechanism, he envisaged an early stage when the genetic code was not universal. H. Harris then reported on the nature of the short-lived RNA of the cell nucleus. He thought that most of the messenger type RNA produced in the cell was quickly degraded but occasionally mutations occur in the unstable *m*RNA conferring stability on it, thus allowing it to function in the cytoplasm should it produce an evolutionary desirable protein. To close this session N. Sueoka discussed the evolution of informational macromolecules. He also suggested that the accepted scheme for the translatory mechanism was established before the ramification of organisms.

M. Demerec commenced the final session and concluded that genes might be homologous with respect to their function yet differ in their molecular structure. Next, V. Moses and M. Calvin discussed the possible evolutionary significance of molecular regulation with particular reference to the consequences of differential *m*RNA stability. Studies of the relation of *r*RNA to the genome carried out by S. Spiegelman and S. A. Yankovsky are reported in detail. They concluded that a multiplicity of cistrons exists for *r*RNA and also located *Drosophila* DNA complementary with *r*RNA in the region of the nucleolar organizer. The work of R. B. Roberts and his colleagues was discussed with reference to the evolution of polynucleotides. Finally, the symposium ended with R. Sager pursuing the possible paths of the evolution of genetic material from the consideration of work on the recombination of non-chromosomal genes.

Clearly the mass of data collected together in this volume makes it a necessity for specialists in this field and provides a background for those wishing to enter the field. It will be readily understood by graduates trained in biochemistry. Because of the range of articles, which includes reviews and the presentation of new results, I have commented only on what I consider to be the more interesting points made by the various speakers. No modern library for biological chemistry should be without it.

B. F. C. Clark

SENSORY DEPRIVATION

Inside the Black Room

By Dr. Jack A. Vernon. Pp. xvii + 203. (London: Souvenir Press, Ltd., 1965.) 21*s*. net.

INSIDE the Black Room is an attempt to give a popular account of the work done on sensory deprivation by a number of research workers at Princeton, and was first published in the United States in 1963. The project was made possible by grants from the Office of the Surgeon General of the U.S. Army and from the National Science Foundation, and the type of research followed was that previously initiated by Dr. D. O. Hebb and his associates, working at McGill University, where the term 'black box' was first incorrectly used to indicate the room in which the experiments were conducted. At McGill

the cubicle in which the subjects were confined was lighted but rendered semi-soundproof: at Princeton the room was lightproof and soundproof.

Examination of the effects of sensory deprivation was originally intended to throw light on the reduction of variability and quantity of sensory stimuli as a potential hazard in space flight, but even before these experiments unusual sensory effects had been recorded with radar observers and other workers engaged in very monotonous and routine jobs. As observations multiplied, so the whole subject became connected with what was called 'brain-washing' and this was the main point dealt with by the experiments at McGill.

Dr. Vernon's arrangements were more severe than those of Dr. Hebb, although both used only male subjects, those assisting Vernon being about one hundred Princeton graduates aged between twenty and thirty-two. Confinement was usually continued up to ninety-six hours, although the subject could obtain release at any time should he find the situation intolerable by pressing a button, although no continuation of the test was permitted once he had left the cubicle. The usual arrangements were made for providing food and toilet facilities.

As a result of the experiments a number of facts relating to conditions of sensory deprivation were found. About one out of five subjects used the release button before their time was up; and although they were forbidden to smoke they found to their surprise that they no longer wanted to do so. It was found that, using the body-sway test, suggestibility could be increased and the under-estimation of time was a marked characteristic. Effects on thinking varied: in some cases it remained clear, but for most subjects connected thought became weaker the longer confinement was continued.

As regards physical effects, it was found that confinement had an undesirable effect on some simple motor tasks, although no effect on reaction time was observed. Sensitivity to pain was increased, and what was more unexpected was a loss of weight due to the fact that the subjects did not consume enough to prevent it.

Dr. Vernon's study is a clear and interesting exposition of the general effects of sensory deprivation under certain well-defined conditions. But what is now clear is that much more work will have to be done, including tests with less educated and with female subjects and with persons of widely differing age groups. It seems that man does not well endure such conditions, since they afford no variation in the few stimuli presented to him. Even the simplest stimulus is received with pleasure and becomes a subject for study. If the results of sensory deprivation are to be accepted, then the dependence of modern man on the external world and the limitations of his own self-sufficiency have been exemplified. E. J. DINGWALL

PARASITES OF FRESHWATER FISH

Key to Parasites of Freshwater Fish of the U.S.S.R.

Compiled by I. E. Bykhovskaya-Pavlovskaya *et al.* Translated from the Russian. Pp. viii + 919. (Jerusalem : Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 180*s*.

KEY to Parasites of Freshwater Fish of the U.S.S.R. was originally published in 1962. The book contains keys to the identification of 1,211 species of parasites from freshwater fish of the U.S.S.R. The parasites belong to seven phyla and fifteen classes of invertebrates. The keys are constructed on a dichotomous pattern, and require comparisons of characters of the parasite to be made. A description of each species of parasite is given.

The Protozoa occupy 231 pages of the translation; the Coelenterata, 2 pages; the Platyhelminthes Monogenoidea, 207 pages; Cestoidea, 66 pages; Trematoda (Digenea), 104 pages; the Nemathelminthes Nematoda, 67 pages; Acanthocephala, 45 pages; the Annelida Hirudinea, 13 pages; the Mollusca, 4 pages; the Arthropoda Crustacea, 57 pages; and Arachnoidea, 5 pages. There is a twelve-page index of common and Latin names of the fish, followed by a thirty-nine-page list of parasites of the freshwater fish of the U.S.S.R., arranged under species of host. The bibliography gives references to 249 Russian and 116 foreign (non-Russian) publications. A list entitled "Basic Manuals, Keys and Papers on Fish Parasites" contains thirty titles, all by non-Russian authors.

The index of Latin names of the parasites is photographically reproduced from the original publication and occupies the final thirty-three pages of the book. Unfortunately, the page numbers of the original book are given, and although these are shown at the margins of the text of the translation, the index is inconvenient to use at first. Thus the text of p. 665 of the Russian original appears on pp. 784 and 785 of the translation.

According to the introduction, the book is for the use of persons of biological background who are not necessarily parasitologists. Each section commences with an introduction which describes the morphology, biology, phylogeny and epizootological significance of the parasites being considered. This is an excellent feature, but I am doubtful if a person inexperienced in the study of some of the groups of parasites would be able to find and correctly interpret some of the structures. It is strongly recommended that identifications made by inexperienced workers be referred to an expert for confirmation. This comment is not intended to condemn the selection of key characters, or the construction of the keys. It is a warning that identification is not necessarily an easy process, and that incorrect determinations can be easily made no matter how good the keys.

The descriptions given for each species of parasite are brief and to the point. The illustrations are largely from the world literature, although some are original. On the whole they are good, but some have suffered in quality owing to their manner of reproduction in the translation.

The book is a key to the parasites of the fishes of the U.S.S.R., and species not found in the U.S.S.R. are omitted. This is an obvious point, but, as the translation will be used outside the U.S.S.R., it is an important one. If the keys do not work for a parasite, it must be remembered that it may be a species not recorded from the U.S.S.R.

The translation from the Russian appears to be accurate. A comparison of the part of the book dealing with the class Cestoidea was made with a translation of the same part prepared by the Translations Section of the National Lending Library[1]. The comparison was favourable, as the two translations were identical in factual content and interpretation of the Russian original.

The editor and authors of the original publication are to be congratulated on the excellent compilation. As they point out in the foreword, the authors are under no illusion that the work is complete, or free from deficiencies. They invite critical remarks and supplementary material for incorporation in future editions. The translators should also be complimented on the production of the English version in the relatively short time since the appearance of the original.

While not everyone will agree with the taxonomic treatment given to some of the groups of parasites, this in no way affects the value of the book as a key-work. The book is highly recommended, and should be an invaluable aid to the identification of freshwater fish parasites. The keys were tested with a variety of parasites from several groups, and the correct identifications achieved. JAMES C. CHUBB

[1] Dubinina, M. N., *Class–Tapeworms*. Translation of pp 384-438 Opredelitel Parazitov Presnovodnich Rib S S S R. Akad. Nauk S S.S.R Moscow-Leningrad (National Lending Library for Science and Technology, December, 1964)

The Works of William Harvey, M.D.

Translated from the Latin with a Life of the Author by Robert Willis. (The Sources of Science, No. 13.) Pp. xcvi+624. (New York and London: Johnson Reprint Corporation, 1965.) 25 dollars.

THE Johnson Reprint Corporation are publishers of an excellent series, "The Sources of Science", in which they reprint the original work of pioneers, such as Boyle, Newton, Kepler and Grew. This compendious volume contains the works of William Harvey; a reprint of *De Motu Cordis*, *De Circulatione Sanguinis* and *De Generatione Animalium*, which were prepared for the Sydenham Society by Robert Willis and published in 1847. Willis also included some of Harvey's letters to foreign medical correspondents, the report on Thomas Parr, a transcript of Harvey's will and a memoir of Harvey.

Robert Willis, M.D. Edinburgh, was Librarian of the Royal College of Surgeons from 1828 to 1845. After this he was in general practice at Barnes. A good linguist, he translated Wagner's *Physiology* from the German and *Rayer on Skin Diseases* from the French. He died in 1878.

Willis had had a classical education, and his translations of Harvey's works were an improvement on those of previous translators. Harvey wrote excellent Latin, *pace* Aubrey, but his English translators, including Willis, are not sufficiently accurate for those doing either historical or experimental research. Willis's translations of *De Motu Cordis* and *De Circulatione Sanguinis* have been superseded by the scholarly translations of Prof. Kenneth J. Franklin (now obtainable in the Everyman edition). Prof. Franklin has exceptional gifts for translating the works of Harvey, being a physiologist, classical scholar and medical historian. This reprint of Willis's English translation is, therefore, chiefly of historic interest, although until Prof. Franklin's translation is published it is the best available of *De Generatione*.

ARTHUR MACNALTY

Le Message Héréditaire

Par Jean de Grouchy. Pp. xi+196. (Paris: Gauthier-Villars, 1965.) 26 francs.

IN the past ten years molecular genetics has advanced in a spectacular way, and several people have felt the urge to communicate these developments to a wider public. There has been a spate of books written at various levels which should ensure that the present generation of sixth formers and undergraduates can easily assimilate the major developments. Even the youngest of the masters, James Watson, has recently given us a stimulating and informative summary, *The Molecular Biology of the Gene*.

Le Message Héréditaire is written at an intermediate level, suitable for students in general biology, and—with its several examples from human disorders—for medical students. It follows the general pattern set by Dr. de Grouchy in 1958 with his *L'Hérédité Moleculaire*. Comparison of the two books is instructive because it reminds us how radically views have changed during the past seven years. During this time the roles of transfer-RNA, messenger-RNA, polyribosomes, enzymatic transcription and coding have all been clarified, and a beginning has been made in the analysis of mechanisms controlling expression of genes. Dr. de Grouchy conveys his message with simplicity and clarity, quoting most of the classical examples from microbial and human genetics. Even controversial subjects such as control mechanisms are handled tactfully. The Jacob–Monod system is fully presented, but some recent microbiological evidence which has led to conclusions at variance with the Parisian orthodoxy are mentioned. Suggestions that a number of human disorders are due to abnormalities of control mechanisms are quoted, even though one does not, unfortunately, know in any single case whether this is the correct explanation of the observations.

The material is adequately illustrated and systematically presented, so the absence of an index is no great handicap. The book is accurate and well written, and it will doubtless be widely read by French students; but the language barrier, and the fact that excellent alternatives are available in English, will probably limit its use in Britain and the United States.

A. C. ALLISON

Outline of Endocrine Gland Syndromes

By Prof. T. S. Danowski. Pp. ix+374. (Baltimore: The Williams and Wilkins Company, 1965. Distributed in the U.K. by E. and S. Livingstone, Ltd., Edinburgh.) 50*s*. net.

OUTLINE of Endocrine Gland Syndromes provides a synopsis of present knowledge regarding the aetiology, clinical features, differential diagnosis, prognosis and therapy of endocrine diseases with the exception of diabetes mellitus which is discussed in a companion volume. Although the style of writing is in places somewhat cryptic, most of the relevant information is presented, the sections dealing with the thyroid, parathyroids and adrenal cortex being more comprehensive than those concerned with the anterior and posterior pituitary and the gonads. The bibliography on purely clinical topics is generally adequate and includes many recent references. However, the value of hormone assay procedures in the diagnosis and management of endocrine diseases is not greatly emphasized, and it is to be hoped that in later editions of the volume this aspect of the subject will be expanded. The book in its present form should prove valuable not only to the clinical endocrinologist but also to the general physician who is anxious to acquaint himself with recent developments in this speciality.

JOHN A. LORAINE

Bibliography of Statistical Literature

1940–1949. By Dr. Maurice G. Kendall and Alison G. Doig. Pp. 190. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 55*s*.

THIS is the second volume to appear of the projected three-volume bibliography which is intended to provide a complete bibliography of statistical literature from its earliest beginnings until 1958, when the *International Journal of Abstracts of Statistical Theory and Method* commenced its coverage. The volume for the period 1950–58 appeared in 1962, and will already be familiar to many statisticians. The high standard set by this earlier volume has been maintained. In each volume papers are listed alphabetically by the name of the author followed by the title and exact journal reference.

Since there is at present no indexing by subject, the bibliography as it stands is of little direct use to the statistician, but the availability of this almost exhaustive list of statistical articles will make it easy to compile specialized bibliographies for any particular purpose. There is also a reasonable hope that a subject index, such as that already prepared by M. J. R. Healy (described in the *Journal of the Royal Statistical Society*, series A, 126, 270; 1963), will eventually be published. The work is therefore likely to gain in value as time passes.

A serious defect of the work is that quite a high proportion of the titles in foreign languages are not translated into English, although in some cases a French or German translation is given. It is often fairly easy to guess the meaning of those in Portuguese or Italian, but occasional Swedish titles, for example, may be completely baffling. No doubt the authors, with inadequate resources and limited time, have done far better than could reasonably be expected. The fault lies rather with the authorities who failed to provide assistance when it would have done most good.

D. F. KERRIDGE

ON THE NATURE OF THE QUASI-STELLAR SOURCES

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In a recent paper, under the same title as this article[1], two of us have discussed both the usual cosmological interpretation of the red-shifts of the spectrum lines of the quasi-stellar objects and also the possibility that these objects are comparatively 'local' to our Galaxy, with distances of 1–10 Mpc. The work was begun with the conventional prejudice toward the cosmological interpretation, the initial aim being to disprove the local hypothesis. In the outcome, however, we were not able to satisfy ourselves that the local hypothesis must be discarded—in fact we arrived at a balanced position between the two hypotheses. In this communication we do not propose to repeat the former arguments but to put forward new considerations which seem to us to point toward the local hypothesis.

The new argument turns on the Compton process in which relativistic electrons are inelastically scattered by low-energy photons. The importance of this process was realized by Greenstein and Schmidt[2] and also discussed by Ginzburg, Ozernoi and Syrovatsky[3]. Like synchrotron radiation, the Compton process depends on the presence of electrons of relativistic energies. The electrons lose energy by the synchrotron process and by the Compton process in accordance with the ratio:

$$\frac{\text{Loss by Compton scattering}}{\text{Loss by synchrotron scattering}} = \frac{U_{\text{rad}}}{H^2/4\pi} \qquad (1)$$

where U_{rad} is the energy density of the radiation field. In order to apply the relation (1) to the Compton process it is necessary to discuss two important questions: (*a*) What is the intensity of the synchrotron emission from quasi-stellar objects? (*b*) What are the dimensions of the emission regions? We begin with a discussion of (*a*).

The observed radiation from quasi-stellar objects is thought to be derived from two processes: (i) synchrotron radiation and (ii) radiative recombination of an ionized gas. The most detailed studies have been carried out for 3*C* 273 and 3*C* 48. There is no question but that the radio frequency radiation from the quasi-stellar objects is generated entirely by the synchrotron process. However, as will become clear from the later discussion, it is the high-energy density of radiation which comes almost entirely from the flux emitted in the optical and infra-red spectrum which is of critical importance here.

Matthews and Sandage[4] ascribed the whole of the continuous radiation from 3*C* 48 in the wave-length range $3{\cdot}0$–$8{\cdot}3 \times 10^{14}$ c/s to the synchrotron process. Greenstein and Schmidt[2] and Oke[5] have both discussed the optical spectrum of 3*C* 273 in terms of radiative recombination of a hot gas. In Fig. 1 we compare the observed continuous energy distribution in the optical and near-infra-red regions published by Oke and by Johnson[6] with the theoretical radiative recombination spectra of the two models discussed. The two theoretical spectra have been given an arbitrary vertical shift to facilitate comparison of the forms of the continua.

Oke chose a value of $T_e = 160{,}000°$, which gives the observed Balmer discontinuity, *B*; however, the Paschen continuum must then be ascribed to a component of unknown origin (which Oke supposed to be synchrotron radiation) which rises steeply into the near infra-red. As Oke pointed out, Greenstein and Schmidt's model with $T_e = 16{,}000°$ requires Balmer and Paschen discontinuities much larger than those observed; thus in their case also a spectrum rising steeply into the infra-red must be added to the recombination spectrum. Searle *et al.*[7] have shown that in each case the spectrum which must be added has the same slope as those of the Crab nebula and 3*C* 48. This is very strong evidence that the near infra-red continuum of 3*C* 273*B* is dominated by synchrotron radiation; Dibai and Pronik[8] have also given strong arguments for this conclusion. The presence of a small Balmer discontinuity together with the Balmer emission lines shows that a hydrogen recombination spectrum is also present and begins to dominate below λ4,000.

Extension of the observations of 3*C* 273 into the infra-red ($\nu = 3 \times 10^{13}$–$1{\cdot}4 \times 10^{14}$ c/s) by Johnson and Low[9] and into the millimetre range ($\nu = 10^{11}$–3×10^{11} c/s) by Low[10] and Epstein[11] has shown that this object is an exceedingly powerful emitter in these regions. For other quasi-stellar objects there are no published observations of fluxes in the infra-red, but indications that the fluxes are rising in the near infra-red suggest (because of the effect of the red-shift) that a synchrotron contribution may be important in the optical region in some objects.

An integration of the energy distribution curve of 3*C* 273 shows that the total flux received at the Earth in the frequency range 10^{11}–5×10^{14} c/s is $5{\cdot}3 \times 10^{-9}$ erg/cm²/

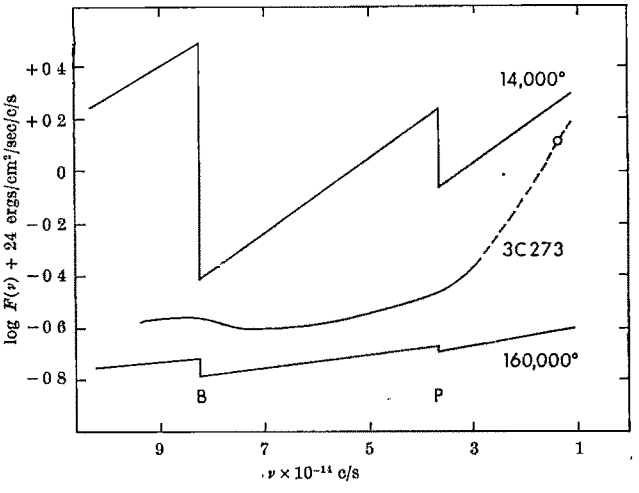


Fig 1. The continuous energy distribution of 3*C* 273 in the optical and near infra-red is compared with the theoretical hydrogen recombination continua appropriate to electron temperatures of 14,000° and 160,000° given by Oke (ref. 5). The continuous line represents Oke's observations of 3*C* 273. The circle represents Johnson's (ref. 6) measurement of the flux at 2·2μ. The ordinate is the logarithm of the observed flux per unit frequency interval from 3*C* 273 at the Earth's surface. The units are 10^{-24} ergs cm^{-2} sec^{-1} (c/s)$^{-1}$. The abscissa is the frequency in units of 10^{14} c/s. Note that the theoretical continua have been given arbitrary vertical shifts

sec. The bulk of this radiation is emitted in the frequency range 10^{11}–10^{13} c/s. For 3*C* 48, Sandage[12] obtained a flux of $1{\cdot}1 \times 10^{-11}$ erg/cm²/sec for this frequency range.

It has often been pointed out that detection of a significant degree of polarization in the radiation might add weight to the circumstantial evidence for the flux being synchrotron radiation. In the case of 3*C* 48, Hiltner found that the degree of polarization in the optical region is less than 2 per cent. Recently, Schmidt[13] has reported that in 3*C* 273*B* there is no measurable polarization in the frequencies near $7{\cdot}5 \times 10^{14}$ c/s, but at 6×10^{14} c/s about 4 per cent polarization is present. This is consistent with a steadily increasing proportion of synchrotron radiation at lower frequencies.

We conclude that the bulk of the energy emitted from 3*C* 273 and probably from the quasi-stellar objects in general is synchrotron radiation. We consider next the dimension of the source from which this radiation is emitted.

It is well known that it is the flux variations from the source which set a much stronger upper limit for the size than the 'quasi-stellar' characteristic. 3*C* 273*B* has been shown to vary in the optical region in time scales of years and months[14]. Recent observations in frequencies near 3×10^{11} c/s (ref. 9) suggest that there have been large variations in periods of months. In other quasi-stellar objects variations in the optical flux of similar and sometimes shorter time scales are seen. In the case of 3*C* 345 significant optical and spectroscopic variations on a time scale of weeks have recently been reported[15,16] though no observations of the infra-red flux in this object are available. Thus a reasonable scale to take for the size of the region in which this synchrotron flux is generated is $\sim 10^{17}$ cm or about a light month.

We return now to our discussion of the inverse Compton process. In attempting to estimate the relative importance of the synchrotron process and the Compton scattering process, we first considered that the dimension of a 'coherent' emission region is such that a relativistic electron can travel in its lifetime across the whole region. For a sphere of radius R this requires $\tau > R/c$, where τ is the electron lifetime against synchrotron radiation. Calculating τ from the synchrotron process alone, we have:

$$\tau = 10^{12}\nu^{-1/2}H^{-3/2} \text{ sec} \qquad (2)$$

where H is the magnetic intensity in gauss and ν is the critical frequency in c/s of the electron in question. From the inequality $\tau > R/c$, we obtain:

$$H^{3/2} < 3 \times 10^{22}\nu^{-1/2}R$$

giving:

$$\frac{H^2}{4\pi} < \sim 10^{29}\nu^{-2/3}R^{-4/3} \qquad (3)$$

R being in cm.

Suppose first that the whole emission comes from just one such coherent region. Then, if d is the distance to the source and s is the flux in ergs cm^{-2} sec^{-1} at the Earth, $L = \pi R^2 c\ U_{\text{rad}} \simeq 4\pi d^2 s$, neglecting a red-shift factor, so that:

$$U_{\text{rad}} \approx \frac{4d^2 s}{R^2 c} \qquad (4)$$

Taking the ratio of (4) to (3):

$$\frac{U_{\text{rad}}}{H^2/4\pi} = \frac{4d^2 s}{10^{29}c}\left(\frac{\nu}{R}\right)^{2/3}$$

If we substitute for s the value of $5{\cdot}3 \times 10^{-9}$ erg/cm²/sec, then:

$$\frac{U_{\text{rad}}}{H^2/4\pi} = 63{\cdot}6\ D^2\left(\frac{\nu}{R}\right)^{2/3} \qquad (5)$$

where D is measured in megaparsecs (Mpc) ($d = 3 \times 10^{24} D$). Putting $D = 470$ Mpc, the cosmological distance for 3*C* 273, we have:

$$\frac{U_{\text{rad}}}{H^2/4\pi} = 1{\cdot}3 \times 10^7\left(\frac{\nu}{R}\right)^{2/3}$$

Thus, if $R = 10^{17}$ cm and $\nu = 10^{14}$ c/s (the near infra-red):

$$\frac{U_{\text{rad}}}{H^2/4\pi} = 1{\cdot}3 \times 10^5 \qquad (6)$$

It is of some interest to apply a similar argument to the case of 3*C* 345. This quasi-stellar source which has a red-shift $z = 0{\cdot}595$ (ref. 17) has varied significantly in a time scale of $\sim$ light week or $\sim 10^6$ sec (ref. 15). In this case we do not have such strong evidence that a major part of the flux is synchrotron radiation. The flux that is seen is ultra-violet radiation and we have no knowledge concerning the flux in the frequency range discussed for 3*C* 273 because of the much larger red-shift. However, we shall assume that the flux is synchrotron in origin. Taking an apparent magnitude of $17^{\text{m}}{\cdot}0$ for 3*C* 345, we find that for this object $s = 8{\cdot}4 \times 10^{-12}$ erg/cm²/sec. Thus for this source, going through the same steps as before:

$$\frac{U_{\text{rad}}}{H^2/4\pi} = 1{\cdot}0 \times 10^{-1}D^3\left(\frac{\nu}{R}\right)^{2/3}$$

The cosmological distance, D, is equal to 1,770 Mpc. and thus:

$$\frac{U_{\text{rad}}}{H^2/4\pi} \approx 3{\cdot}1 \times 10^5\left(\frac{\nu}{R}\right)^{2/3}$$

Putting $\nu = 6 \times 10^{14}$ c/s, $R = 3 \times 10^{16}$ cm:

$$\frac{U_{\text{rad}}}{H^2/4\pi} \approx 2{\cdot}3 \times 10^4 \qquad (7)$$

Thus, provided that the ultra-violet radiation from 3*C* 345 is synchrotron in origin, we arrive at much the same ratio as for 3*C* 273.

The results (6) and (7) give a manifest contradiction because U_{rad} in (6) and (7) was calculated from the synchrotron process alone. Our results would therefore require the energy density of radiation from the Compton process to be greater than that from the synchrotron process by the large factor $\sim 10^5$, yielding a divergence in which the inverse Compton process acts on itself, giving a repeated stepping up of the energies of quanta initially generated by the synchrotron process. When U_{rad} is estimated from the synchrotron process, the right-hand side of (1) must not be greater than unity. It may be noted that this requirement prevents one from arguing that the optical and infra-red emission arises from the Compton process acting on radio-frequency quanta. For, since the energy of the former much exceeds the latter, it would be necessary on this idea for the Compton process to multiply the energy of the synchrotron process by a large factor. The same process would then multiply itself by a similar factor, giving an impossible divergence.

Reference to (4) shows that these difficulties are avoided if the distances of the quasi-stellar objects are reduced by a factor $\sim 10^2$, that is to say, from characteristic distances of 1,000 Mpc to characteristic distances of 10 Mpc. It seems to us rather unlikely that the cosmological interpretation of the distances can be saved by any modification of our argument except perhaps by modifying our assumption that the emission comes from just one coherent region—in the sense we have used coherence (compare equations (2) and (3)). We continue our discussion by examining the consequences of denying this assumption.

Suppose there are n coherent sub-regions each of radius R. Proceeding in the same way as before, but now for a single sub-region, we find that $4\pi U_{\text{rad}}/H^2$ is reduced by n. Evidently, for n large enough this ratio can be reduced to a value of order unity—even when D is a cosmological distance. However, since the fluctuation data require all n sub-regions to be fitted into an overall dimension of $\sim 10^{17}$ cm, it is clear that for $n \gg 1$ the radius R of each region must be taken substantially less than 10^{17} cm. Thus the factor $(\nu/R)^{2/3}$, which appears in the analysis,

cannot now be taken much greater than unity. It follows that n must be still larger than the numerical values appearing in equations (6) and (7)—$n \geqslant \sim 10^6$ is needed.

The problem is then to explain how so many sub-regions can vary in phase with each other, as they must do if their combined radiative output is to vary at all appreciably in a time scale of a few weeks. A correlation between the sub-regions is needed—they cannot be autonomous. We might think of a magnetic field maintaining a connexion from one region to another, but for the connexion to be strong enough to co-phase the regions the magnetic intensity could not be significantly less in the inter-region zone than it is within the regions themselves. The concept of separate sub-regions then becomes of doubtful validity and it seems better to return to a single coherent region of dimension $\sim 10^{17}$ cm. However, we can now regard the 'coherence' as being maintained by a magnetic field rather than by a diffusion from place to place of the relativistic particles. In terms of our previous analysis, we can increase the value of H^2 until the ratio $4\pi U_{rad}/H^2$ falls to unity. The effect of such an increase is to give extremely short lives to the relativistic electrons. Instead of a few weeks, the lifetimes are only $\sim 10^3$ sec—this is for the electrons which radiate the main energy, at $\nu \simeq 10^{14}$ c/s.

It would seem then that the assumption of cosmological distances for the quasi-stellar objects leads to the requirement that relativistic particles are generated *in situ*. From what? Perhaps from oscillations in the magnetic field. In order to explain the optical fluctuation data such oscillations must propagate with essentially the speed of light—the oscillations would need to be electromagnetic, not hydromagnetic.

A mechanism for generating electromagnetic waves has been discussed by Hoyle, Narlikar and Wheeler[18]. The waves were regarded as being emitted in the dynamical oscillations of a highly collapsed star, the wavelength being a few kilometres. Such waves do not propagate unless the electron density is very low. Hence it would be necessary for an inner region of dimension $\sim 10^{17}$ cm surrounding a collapsed object to be largely devoid of matter. To prevent the waves simply radiating freely into space it would also be necessary in such a model to trap the waves within the inner region, by surrounding the region with a gas cloud of comparatively high density. It may be added that the distinction between synchrotron emission and the Compton process is now lost; the two processes are the same in a purely electromagnetic field.

Difficulties remain, however. The most immediate is that the outer gas cloud must have a characteristic dimension of the same order as the radius of the inner region, $\sim 10^{17}$ cm. For a dimension as small as this it is hard to obtain sufficient line emission (for example, for Hβ in 3*C* 273) without requiring the electron density to be so high that the cloud is very opaque in the continuum.

We conclude that either the quasi-stellar objects are at distances of ~ 10 Mpc or less, or the physical model associated with these objects must be substantially different from the theories at present in vogue.

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ANGULAR DIAMETERS OF SOME RADIO SOURCES WITH ANOMALOUS HIGH-FREQUENCY SPECTRA

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DENT and Haddock[1,2] have recently directed attention to the existence of a class of radio sources in which the flux densities at frequencies above 3,000 Mc/s are greater than would be predicted by a linear extrapolation of the low-frequency spectra. Altogether seven such sources are listed in refs. 1 and 2. The present communication discusses the dimensions derived from observations of five of these sources (3*C* 84, 3*C* 273, 3*C* 279, 3*C* 345 and 3*C* 380) using an interferometer of very high resolving power, operating at a frequency of 1,422 Mc/s.

Previous authors[3,4] have discussed a connexion between the angular diameter of a source and the existence of a low-frequency cut-off in the spectrum. The new information on angular sizes presented here seems to indicate that anomalous spectra of the type reported by Dent and Haddock are also associated with sources of extremely small diameter. Interferometric observations were made during May and June 1965 using the Mark I 250-ft. radio telescope at Jodrell Bank and one of the 82-ft. radio telescopes of the Royal Radar Establishment. The Royal Radar Establishment instrument is at Defford, near Malvern, and effective baselines of up to 604,000 wave-lengths were achieved. The equipment has been described by Adgie *et al.*[5], who have also given details of the normalization procedure and calibration of the absolute sensitivity of the system.

Two of the sources (3*C* 273, 3*C* 380) were observed at all hour angles at which they were above the horizon, and a third (3*C* 84) was observed over a large but incomplete range of hour angles. The remaining two sources

(3C 279, 3C 345) could be observed only very briefly because of the limited period during which both telescopes were available for this work. Observations made at elevations less than 15° have not been included in the analysis because of the large correction factors involved[5]. The results of the measurements are summarized in Table 1, which also contains some earlier data for comparison. It can be seen that, for all five sources, the angular dimensions at 1,422 Mc/s are smaller than at the lower frequencies.

Each of the five sources gave a clear fringe pattern at all the hour angles at which it was observed, indicating that in every case a substantial part of the emission comes from a region or regions smaller than 0·1 sec of arc. As reported by Adgie *et al.*, the source 3C 273B shows a dependence of fringe amplitude on hour angle which indicates that it probably contains two such small components. The less intense source 3C 380 also shows some changes in fringe amplitude which are believed to be significant and suggest a more complex structure than that attributed to 3C 273B. More measurements are required in order to confirm this interpretation. The observations of the remaining sources did not reveal any significant changes of fringe amplitude with hour angle. It is not possible, therefore, to derive any detailed information about the angular structure of 3C 84, 3C 279 and 3C 345.

Four of the sources discussed here have been identified with quasi-stellar objects[14,15] and the remaining one (3C 84) with the Seyfert Galaxy *NGC* 1275 (ref. 16). Three of the quasars, 3C 273B, 3C 279 and 3C 345, are known to have variable optical luminosities[17–19]. Variations of radio emission from these three quasars and from 3C 84 have also been observed by Dent[9,20] at both 8·0 and 16·5 Gc/s. Out of thirty-five radio sources which he examined, these four were the only extragalactic sources for which he reported variations in flux density, and it is interesting to note that all four of them appear to have a component smaller than 0·1 sec of arc at 1,422 Mc/s. (The reported radio variability of *CTA* 102 (ref. 21) is still controversial[22].)

A value for the optical red-shift has been published for each source[14,18,23,24]. If it is assumed that the red-shifts are due to the general expansion of the Universe and a particular cosmological model is adopted, the angular size measurements reported here can be used to calculate an upper limit to the linear dimensions of the radio-emitting regions. These linear dimensions, and information on optical and radio variability, are presented in Table 2. Variations on time scales such as these have previously been taken to imply that these sources have components of very small linear dimensions, and a similar conclusion follows from the present observations, even though the

Table 1

Source	Previous measurements			Present measurements at 1,422 Mc/s			
	159 Mc/s E.–W. baseline Allen *et al.* (ref. 6)	960 Mc/s Maltby and Moffet (ref. 7)	Others	Hour angle (h)	Effective baseline (in millions of wavelengths)	Visibility, γ*	Suggested interpretation
3C 84	Diameter ~ 1′ possibly with 20% core < 1″	Halo diameter ~ 4 5′. 85% of flux from core < 24″	⩽10″ at 1,420 Mc/s (ref. 10) ⩽10″ at 4,675 Mc/s (ref. 11)	0300–0900 and 1300–2400	0·60–0·30 and 0·18–0 59	0·7. No variation with hour angle	Approximately 70% of flux from core < 0·1″ diameter
3C 273	Dimensions ~ 20″	d < 24″	Double source, separation 20″ (ref. 12, 13)	1800–0600	Between 0 38 and 0 60	Component B unresolved at some position angles (ref. 5)	Component B double, separation ~ 0·4″; component sizes < 0·1″ diameter
3C 279	23″ < d < 47″	d < 36″ E.–W.	—	0230–0500 and 2030–2230	0 46–0·59 and 0·48–0 35	⩾0 7 at a resolution of 0·47 × 10⁸ wavelengths and two position angles	Probably 70% of flux from core < 0·1″ diameter
3C 345	Dimensions ~ 10″ (visibility drops sharply to < 0·3 at 32,000 wavelengths)	d < 42″ E –W	d ⩽ 10″ at 4,765 Mc/s (ref. 11)	1400–1600	0 29–0 47	~ 0·8 at a resolution of 0·46 × 10⁸ wavelengths	Some structure with dimensions ⩽ 0 1″
3C 380	5″ < d < 11″ possibly with 10% core	d < 24″ E.–W.	—	0000–2400	Between 0·12 and 0·60	0·4 at most hour angles. Variations at relatively low resolutions	Complex structure with 40% of flux in core < 0·1″ diameter

* Fluxes taken from Kellerman (ref. 8). No corrections have been made for possible secular variations in flux (ref. 9)

Table 2

Source	Identification	Red-shift z	Linear diameter* corresponding to an angular diameter ⩽ 0·1″ (parsecs)	Brightness temperature of unresolved core † (° K)	Variability	
					Optical	Radio
3C 84	Seyfert Galaxy *NGC* 1275	0·018	⩽25·3	⩾8·0 × 10⁸	—	40% increase in the 8,000 Mc/s flux density in ~ 4 months. Also variable at 16,500 Mc/s (ref. 20)
3C 273B	Quasi-stellar radio source	0·158	⩽172	⩾2·6 × 10⁹	Gradual decrease in optical brightness ~ 0·2 magnitude/century. Major fluctuations over periods ~ 10 yr. Light flashes lasting for periods of a few days (ref. 17)	42% increase in the 8,000 Mc/s flux density in 3 years. ~ 35% increase in the 16,500 Mc/s flux density in 1 year (ref. 20). Also strongly variable at wavelengths of 3·4 mm (ref. 25) and 1 mm (ref 26)
3C 279	Quasi-stellar radio source	0·536	⩽234	⩾4·4 × 10⁸	Decrease in brightness ~ 1 magnitude in 9 years (ref. 18)	48% decrease in the 8,000 Mc/s flux density in 2 years. Also variable at 16,500 Mc/s (ref. 20)
3C 345	Quasi-stellar radio source	0 594	⩽340	⩾4·4 × 10⁸	Increase in brightness ~ 0·4 magnitude in about 20 days (ref. 19)	36% decrease in the 8,000 Mc/s flux density in 2 years. Also variable at 16,500 Mc/s (ref. 20)
3C 380	Quasi-stellar radio source	0 691	⩽350	⩾4·4 × 10⁸	—	—

* Calculated on the assumption of a zero cosmological constant and a deceleration parameter $q_0 = +1$ (ref. 27). If $q_0 = 0$ these values must be multiplied by (1 + 0 5z). Hubble's constant, H, has been taken as 100 km/sec/megaparsec.

† Epoch 1965·5.

observing frequency is lower than those at which variability has generally been observed.

It is apparent that in respect of their unusual radio spectra and variations in flux density the quasars 3*C* 273, 3*C* 279 and 3*C* 345 are remarkably alike. They are also among the small number of radio sources known to have very high brightness temperatures (see Table 2). The radio luminosity of 3*C* 84 is smaller than that of the quasars by two or three orders of magnitude, but with regard to the three radio properties mentioned here, there is a close similarity. These measurements therefore provide support for the views of Shklovskii[28] and Hoyle and Burbidge[29] that similar explosive processes to those in some, presumably young, quasars are occurring in this Seyfert Galaxy. The measurements also support the suggestion of Hoyle and Burbidge[29] that variations in optical brightness may be expected in 3*C* 84. Further, in view of the anomalous spectrum of 3*C* 380 and the high brightness temperature at 1,422 Mc/s, this source might also be expected to show variations in optical and radio brightness. It has so far not shown any radio variations[20], but it would be interesting to extend the search to cover a longer period of time, and to include optical as well as radio wave-lengths.

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IMPACT OF PLASMA ON A DIPOLE MAGNETIC FIELD

By Dr. J. B. G. ROBERTS and J. P. TURNER

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THE interaction of the solar corpuscular stream with the Earth's magnetic field has been the subject of many theoretical and experimental investigations; but despite the great volume of data provided by artificial satellites and space probes, the structure of the magnetosphere and the mechanisms producing magnetic storms and aurorae are not yet well understood. Observations from space vehicles do not provide a complete picture of the magnetosphere and it is also difficult to distinguish spatial and temporal variations. In these circumstances a laboratory simulation under controlled conditions can be of considerable value in demonstrating the type of interaction that may occur. In the experiments described here plasma was made to impinge on a dipole magnetic field; and to correspond with the solar wind, the values of the plasma velocity, V, and conductivity, σ, were chosen so that, for a convenient experimental scale length, L, the product $4\pi\sigma LV$ (the magnetic Reynolds number) was much greater than unity. With these conditions, the incident plasma would be expected to compress the field in front of it.

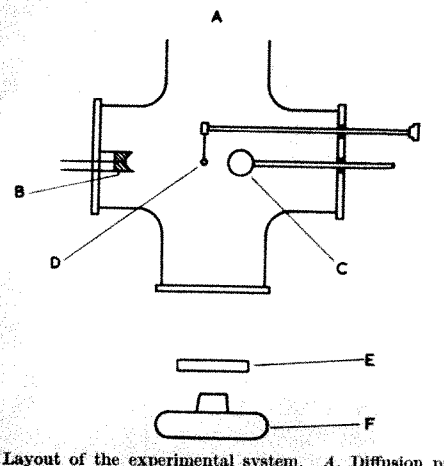
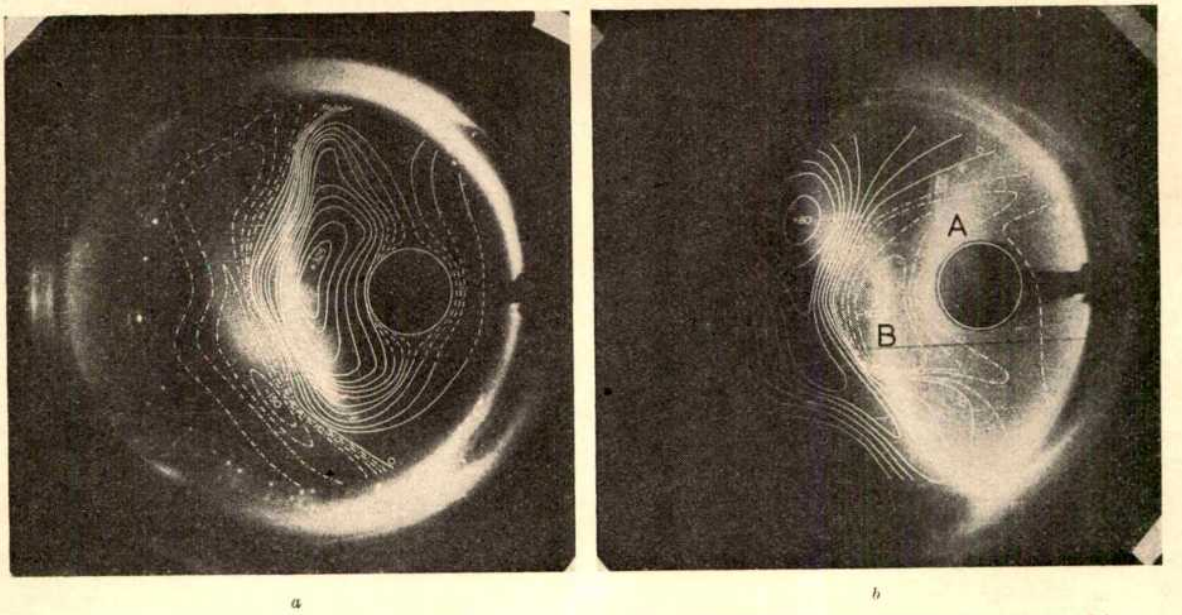


Fig. 1. Layout of the experimental system. *A*, Diffusion pump; *B*, plasma gun; *C*, dipole coil; *D*, search coil; *E*, Kerr-cell shutter; *F*, camera

The experimental arrangement was as shown in Fig. 1. A co-axial plasma gun, powered by the discharge of a 16 μF capacitor from 10·5 kV, was used to propel plasma through a vacuum chamber at a velocity of about 4×10^6 cm/sec towards the quasi-static magnetic field surrounding a dipole of moment $1{\cdot}0 \times 10^5$ gauss cm^3, with the axis of the dipole perpendicular to the plasma flow. The current through the plasma gun was prevented from oscillating by means of a fuse designed to melt after the first half-cycle of the current waveform. The dipole field was produced by the discharge of a 328 μF capacitor at 2·5 kV through a spherical coil 4·2 cm in diameter surrounded by an earthed copper screen. The firing of the plasma gun was timed to occur near the first peak of the dipole field when the field was varying only very slowly because of the long period of oscillation of the coil current.

The effects observed were reproducible, so by using several firings the sequence of events could be photographed at various pre-set delays using a Kerr-cell shutter which gave exposure times of 1 μsec. Photographs were taken looking perpendicular to the equatorial plane; and in order to determine the three-dimensional structure, others were taken with the dipole re-orientated to display the meridian plane. The temporal variations of the component of the magnetic field in the axial (north–south) direction were measured at many points in the equatorial plane by a small mobile search coil connected to an integrating circuit. The magnetic field changes were displayed on an oscilloscope and recorded photographically. The field variations were corrected for the slow change in the dipole field, which was separately observed with the search coil when the plasma gun was not operated.



a *b*

Fig. 2. Contours of equal magnetic field change superimposed on 1-μsec exposures of the plasma. *a*, 7 μsec after the plasma gun firing; *b*, 19-μsec delay

The method of presentation of the results has been chosen to show the changes in magnetic field, produced by electric currents induced in the plasma, and to compare these with the pictures of the glowing plasma obtained with the Kerr-cell camera. The values of the magnetic field perturbation at all locations at any chosen time were interpolated to build up a contour map showing lines of equal field change (rather than total field) at this time. The field-change distribution is caused by currents in the plasma, and the contours can therefore meaningfully be superimposed on to the Kerr-cell picture for the same delay time. A set of such comparison photographs were made for a succession of delay times between 4 and 27 μsec after the firing of the gun. The two photographs shown in Fig. 2, corresponding to delays of 7 and 19 μsec, illustrate important stages of the interaction and exemplify an interesting reversal of the induced field. In Fig. 2 the plasma advances from the left and the camera is situated on the dipole axis facing the south-seeking pole (corresponding to the northern terrestrial pole). The contours are at intervals of 25 gauss in Fig. 2(*a*) and 10 gauss in Fig. 2(*b*). The contours corresponding to negative field changes are shown chain-dotted and the numbers label the maximum and minimum contours. The dashed lines close to the dipole show parts of the contours the positions of which have been inferred rather than measured.

In the early stages of the interaction there was an increase in magnetic field strength near the dipole, while farther out towards the plasma source the field strength was depressed. This field distribution conforms to the usual explanation of the initial phase of a geomagnetic storm in which the incident plasma may be thought of as compressing the dipole field because of currents induced in its leading face which change the original magnetic field.

At times later than 16 μsec the general direction of the current in the equatorial plane was reversed to become westward, giving a decrease in field near the dipole but augmenting the undisturbed field at distances greater than about 8 cm from the dipole centre in the direction of the gun. The sequence of magnetic field variations near the dipole is strongly suggestive of the field changes observed at the Earth's surface during a typical magnetic storm.

The form of the induced magnetic field can be used to deduce the way in which the current is distributed in the neighbourhood of the equatorial plane. Certain types of current distribution can easily be distinguished. For example, a magnetic field strength profile resembling a step function is produced by a current sheet. On the other hand, if the current flow is more localized along a line, the step function would be modified by a rapid decrease of field with distance from the line.

Fig. 3 shows the field-change distribution along the line from the plasma gun to the dipole at the delay times shown. For times earlier than 13 μsec the shape of the curve indicates an eastward current localized near the equatorial plane. At 13 μsec the curve approximates to a step function, showing that the current was then distributed in a sheet extending some distance above and below the plane. After the current reversal which occurred at about 16 μsec, the current was again localized but appeared to be extending into a sheet as the currents decayed with the plasma. The fact that the step functions do not extend equally above and below the zero line is a consequence of the curvature of the current sheets about the dipole axis.

Referring again to Fig. 2 and ignoring the bright regions where the plasma has been intercepted by the glass walls of the vacuum chamber, we interpret the luminous areas as regions of enhanced recombination rate where the plasma density (and probably temperature) was raised. At 7 μsec, the light emission was from the region of maximum current density as indicated by the field-change contours. After 19 μsec delay the bright plasma regions were mainly nearer to the dipole than the high current density region. The bright area *A*, which seems to be in contact with the dipole coil in Fig. 2(*b*), was shown, by a meridian plane photograph taken at 19 μsec delay, to be, in fact, well removed from the equatorial plane with which we are mainly concerned here. A region of field-aligned luminosity passing through the equatorial plane is seen in region *B*, but the field-change contours show that it cannot be identified with a high current density as could be the luminosity in Fig. 2(*a*). In the early stages of the interaction, these observations are consistent with behaviour predicted by the Chapman–Ferraro model in that the plasma flow was held off, and near the equatorial plane an eastward current was induced in the advancing plasma front. Afterwards the current extended itself into a sheet at a stand-off distance of about 8 cm from the centre of the dipole. Later some of the plasma seemed to penetrate farther towards the dipole, into the 'magneto-

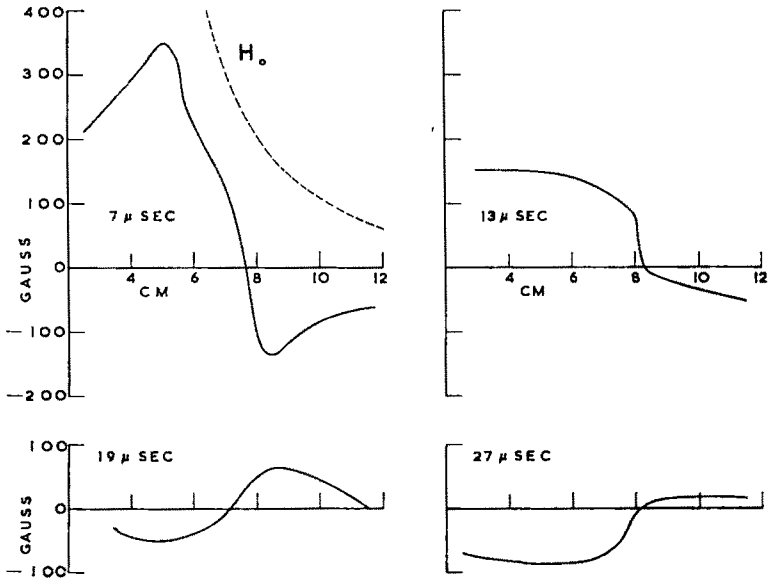


Fig. 3. Profiles of the change in magnetic field along the line from the dipole to the plasma gun at the delay times indicated. The abscissae represent distance from the dipole axis towards the gun. The undisturbed dipole field H_0 is also shown for comparison

sphere' and a westward current was set up at about the same stand-off distance. This current may be analogous to the ring current proposed in the explanation of the main phase of geomagnetic storms.

There was no strong gradient in the field-change within the region B, which thus showed that the net current there associated with the particle motion was small. This indicates, in accordance with meridian plane photographs, that B was a belt of charged particles trapped by the magnetic field. A net current carried by this belt could only be due to an approximately collisionless drift[1] produced by the field gradient, and the drift current is shown by the observed field-change distribution to be negligible compared with the west-flowing current surrounding the plasma. It appears that the dominant effect here was the injection into the initially compressed field of the 'magnetosphere' of diamagnetic plasma which lowered the magnetic flux in this region, thus necessarily maintaining a circulating current around itself and the dipole in a westward direction.

The experiment exhibits several features matching geophysical observations. A typical geomagnetic storm is probably caused by a sudden gust in the solar plasma stream passing the Earth. The initial phase of the storm is observed at the Earth's surface as an increase in the local magnetic field and is adequately explained by magnetosphere models following the ideas of Chapman and Ferraro[2,3]. The main phase of the storm, during which the terrestrial field strength stays below its undisturbed value, has proved more difficult to explain. Our experiments suggest that the decrease in field intensity could be caused by penetration of solar plasma containing little magnetic flux into the magnetosphere. The meridian plane photographs indicate that there is appreciable plasma injection along the magnetic field lines passing through the so-called neutral points over the polar regions, where even a 'closed' model of the magnetosphere is open to the plasma stream.

Bostik *et al.*[4] in a similar experiment have concluded that vortices present in the plasma flow enable plasma to break through the magnetosphere boundary. This has possibly occurred in the experiments described here and might also be important in geophysical phenomena since observations[5] have indicated that in the neighbourhood of the Earth's orbit the solar wind is highly irregular. The scale of the irregularities dominating the flow is probably between 10^2 and 10^4 km (less than one-tenth a typical magnetosphere dimension) so that the magnetosphere is set up by the average wind. If the plasma irregularities are vortices and contain closed magnetic field lines, some of these might link with the Earth's field so that penetration of the interplanetary plasma would be facilitated.

A more detailed analysis of the experimental results is proceeding and it is hoped to determine whether the characteristics of the experimental magnetosphere most resemble those predicted by the Chapman–Ferraro model and its derivatives or those expected on the basis of the theories of Alfvén[6] and Karlson[7].

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OBITUARIES

Prof. S. G. Wilson, C.B.E.

THE untimely death of Prof. Samuel Geoffry Wilson came as a great shock to the veterinary world and as a still greater one to his friends and colleagues

Geoff, as he was called by those who knew him, was born in Donaghmore, County Tyrone, Northern Ireland, educated at the Royal School, Dungannon, and then went to Queen's College, Belfast, where he took a B.Sc. degree in agriculture. From there he moved to the Royal (Dick) Veterinary College, Edinburgh, and obtained the degree of M.R.C.V.S.

In 1936 he was awarded a colonial scholarship and went to Nyasaland (now the Malawi Republic) where, among other duties, he studied ticks and tick-borne diseases; for these researches he was awarded a Ph.D. degree in 1946. In fact, he was a recognized authority on ticks in Africa.

At the same time he had a very keen interest in tsetse and trypanosomiasis problems and in 1947 transferred to Uganda as chief veterinary research officer at Entebbe. In 1949 he took up the post of chief field zoologist in the Tsetse and Trypanosomiasis Control Division of the Kenya Veterinary Department. In 1952 he became director of veterinary services in Northern Nigeria. He retired from Government service in 1962.

Later in 1962 he was appointed professor and director of the Institute of Tropical and Protozoal Disease in the Veterinary Faculty of the State University of Utrecht. There he remained until he died in September 1965. He was 55 years old.

Geoff Wilson was full of drive, enthusiasm and energy. He was also bold and quick to see the practical application of new developments—the ideal man to introduce modern and progressive methods.

In Uganda he studied trypanosome infections in cattle, sheep and goats, carried out some early trials with the new trypanocidal drug 'Antrycide' and investigated drug

resistance acquired by trypanosomes resulting from underdosing with 'Antrycide'.

In Kenya he started an insecticidal tsetse eradication scheme in the Nyando river basin near Lake Victoria, in collaboration with the Tropical Pesticide Research Institute. This project was to have far-reaching effects on the sleeping sickness problem in that country, for it was the first successful large-scale residual insecticidal project undertaken against *Glossina* in East Africa.

When he went to Nigeria he immediately recognized the urgent necessity of 'Nigerianizing' his department and began at once to do so. Again, with his characteristic foresight and drive, he lost no time in forming a Veterinary Tsetse and Trypanosomiasis Unit, staffing it with the best men he could find, and sent an officer to Kenya to examine the methods in use there. The next step was the Komadugu Ghana scheme, the biggest, boldest, most successful and cheapest residual insecticidal spraying operation ever undertaken against the tsetse flies *Glossina morsitans submorsitans* Newstead and *G. tachinoides* Westwood in the Sudan zone of Northern Nigeria. This work was followed up by even larger schemes and to-day the Northern Nigerian Veterinary Tsetse and Trypanosomiasis Unit ranks among the best and most up-to-date organizations of its kind in Africa. In recognition of his outstanding services he was awarded the C.B.E. by Her Majesty the Queen.

Geoff Wilson was a man of many parts, and two years after his appointment as professor at Utrecht he gave an inaugural address entitled "The Problems of Trypanosomiasis Control in Cattle in Africa", ending up by speaking for 10 minutes in the Dutch language. But he had lost his heart to Northern Nigeria, and through his influence the 'Zaria Project' involving a part of the Veterinary Faculty of the new Ahmadu Bello University, supported by Dutch aid, came into being.

Apart from his numerous scientific interests and achievements, Geoff was an accomplished sportsman. As a boy he played rugby for the Ulster Schools side and later won his boxing blue at Edinburgh—he was Scottish Universities Welterweight Champion for 1933–34.

Besides his wife, Maureen, he leaves behind two sons and a daughter. His eldest son is a veterinarian following in his father's footsteps, for he has recently taken up a post with the East African Trypanosomiasis Research Organization at Tororo in Uganda.

Geoff was Irish to the finger-tips—kind, generous and considerate to his friends and a thorn in the side of his enemies—he will be sorely missed. P. E. Glover

Prof. S. K. Allison

Samuel King Allison, director of the Enrico Fermi Institute for Nuclear Studies and the Frank P. Hixon distinguished service professor in the Department of Physics at the University of Chicago, died on September 15, 1965, in England.

Prof. Allison was serving as the United States delegate to the Plasma Physics and Controlled Nuclear Fusion Research Conference under the sponsorship of the International Atomic Energy Agency in Culham, near Oxford. He suffered an aortic aneurism on September 6, and underwent surgery at the Radcliffe Infirmary, Oxford. Death was attributed to a pulmonary embolism.

Allison was one of the best known and best loved of American scientists. Born in Chicago in 1900, the son of a Chicago public school principal, he was a product of the community of which the University of Chicago is a part. He was educated in the Chicago public schools, received his B.S. degree from the University of Chicago in 1921 and his Ph.D. degree two years later. Following a period as National Research Fellow at Harvard University (1923–25) and as Fellow at the Carnegie Institution (1925–26), he joined the faculty at the University of California, Berkeley, but returned to the University of Chicago in 1930 after having achieved the rank of associate professor.

Allison's early researches at Berkeley and Chicago were in X-rays. His major work involved the design and construction of a high-resolution double crystal spectrometer, its application to the measurement of the widths and intensities of X-ray lines, and to the experimental confirmation of the C. G. Darwin–P. P. Ewald dynamical theory of X-ray diffraction. The depth of his understanding of the field of X-rays is evident from the book he wrote jointly with A. H. Compton, *X-rays in Theory and Experiment*, a classic text in the field.

His interest turned to nuclear physics during the middle 1930's. He concentrated on the reactions among the light elements that could be examined with accelerators of relatively low energy. The Cockcroft–Walton accelerator which he built himself and operated with his students developed a maximum of 400 kV. He dubbed it *Kevatron* to emphasize its modest capabilities at a time when his associates were constructing machines in the million- and then the billion-volt range. Yet the *Kevatron* kept turning out a continuous stream of interesting research and a continuing succession of able graduate students. Much of the research which he carried out together with his students concerned precise measurements of the energy released in proton-induced reactions. In later years this interest shifted to the reactions induced by heavier ions. He was particularly successful in developing sources of lithium ions. To pursue these reactions more extensively, he needed higher energy machines. The University made available to him its 2-MeV Van de Graaff, acquired originally for biological studies. In the end he obtained a 4-MeV machine with a grant from the National Science Foundation.

A subsidiary interest which grew into a major activity was the investigation of the energy loss of charged particles moving through gases. This developed into an extensive investigation of the mechanism of capture and loss of electrons by the ions, a subject that turned out to give important insights into plasma physics and the quest for ways to harness thermonuclear fusion. For these studies the *Kevatron* continued to play a central part until the end.

Allison's emergence into public life had its origins in the secret wartime enterprise that developed the atomic bomb. He began, in January 1941, to investigate the suitability of beryllium as a moderator of slow neutrons for the chain reaction. The work was done with a grant of 9,000 dollars, obtained from the U.S. Office of Scientific Research and Development. Writing about this later, he commented, "I remember being aghast at the ease with which this seemingly colossal sum was placed at my disposal". This work has remained of fundamental interest and serves now as the basis for certain major lines of nuclear reactor development. Although beryllium, because of its scarcity, did not occupy a prominent place in the initial development of the chain reaction, Allison's early effort served to focus that development at the University of Chicago. His group at Chicago was joined by Fermi's Columbia group and Wigner's Princeton group when, in February 1942, the "Metallurgical Laboratory" was organized under the leadership of A. H. Compton to develop a method for producing plutonium as a possible material for the atomic bomb. It was this Laboratory, at the University of Chicago, that successfully achieved the first nuclear chain reaction in December 1942.

Allison liked to recall his role on the occasion of the first chain reaction experiment. He was the leader of the "suicide squad". It was his brawn, not his brain, he insisted, that qualified him to be entrusted with the ultimate, last-ditch measure. It was his idea to prepare a huge jug of cadmium solution, and he stood ready to pour it over the chain-reacting pile in the event of all other control mechanisms failing to operate.

The work of the Metallurgical Laboratory led directly to the large-scale production of plutonium, out of which

the first nuclear bomb was constructed. Allison served in this project from its beginning until 1944. He served as director of the Laboratory during the final year of this period. He jested that his main job was to sign the huge stacks of blueprints and documents having to do with the construction of the great nuclear reactors at Hanford.

In November 1944, he went to the Los Alamos Laboratory to work more directly on the development of the atomic bomb. He served as chairman of the Technical and Scheduling Committee which scheduled the final stages of the test of the first atomic bomb on July 16, 1945. He made history when his voice sounded across the New Mexico desert, counting off seconds to zero on that memorable dawn that witnessed the first nuclear explosion.

September 1, 1945, was the day on which the University of Chicago announced the formation of its new research institutes with Allison as the newly appointed director of the Institute for Nuclear Studies. Such luminaries as Enrico Fermi and Harold Urey were already enlisted as members. It was on this occasion that Allison made what came to be known as "Sam's butterfly speech". He warned that if the exchange of scientific information was prohibited by military regulation, scientists in America would leave the field of atomic energy and devote themselves to such subjects as the colours of butterfly wings. The speech became the opening salvo in the campaign the scientists fought to prevent military control of the peacetime development of atomic energy.

He served as the first director of the Institute of Nuclear Studies (later renamed for Enrico Fermi) until his death, except for one five-year interlude. The Institute was devoted to the idea that physicists, chemists, and astrophysicists had complementary interests and could work together under one roof with mutual benefit. Under Allison's stewardship the Institute flourished. The seminars were brightened by such men as Fermi, Teller, Urey, J. Meyer, Wentzel, Chandrasekhar, Gell-Mann, and Dalitz. Many new ideas were developed, among them two that have already won Nobel prizes: radio carbon dating (W. F. Libby) and the nuclear shell model (M. G. Mayer).

Allison was awarded the Medal of Merit for his work on the atomic bomb: he was a founding member of the Board of Sponsors of the *Bulletin of the Atomic Scientists*. He was elected a member of the National Academy of Sciences in 1946 and served as chairman of its Physical Section from 1960 until 1963. He served as guest lecturer and adviser in nuclear physics matters on several occasions in Spain, in Argentina, and in the United Arab Republic.

Allison was a large man and his tall stature and silvery-white hair gave him a striking presence in any gathering among men. It was difficult to disturb his sense of equilibrium and he had an air of imperturbable calm that made doubly effective the sound common sense he could interpose at the propitious moment in colourful but clear and quotable language. He never failed to find a humorous side to every situation, however tense.

He enjoyed the life of teaching and research. He never lost an opportunity to work in the laboratory with his own hands. He had a natural talent and the requisite patience for making his apparatus work and he took a particular pride in displaying these skills to his students.

He was a man who asked little but gave much. His friends, colleagues and former students mourn their loss.

H. L. ANDERSON

Dr. H. R. Lang

DR. HERBERT RAPHAEL LANG, who died suddenly on November 18 at the age of sixty-one, had been secretary of the Institute of Physics, and afterwards of the amalgamated body of the Institute of Physics and the Physical Society, since 1932. His connexion with the Institute went even further back than this and he was present as a schoolboy at the inaugural meeting in 1921. Lang read physics at the Imperial College of Science and Technology, and after his first degree continued as research student and demonstrator, gaining his Ph.D. for work on the investigation of the effect of temperature on the specific heats of liquids. His work on the thermal properties of liquids continued after his appointment to a research Fellowship by the Institute of Petroleum. He could have made an outstanding career in research or in industry but, fortunately for the physics profession, he was persuaded to become secretary of the Institute. It is difficult to imagine anyone better suited for such a post. Apart from his deep interest in physics he had a rare combination of wisdom, realism, determination and loyalty, as well as an enormous capacity for hard work, and above all a kindly and unselfish nature. No task associated with physics was too great or too small for him, whether it was organizing an international conference, forming a new subject group, giving professional advice to a junior colleague or operating the benevolent fund.

Lang was, by nature, shy and retiring. Although most members of the Institute and Society realized that he played a very large part in its affairs, it was only those who were privileged to work closely with him who fully appreciated the extent of his efforts and devotion. His work was conducted largely behind the scenes and he sought no personal credit. New developments and policies which were largely inspired by him were usually presented to the members through the honorary officers. At the same time, he was always ready to give credit to his own staff.

When Lang took office in 1932 the physics profession, as such, barely existed. To-day there are more than eleven thousand members, drawn from academic establishments, industry and government service. Under Lang's guidance, this vast number of pure and applied physicists, distributed throughout the United Kingdom and abroad, have developed, through the branches and specialist groups, common interests and activities, and a sense of belonging to the profession. From his early days in the Institute of Physics, Lang maintained close contact with the Physical Society, and it was due in no small measure to his untiring efforts during many years that amalgamation of the two bodies was effected in 1960.

Physics owes much to Lang and he will be greatly missed. He leaves a widow and three sons.

M. R. GAVIN

W. B. Alexander

W. B. ALEXANDER, formerly director of the Edward Grey Institute of Field Ornithology, University of Oxford, died on December 18, 1965, at the age of eighty. He was educated at Bootham School, York, Tonbridge School, and King's College, Cambridge. The Cambridge degree ceremony then involved a procession, headed by the first-class honours men (in alphabetical order) from the college providing the Vice-Chancellor, and in 1909 it was led by W. B. Alexander and the man he referred to as 'young Brooke'. After brief appointments in the Cambridge Museum of Zoology and then as assistant naturalist to the Board of Fisheries (North Sea), he accepted, in 1912, the post of assistant in the Western Australian Museum, Perth, and was Keeper of the Biological Department there from 1914 to 1919 (apart from two years in Melbourne on war work as a science abstracter). In this period he revitalized the Perth museum. After a brief return to England, he was biologist to the Prickly Pear Board from 1920 until 1925, and thus a prominent member of the team which effected one of the most successful examples of biological control of a pest ever recorded, Alexander being the man who first brought live *Cactoblastis* from South America to Australia. While in Australia he also played a prominent part in the Royal

Society of Western Australia and the Royal Australian Ornithologists' Union, and he is warmly remembered by Australian ornithologists.

On his voyages to England in 1920 and 1926, he was struck by the difficulty of identifying sea-birds, and this resulted in his best-known work, *The Birds of the Ocean*, published in 1928, which may fairly claim to be the first ornithological field guide. After 3 years without a regular post (he was a bachelor with modest private means), he was in 1929 appointed superintendent of the Tees survey of the Marine Biological Association. He left this post in October 1930 to become director of the Oxford Bird Census, founded by E. M. Nicholson, the late B. W. Tucker and the Oxford Ornithological Society, which, after the creation of the British Trust for Ornithology in 1933–34, became the Institute of Field Ornithology, and in 1938 the Edward Grey Institute. In his period as director, he played a valuable part in encouraging undergraduate ornithologists, and it was after taking undergraduate parties to trap migrants on Holy Island in 1931 and 1932, and a visit with senior ornithologists to Heligoland in 1933, that Alexander stimulated the founding of the first British Bird Observatories, on Skokholm and the Isle of May. His other main service was to build up the library of the Edward Grey Institute, much of it from his own pocket, as funds were very low. When he retired as director in 1945, he remained as librarian for a further ten years, and the library was named after him. His remarkable memory, usually precise to the part of a page, helped all in search of information. By now, the Alexander Library is probably the most complete ornithological library in the world, and the residue of Alexander's estate has been bequeathed to it.

D. Lack

NEWS and VIEWS

The Academy of Sciences of the U.S.S.R.: Foreign Members

Thirty-five scientists were elected Foreign Members of the Academy of Sciences of the U.S.S.R. at the recent meeting in Moscow. Included among these were four British scientists: Lord Florey, past-president of the Royal Society, and Provost of The Queen's College, University of Oxford; Prof. P. M. S. Blackett, president of the Royal Society and emeritus professor of physics, University of London, and part-time scientific adviser to the Ministry of Technology; Sir Robert Robinson, past-president of the Royal Society and emeritus professor of chemistry, University of Oxford; and Sir Geoffrey Taylor, formerly Yarrow research professor of the Royal Society. The total number of Foreign Members of the Academy of Sciences of the U.S.S.R. is now seventy-three.

Honorary Fellowship of the Institute of Mathematics and its Applications: Sir Geoffrey Taylor, F.R.S.

The Institute of Mathematics and its Applications has elected Sir Geoffrey Taylor as its first Honorary Fellow. Sir Geoffrey, who will celebrate his eightieth birthday on March 7, 1966, has made distinguished mathematical and experimental contributions to the mechanics of fluids and solids and their technological and geophysical applications. He was elected to Fellowship of the Royal Society in 1919 and received the Copley Medal in 1944. From 1923 until 1952 he was a Yarrow research professor of the Royal Society. Sir Geoffrey Taylor has been honoured by universities, academies and societies throughout the world. A formal presentation of the certificate of honorary fellowship will be made at the Imperial College of Science and Technology, London, in the Department of Physics on February 23, immediately preceding a lecture to the Institute by Dr. J. W. S. Cassels on "What are p-Adic Numbers?"

The Scottish Horticultural Research Institute, Mylnefield, Invergowrie: Dr. T. Swarbrick, C.B.E.

Dr. T. Swarbrick retired from the post of director of the Scottish Horticultural Research Institute, Mylnefield, Invergowrie, on January 31, 1965. He was appointed as the first director of the Institute on its establishment in 1951 and his retirement after 14 years thus marked the end of the first phase in the development of this important centre of horticultural research in Scotland. Dr. Swarbrick received his scientific training at the University of Leeds, where he graduated in agriculture and botany (1923), B.Sc. Hons. in botany (1924) and Ph.D. in plant physiology (1927), after holding a Ministry of Agriculture scholarship at Leeds, Long Ashton and Wisconsin (U.S.A.). On completing his training, he served on the staff of the Long Ashton Research Station first as physiologist and later as pomologist until 1945, when he joined the scientific staff of the Royal Dutch Shell Group, with headquarters in The Hague and Amsterdam. This was followed in 1951 by his appointment at Mylnefield, where he had the task of establishing and developing this new research centre to serve the special needs of the Scottish horticultural industry. In developing the research programme there, Dr. Swarbrick used as a basis the research units of the Department of Agriculture for Scotland for the investigation of diseases of raspberries and strawberries at Dundee and Auchincruive, and these subjects are still prominent in the present programme. Other important subjects of investigation include the breeding of raspberries, strawberries and vegetables, and the occurrence of natural growth substances in the strawberry in relation to environmental factors and developmental effects. Mylnefield, under Dr. Swarbrick's able guidance, has become a national research centre of repute and is a fitting memorial to his scientific ability and vision, his drive and enthusiasm, and his high personal qualities. His distinguished work there was officially recognized by the award of a C.B.E. in 1964; he has received the 1965 Scottish Horticultural Medal.

Organization for Economic Co-operation and Development

The Organization for Economic Co-operation and Development (O.E.C.D.), successor to the Organization for European Economic Co-operation (O.E.E.C.), was established in 1961; it comprises all the industrialized countries of North America and Western Europe, and also Japan. Three major aims guide its policy: first, promotion of the highest sustainable economic growth and employment and a rising standard of living in the member countries; secondly, support for sound economic expansion of member and non-member nations in the process of development; and thirdly, furtherance of the expansion of world trade on a multilateral, non-discriminatory basis. This Organization functions primarily as a forum for policy-making representatives of member Governments, and a brief account of its work has previously been noted in these columns (*Nature*, 205, 1098; 1965). To take one example, agriculture, ". . . Because of the difficulties encountered in adapting national agricultural policies to the requirements of fuller co-operation, agriculture has often been considered an obstacle to co-operation. So far

as agriculture is concerned, it is the function of O.E.C.D. to promote the transformation of agricultural policies to meet changes in the position of agriculture within and between countries".

This and much other information on the work of the Organization in this field is contained in an article, "Agriculture in an Industrial Economy", contributed by the Directorate for Agriculture, O.E.C.D., Paris (*Span*, 8, No. 3; 1965. Shell International Chemical Co., Ltd., Shell Centre, London, S.E.1). *Span* is an attractive and extremely well-presented magazine, published three times a year, and this particular issue contains a number of articles by different authors, the subjects ranging from pesticides, agricultural extension in the United States, a modern dairy farm in Thailand, Chinese market gardening in Malaya, urbanization and filariasis, tsetse eradication in Uganda, to the work of the Indian Agricultural Research Institute, which in 1965 celebrated its Diamond Jubilee.

Other interests and activities of the O.E.C.D. are chronicled regularly in the bi-monthly magazine, *The O.E.C.D. Observer* (in French and English). In a recent issue (No. 18; October 1965. O.E.C.D. Information Service, Château de la Muette, Paris), subjects dealt with are, *inter alia*: the high-level meeting of the O.E.C.D. development assistance committee; international payments; housing construction policies and techniques in Switzerland; a development plan for the Mediterranean coast of France; agriculture as a positive force in the growth process; and O.E.C.D. experience in efficient use of resources for school building. The Organization is responsible for many other publications covering a wide range of topics, as a list of recent titles in this issue makes clear. The new titles are: "Fish Handling and Preservation"; "Women Workers, Working Hours and Services"; "Wages and Labour Mobility"; "Teaching Physics Today"; and "International Standardization of Fruit and Vegetables", all being evidence of the practical manner in which the fundamental aims of the O.E.C.D. are being furthered and realized.

Scientific Research in France

Le Progrès Scientifique (No. 89; October 1965) contains a broad outline of the fifth Plan for Scientific and Technical Research in France (1966–70), on which 3,600 million francs are to be expended, with 600 million francs for development. This includes all expenditure on fundamental or applied research not under the military or space programmes or on the Atomic Energy Commission. 2,000 million francs are provided for space research, of which 450 million francs are for the European programme. Of the 3,600 million francs, 1,254 million francs are for physics, 590 million francs for biology and medicine, 400 million francs for chemistry, 390 million francs for agricultural research, 315 million francs for mathematics, 284 million francs for the sciences of the Earth, water and atmosphere, 150 million francs for oceanography, 120 million francs for the human sciences, and 162 million francs for construction, public works, urban studies. Some 43,350 research workers and technicians were available in 1963, of whom 11,180 were in the natural and exact sciences, 19,160 in engineering, 8,410 in medicine and pharmacy, and 4,600 in the human sciences. Of the total, 16,530 were in higher education, 17,350 in professional and non-profit institutions, 4,310 in the Centre for Research in Nuclear Science, 2,540 in the Prime Minister's or Defence Ministry, and 2,620 in other technical ministries. The total is expected to increase by 35,000 during the period.

The Netherlands Delta Plan

SINCE time immemorial, the delta area lying south-west of Rotterdam in The Netherlands has been a 'half-open door' through which the North Sea, in its stormier moods, has poured, inundating towns and villages, washing away precious topsoil and choking the land with a surfeit of salt. The tragic floods of 1953, when more than 1,800 people lost their lives, 72,000 people had to flee from their homes south of Rotterdam, 800 miles of dykes were wrecked, 500,000 acres of land overrun, some 200,000 animals destroyed on Dutch farms, to say nothing of 10,000 houses, schools and churches destroyed, all taught a grim lesson. Shortly after this great disaster, what was known as the Delta Commission was formed in The Hague, its task to find a practical means of beating these terrible incursions of the North Sea for ever. One project was to raise the level of existing sea dykes; an alternative was the imaginative scheme of damming up the sea arms of the delta altogether. The Dutch have a fanatical and historical enthusiasm for land reclamation, and it was this second scheme that was recommended by the Commission, adopted by the Minister of Transport and Waterways, eventually approved by the Dutch Parliament, and consolidated under the Delta Act. The Delta Plan, as it is known, is undoubtedly one of the outstanding civil engineering ventures of this or, for that matter, any other age; it is estimated that it will take 25 years to complete; it will shorten the country's coastline by more than 400 miles, at an estimated cost of some £250 million.

In a strikingly illustrated article entitled "Against the Sea" (*Esso Magazine*, 4, 14; Autumn 1965. Esso Petroleum Co., Ltd., London, S.W.1), some details are given of this ambitious undertaking, including a map of the Delta Plan, giving the dates of starting and completing the major projects. The seven main constructions are Haringvliet, between Voorne and Goeree (1957–69); Brouwershavense Gat, between Goeree and Schouwen (1962–72); Oosterschelde, between Schouwen and Noord Beveland (1965–78); Veerse Gat, between Noord Beveland and Walcheren (1956–61); Zandkreek, between Noord Beveland and Zuid Beveland (1958–60); Grevelingen, between Overflakkee and Duiveland (1958–64); and Volkerak, between Hoeksche Waard and Overflakkee (1957–67); the years in parentheses are starting and finishing dates. The dams in the Haringvliet, Brouwershavense Gat and Oosterschelde are all major sea walls, the last-mentioned being the longest and heaviest dam in the whole scheme. The Haringvliet is the estuary through which more than half the waters of the Rhine and the Meuse empty into the North Sea. The Dutch want to hold and store as much of this water as possible in large fresh-water lakes, which will be constructed when the Delta Plan is complete, but at the same time they want to control the efflux of surplus water into the North Sea by means of seventeen sluices, each 185 ft. wide.

The Delta Plan will eventually secure the safety of the existing land; it will combat salination of rivers and adjoining channels, thus increasing agricultural production. The islands of the Delta will no longer be isolated; new roads will run on top of the new dykes, providing fast links with Belgium, Rotterdam and towns beyond in The Netherlands. A new natural playground will be created in this region, a country of wooded lakes and sandy beaches, an added attraction to tourists. The one disadvantage of this Delta Plan is that with the exclusion of the sea, once it is completed, the fisheries, oyster and mussel beds, so long characteristic of Zeeland, will disappear; but, it is argued, this is perhaps a small price to pay for beating the destructive power of the North Sea for ever in this enterprising country.

Research in Chemical Information

ACCORDING to *Scientific Information Notes* (7, No. 5; October–November 1965), the National Bureau of Standards, in connexion with the Chemical Information Programme of the National Science Foundation, is to investigate fragmentation codes for preparing indexes of chemical structures, and searching techniques for specific and generic chemical structures represented as connexion

tables. The Bureau will also investigate file organization, to develop means for storing specific categories of data, and will continue previous work on extending and mechanizing various linear notations. The research group will work in close co-ordination with the research and development group of the Chemical Abstracts Service, which is also supported by a contract from the Chemical Information Programme.

Jena Review

The *Jena Review* (*Jenaer Rundschau*, as it is more widely known) was first published in 1956 and has recently celebrated its tenth anniversary in a number to which no less than seven Nobel Prizewinners contribute (*Jena Review*. VEB Carl Zeiss Jena. VEB Verlag Technik, Oranienburger Str. 13/14, Berlin C 2. No. 5; 1965). This enterprising house journal is now available in English, French, Russian and Spanish, besides the original German. Following the editorial by Dr. H. Schrade entitled "Ten Years of Jena Review", articles by the seven eminent scientists are: "The 'False Trans-uranic' Elements", by Dr. O. Hahn, Göttingen; "Stable Isotopes", by Dr. G. Hertz, Berlin; "Symmetry and Invariance", by Dr. E. P. Wigner, Princeton, New Jersey; "The Part played by Pure Science and the Progress in High-Energy Physics", by Dr. C. P. Powell, Bristol; "Critical Opalescence", by Dr. P. J. W. Debye, Ithaca, New York; "Seeing and the Nature of Light", by Sir C. V. Raman, Bangalore; and "Celebration of the Award of the 1964 Nobel Prizes", by Dr. A. Tiselius, Uppsala. Other contributions are "Structure of Liquids and the Formation of Glass", by Dr. W. A. Weyl, Pennsylvania State University; "The Importance of Walter Nernst", by Dr. K. Schwabe, Dresden; and an obituary notice of the late Dr. Hans Boegehold, for 57 years with Zeiss, by Dr. H. Schrade. *Jena Review* appears bi-monthly; it is always well printed and produced, and the illustrations, both in black-and-white and in colour, are of consistently high quality. Although originally founded to be ". . . a bridge and a linking of all the friends of the scientific and technical work of VEB Carl Zeiss Jena to all users of Zeiss equipment in every country throughout the world", in which aspiration it has certainly succeeded, this magazine is never a collection of explanatory advertisements; it is a welcome vehicle of up-to-date technical information, especially in the field of optical instruments and physics.

The Museums Journal

The December issue of *The Museums Journal* follows the pattern of previous issues and is mainly devoted to a single theme (65, No. 3; 1965). The articles help to define some areas of work with art collections. One concerns a regional art collection and the problems it poses of balance and specialization, while another depicts the work of an art dealer from his side of the desk. The applied arts are no less complex than the fine arts, and a paper on furniture introduces a field of study which has recently generated a new society devoted to its history. The new processes used for the reproduction of relatively large numbers of lithographs are described. All these articles will help reflection on the role of a curator in an art gallery. The concluding article describes museum and art gallery buildings erected between 1845 and 1914.

Sarawak Museum

The Sarawak Museum Journal, edited by the curator, Mr. Tom Harrisson, includes an interesting set of papers, among which is a comprehensive study of the Sarawak Malays by the editor (11, Nos. 23–24. (New series.) Pp. xii+341–666. July–December 1964. 5 dollars; 12*s.* 6*d.*). This paper will form the opening chapters of a book on the subject to be published in 1966. Other articles include notes on the descent of some Saribas Malays, Sarawak Malay pottery, some Baram coffin burials, Papuan stone adzes, an introductory vocabulary of Suluk, Sarawak relations with Britain 1858–70 and Borneo maps of sixteenth and seventeenth centuries.

Transvaal Museum

The Annals of the Transvaal Museum comprises an account by Hans John (Germany) of the species of the coleopterous family Discolomidae collected between 1960 and 1962 during field research on the humicolous fauna (25, No. 1: *Sudafrikanische Discolomidae* (*Coleoptera*). Von Hans John. Pp. 19. Pretoria: Transvaal Museum, 1965). Included also is a paper by Jean Thérond (France), detailing the species collected in Southern Africa during recent Transvaal Museum expeditions (25. No. 2. Pp. 14).

The National Central Library

The forty-ninth annual report of the Executive Committee of the National Central Library covers the year ended March 31, 1965, in which the number of applications increased to 119,468 (107,742 in 1963–64), 77 per cent being satisfied, compared with 78·75 per cent in 1963–64 (Pp. 24. London: The National Central Library, 1965). Of the applications, 92,844 were for books and 26,844 for periodicals: of those satisfied, 38,318 came from public libraries, 20,895 from university libraries, 12,007 from special outlier libraries, 2,608 from Government libraries and 4,727 from overseas libraries. British libraries made 4,150 loans to overseas libraries (3,739 in 1963–64) and borrowed 3,863 items from overseas (3,488 in 1963–64); 577 photocopies were supplied to foreign libraries and 1,417 obtained. Of the fifty-eight lending countries, West Germany was the chief, followed by the Soviet Union, the United States, East Germany and France. West Germany was also the principal of 36 borrowers, followed by Hungary, the Soviet Union, Denmark, Czechoslovakia and East Germany. The United States was the chief source of photocopies and Bulgaria the chief recipient. The 384 outlier libraries satisfied 21,278 applications, and other special libraries another 8,313. In addition to 24,468 loans made at the direct request of the National Central Library, university libraries lent 17,445 items as members of regions. A further 15,000 books were catalogued and housed at the Woolwich building, bringing the total to 82,000, and the *Survey of Applications, January 1–June 30, 1964*, is now being examined by the Executive Committee. The first stage of the unification of the catalogues was completed and the British Union Catalogue of Periodicals was continued from 1960 on a new basis. The Treasury grant for the year was £105,000 (£95,000 in 1963–64). The National Committee on Regional Library Co-operation and the Implementation Committee both met twice during the year.

Indexes and Indexing

Of how many authors of scientific books can it be said with Goldsmith that "He writes indexes to perfection"? Time and again reviewers are critical of the adequacy (or lack of adequacy) of the index to a volume crammed full of academic or technical detail, of which the contents page or chapter headings suffice for direction, with perhaps only a skeleton index to the main subjects to assist the reader. Codes of practice exist as guidance to authors in preparing scientific papers for publication, on the style in which they should be presented, on the proper arrangement of references and quotations, even to instructions on desirable forms of illustration; but there are still no precepts generally accepted, let along followed, on the subject of indexing for its own and especially for the reader's sake; this, despite a British Standard on *Alphabetical Arrangement*, which is a set of rules designed to secure uniformity in the alphabetical arrangement of items in indexes, catalogues and lists of all kinds (1749;

1951. Pp. 12. British Standards Institution, London). After all, many scientific and technological books are, in fact, a series of related papers, treating logically of various phases of a specific subject; as such, they merit a proper and comprehensive index, not only for the student of the subject, but even more for others, perhaps in different disciplines, who have to refer quickly to relevant author or subject details. At the other extreme, there is the type of index which is self-defeating because it emphasizes the wrong word, for example, the adjective, not the noun.

A case in point is emphasized by the writer of a notice of a recent British official technical publication, *Research on Road Traffic* (Pp. 517. London: H.M.S.O. 42*s*.) in the *Road Research Unit Newsletter No.* 9 (October 1965. National Roads Board, New Zealand: Wellington, P.O. Box 8024). After paying tribute to this volume, he says: "It is easy to criticize the layout of indexes; yet many criticisms deserve attention. The main charge is against 'hybridization' which supplants the essentials". He comments on ". . . the format technicalities . . . obsessed with system rather than with simple usage" and goes on to say: "Nothing produces such exasperation in a reader as a hunt for relevant figures, diagrams, tables, plates . . . which are each given separate numerical sequences; and therefore 'it's every man for himself' ". Instances are quoted: 'Safety Kerbs' are not shown under 'K' or 'S'; they appear under 'C' and 'R'; 'Barriers' do not appear under 'B', but under 'R' they get two mentions; the list is extended. This writer also makes the foregoing point that indexes so often fail because the author insists on giving preference to an adjective rather than to the basic noun. In many text-books the ideal in indexing is achieved where author and subject indexes are separated; where the subject index really does list the essential nouns in each chapter, whether preceded by capital letter or in lower case, followed by as many qualifying adjectives as may be appropriate. But what is here called 'typographic pedantry' is surely to be avoided at all costs. An example of a clear, concise, rapidly informative subject index is the *Index to N.B.R.I. Information Sheets* recently received (National Building Research Institute, South African Council for Scientific and Industrial Research. CSIR Technical Note *X/BOU* 100; Building Series Dis. 100. Pp. 10. Pretoria, May 1965). But a model for authors is undoubtedly the *Subject Index to List of British Standards*, included in each annual volume of the *British Standards Yearbook*; for the art of indexing and particularly of cross-referencing, this takes a lot of beating.

Check-list of British Lichens

A NEW check-list of British lichens was published by the British Lichen Society in the 1965 number of *The Lichenologist*. It was prepared by P. W. James, of the British Museum (Natural History). The list is notable for aligning the names of the 1,347 British species with those used elsewhere in the world, while ensuring that the nomenclature conforms to the *International Code of Botanical Nomenclature*. Interleaved copies of the check-list are obtainable from the treasurer of the British Lichen Society, Dr. T. D. V. Swinscow, 103 London Road, Knebworth, Hertfordshire, price 7*s*. 6*d*., post free.

Lady Tata Memorial Trust International Grants, Scholarships and Fellowships for Research on Leukaemia and Allied Conditions

THE Trustees of the Lady Tata Memorial Trust invite applications for fellowships, scholarships and grants for research on leukaemia, in the academic year beginning October 1, 1966. In view of the affinity between leukaemia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration. The Trustees specially wish to encourage investigations of the leukaemogenic viruses in mammals, the epidemiology and natural history of leukaemia, and the immunogenic aspects. The awards offered by the Trust are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad. Further information and forms of application can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3. Applications must be submitted before March 31, 1966.

Fulbright Travel Grants

UNDER the provisions of the Fulbright programme, travel grants are available to citizens of the United Kingdom and dependent territories to go to the United States of America for academic or educational purposes, provided that they have adequate financial support in dollars for the proposed period of the visit to the United States. Those applicants who are graduate students must show proof of the admission to an institution of higher learning in the United States for a minimum period of nine months, whereas those who are professors, lecturers and senior research scholars must hold an appointment to undertake lecturing or advanced research at an approved American institution of higher learning. All grants cover the cost of direct round-trip travel between a candidate's home and the American university or institution. For graduate students the departure dates are between June 1, 1966, and July 31, 1966, for which applications must be received before April 1, 1966; and between August 1, 1966, and April 1, 1967, for which applications must be received before May 15, 1966. For professors, lecturers and senior research scholars the departure dates are between June 1, 1966, and July 31, 1966, for which applications must be received before March 16, 1966; and between August 1, 1966, and April 1, 1967, for which applications must be received by June 1, 1966. Application forms and further information can be obtained from the United States–United Kingdom Educational Commission, 71 South Audley Street, London, W.1.

Endeavour Prizes

PRIZES totalling more than £200 are offered by Imperial Chemical Industries, Ltd., publishers of the international scientific review, *Endeavour*, for essays submitted on scientific subjects. In addition to the cash prizes, the prizewinners will receive invitations to attend the whole of the British Association meeting at Nottingham during August 31–September 7. Accommodation will be provided in Nottingham and travelling expenses within the United Kingdom will be paid. As the primary purpose of these awards is to stimulate younger scientists to take an interest in the work of the British Association and to raise the literary standard of scientific writing, the competition is restricted to those whose twenty-fifth birthday falls on or after September 1, 1966. Five prizes will be awarded, namely: a first prize of 100 guineas; a second prize of 50 guineas; a third prize of 25 guineas; and two special prizes of 10 guineas each for competitors who have not passed their eighteenth birthday on September 1, 1966. The subjects for the essays are as follows: (1) mechanisms of morphogenesis; (2) the pattern of sub-nuclear particles; (3) microcircuitry; (4) chemicals in agriculture; (5) the cell surface; (6) recent advances in thermochemistry. The essays, which must be in English and typewritten, should not exceed 4,000 words in length, and only one entry is permitted from each competitor. All entries should be addressed to: the Deputy Secretary, British Association for the Advancement of Science, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1, and the envelope should be clearly marked '*Endeavour* Prize Essay'. The latest date for receipt of entries is June 1, 1966. The essays must be submitted without signature, and the competitor's full name and

address and date of birth should be enclosed in a sealed covering letter attached to the essay and addressed to the Deputy Secretary of the British Association.

The Mathematics of Control Systems

The second residential conference of the Institute of Mathematics and its Applications on "The Mathematics of Control Systems" will be held at Sunderland Technical College during April 3-6. The following lectures have also been arranged: "Non-linear Hydrodynamic Stability" by Prof. P. H. Roberts (Constantine Technical College, Middlesbrough, on February 21); "Reciprocity Theorems in Applied Mathematics" by Prof. G. J. Kynch (Birmingham College of Advanced Technology, on February 22); "Application of Mathematics to Problems arising in the Electrical Manufacturing Industry" by Mr. N. Kerruish (University of Leeds, on February 22); "Mathematics applied to the Large-scale Dynamics of the Atmosphere" by Mr. J. S. Sawyer (University of Strathclyde, on February 24); "Mathematics in Electrical Engineering" by Mr. N. Kerruish (Birmingham College of Advanced Technology, on March 2). Further information can be obtained from the Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex.

University and College News: Belfast

Dr. D. J. Bradley, reader in physics at Royal Holloway College, London, has been appointed to the chair of physics. The following appointments have also been made: *Readerships:* Dr. R. Common (geography); Dr. M. F. Grundon (organic chemistry); Dr. A. P. Roberts (engineering mathematics); Dr. F. J. Smith (applied mathematics); *Senior Lectureships:* Dr. R. M. Hobson (physics); Dr. D. McCloy (mechanical engineering); Mr. N. Stephens (geography); Mr. B. C. S. Wilson (archaeology).

The College of Advanced Technology, Birmingham

Dr. F. M. Page has been appointed professor of chemistry.

London

Dr. B. R. Coles, at present reader in physics at the Imperial College of Science and Technology, has been appointed to the chair of solid-state physics tenable at that College, from October 1.

Southampton

Dr. I. R. Beattie has been appointed to the chair of inorganic chemistry. Dr. J. M. Nightingale has been appointed to the chair of control engineering. Mr. R. A. Farrar has been appointed lecturer in mechanical engineering.

Announcements

Dr. M. A. Tuve, director of the Department of Terrestrial Magnetism of the Carnegie Institution of Washington, has been appointed home secretary of the United States National Academy of Sciences, in succession to the late Dr. H. L. Dryden.

Dr. L. Schiff, executive head of the Department of Physics at Stanford University, has been awarded the 1966 Oersted Medal by the American Association of Physics Teachers, for notable contributions to the teaching of physics.

The address of the Plant Variety Rights Office is now Murray House, Vandon Street, London, S.W.1.

The title of the Fruit and Vegetable Canning and Quick Freezing Research Association is now the Fruit and Vegetable Preservation Research Association.

A symposium on "The Place of the Chemist in Industry", organized by the Royal Institute of Chemistry, will be held at the Oxford College of Technology on March 3. Further information can be obtained from Mr. W. Sabel, Oxford College of Technology, Headington Road, Oxford.

A symposium on "Chemistry and Rheology of Water Soluble Gums and Colloids", arranged jointly by the Colloid and Surface Chemistry Group of the Society of Chemical Industry and the British Society of Rheology, will be held at the School of Pharmacy, University of London, on March 28. Further information can be obtained from Mr. B. Warburton, Department of Pharmaceutics, School of Pharmacy, 29–39 Brunswick Square, London, W.C.1.

A working conference on inter-personal and inter-group relations entitled "Learning and Leadership", arranged by the Department of Adult Education of the University of Leicester in collaboration with the Tavistock Institute of Human Relations, will be held in the University of Leicester during March 25–April 7. Further information can be obtained from Miss D. J. Bostock, Tavistock Institute of Human Relations, 3 Devonshire Street, London, W.1.

Corrigendum. In the article entitled "Isotopic Ages of Minerals from the Pre-Cambrian Complex of the Malverns", by Dr. R. St. J. Lambert and D. C. Rex (*Nature*, 209, 605; 1966), the second sentence of paragraph three, p. 606, line 14, should read "A *Callavia* fauna . . .".

The Night Sky in March

All times are in Universal Time

Moon

New Moon 22d 05h
Full Moon 7d 02h

Conjunctions with the Moon

Venus 17d 22h, 9° N.
Mars — —
Jupiter { 1d 08h, 2° S. / 28d 19h, 3° S.
Saturn — —

Planets

Time of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_g (10⁸ miles)	Zodiacal position
Mercury	*S*	19h 20m	19h 05m	Unfavourable	+1·9	64	Pisces
Venus	*R*	4h 45m	4h 25m	4h 10m	−4·2	47	Aquarius
Mars	—	Unfavourable for observation			—	221	Pisces
Jupiter	*S*	3h 05m	2h 10m	1h 20m	−1·8	472	Taurus
Saturn	—	Unfavourable for observation			—	987	Pisces

D_g is the distance of planet from the Earth on the 15th of the month

Occultations of Stars Brighter than Magnitude +6 at Greenwich

Star	*R*/*D*	Time	Mag.
α Lib	*D*	11d 00h 23·0m	+2·9
δ Lib	*R*	11d 01h 06·4m	+5·3
α Lib	*R*	11d 01h 12·8m	+2·9
τ Tau	*D*	27d 21h 06 5m	+4·3

(*D*, disappearance; *R*, reappearance)

Other Phenomena

7d 02h, Uranus 4° S. of Moon

REVISING THE LAW OF PATENT

THE Patents Liaison Group was originally constituted in accordance with proposals made by Mr. R. Maudling, then President of the Board of Trade, in June 1961, to formulate views on the proposals of the Common Market countries with regard to a common patent system. The Group, at his further request, also advised the Minister on a draft of a Convention on unification of points of patent law proposed under the aegis of the Council of Europe and signed by the United Kingdom and other countries at Strasbourg on November 27, 1963. The Group was reconvened to consider the Convention and to report on those changes in the Patent Act, 1949, which would be involved in the ratification of the Convention by the United Kingdom. For this purpose, the Group, of which Mr. G. W. Tookey was chairman, was enlarged by representatives of the General Council of the Bar and the Law Society, in addition to those from the International Association for the Protection of Industrial Property (British Group), Association of British Chambers of Commerce, Chartered Institute of Patent Agents, Federation of British Industries, National Association of British Manufacturers, Trade Marks, Patents and Designs Federation, and the British National Committee of the International Chamber of Commerce who were originally included.

The report now presented to the President of the Board of Trade* points out that the Group's task was not to translate the provisions of the Convention into a United Kingdom statute. Instead, it was to undertake the complicated business of amending the existing Statute so as to remove parts which are inconsistent with the Convention and to insert in their place new provisions which conform to the requirements of the Convention and are couched in language which fits the general pattern of the Statute and is not ambiguous. The amendments should also make clear which precedents are applicable for the future and which are not. Much case law has been built up from the days of the Statute of Monopolies in 1623, and the definition of 'invention' is still based on the language of that early Statute. This Statute—it has been the subject of much judicial interpretation—requires alteration in certain respects.

The Group's recommendations are collected under four general headings, and those of major scientific interest are to be found under the first and third of these, relating to the novelty and inventive step and to patentable inventions, respectively. In the Convention an invention is stated to be new if it does not form part of the state of the art. This is defined as comprising "everything made available to the public by means of a written or oral description, by use or in any other way, before the date of the patent application or of a foreign application, the priority of which is validly claimed". Some extensive revision of the Patents Act, 1949, will be required to fulfil the obligations on this subject resulting from the Convention. The limitations of place and time in United Kingdom law must give way to the concept of absolute novelty and the law must be revised so that an invention is not regarded as new if in any way it has been made available to the public in any part of the world before the priority date of the patent application for the invention, or is obvious having regard to what has thus been made available to the public.

The group recommends that Section 32 (1) (*e*) and (*f*) of the Patents Act should be amended to read: "(*e*) that the invention, so far as claimed in any claim of the complete specification, already formed part of the state of the art as it existed before the priority date of the claim; (*f*) that the invention, so far as claimed in any claim of the complete specification, was obvious and did not involve any inventive step having regard to the state of the art as it existed before the priority date of the claim".

Further, the definition in Section 101 of the Act should include the following definition of the 'state of the art': "'State of the art' means all matter available to the public in the United Kingdom or elsewhere by means of a written or oral description, by use, or in any other way, so that persons skilled in the art are or can become acquainted with such matter."

It will be necessary to revise Section 7 of the Act so as to remove the provisions which limit the official search to United Kingdom patent applications dated within 50 years of the filing date; to delete the Sections relating to secret use; and to abolish the concepts of "invention of importation" and of the communicated inventions. Section 15 will also have to be revised so as to permit reference to any document published in the United Kingdom or elsewhere. Further amendment will be necessary to the Section protecting inventions displayed at an exhibition certified by the Board of Trade or described in papers read before learned societies, and the Section protecting inventions communicated under international agreements will have to be deleted.

With regard to patentable inventions, the Group considers that there is nothing in the Strasbourg Convention which would prevent the United Kingdom from formulating a new definition of invention. However, none of the proposals which it examined was free from difficulties, and the Group is satisfied that it is preferable not to frame a definition. The field covered by science and technology is already extremely wide, and, with the progress of time, the boundary of patentable subject-matter will inevitably increase. The Group considers it wiser not to seek to put a limit on what should be regarded as an invention and recommends that the new Act should not define 'invention' with any greater particularity than is necessary to conform to the Convention. Deletion of the existing definition and its replacement by wording to the effect that patents may be granted for inventions which can be made or used in any kind of industry, including agriculture, will lead to some uncertainty until the Courts have established some case law, but the Group was convinced that this flexible course was preferable to a restrictive one.

To some extent, the area of uncertainty can be narrowed by inserting in the Act a short list of exceptions. Two such exceptions are stipulated in the Convention itself: inventions contrary to *ordre publique* or morality and inventions in respect of plant or animal varieties. These the Group recommends should be specifically excluded: exclusion of plant or animal varieties from patentability is necessary to avoid overlap with the Plant Varieties and Seeds Act, 1964. The Group doubts, however, whether a distinction can or should be drawn between processes which are 'essentially biological' and those which are not, bearing in mind that any process for treating plants or animals which utilizes the natural functions of living matter will be more or less 'biological'. It recommends, therefore, that there should be no specific exclusion in respect of the plants or animals.

In addition to these exclusions, the Group recommends six other categories of exclusion, which might, in fact, be found by the Courts not to be 'inventions' within the meaning of the Act. These are: (i) methods of calculation: theoretical ideas and scientific principles; (ii)

* Board of Trade. *United Kingdom Patent Law: The Effects of the Strasbourg Convention of 1963.* (Report on legislative changes which would be involved in the proposed ratification by the United Kingdom of the Strasbourg Convention on the unification of certain points of substantive law on patents for invention by the Patents Liaison Group.) Pp. 18. (Cmnd 2835.) (London: H M S.O., 1965) 1*s* 6*d* net

business schemes (for example, methods of office management); (iii) commercial, financial and propaganda schemes; (iv) treatment of human beings; (v) designs or arrangements in which the novelty resides solely in appeal to the eye; (vi) designs or arrangements which serve only to convey information and in which the novelty resides solely in the information conveyed.

The Group recommends that adequate steps should be taken to enlighten agricultural interests as to the changes in the United Kingdom law which are contemplated and to explain the reasons for these changes.

The Group points out that the unification Convention does not completely solve the problem of how to resolve conflict with prior unpublished applications. Article 6 of the Convention broadly requires that parties of the Convention shall not allow double patenting. The present statutory provisions in the United Kingdom rest basically on the principle that double patenting is to be avoided, but the interpretation given to these provisions in recent years has produced serious difficulties. The Group agreed generally that a straightening out of United Kingdom law is necessary to bring some real sense into this subject quite apart from the requirements of the Convention. Generally, the Group is also agreed that a later patent should not be granted if it purports to prevent the working of the invention as claimed in an earlier patent in any manner in which a competent person would normally work it. Some suggested forms of wording for this 'prior claim' proposal are appended to the report. Nevertheless, the Group concludes that there should be no requirement that a patent granted on the earlier application should be granted, or valid if granted.

THE FUTURE OF NUCLEAR POWER

THE John Macrossen Lecture was given by Sir John Cockcroft in the same year as the third International Conference on Atomic Energy was held at Geneva. The Lecture is a very brief statement of the prospects of the economic development of nuclear power at that date, a subject very fully dealt with at Geneva. The capital costs per kW of electricity generated by the stations built by industry for the Central Electricity Generating Board will almost have halved in the 6 years between the completion of the 275-MW Berkeley station and the 1,180-MW Wylfa nuclear station, each of them containing two reactors. The fall is due in large part to the increased size of the unit; fuel costs have also fallen, but the overall cost of power by any calculation is still higher than that from coal-fired stations of the same dates.

The advanced type of gas-cooled reactor was expected in 1964 to cost less than a coal station of the same date, and within a year of the delivery of this Lecture the firm tender prices for the Dungeness *B* nuclear station showed that this was, in fact, the case, provided the station is built at the tender price.

In the United States similar dramatic falls in costs have been experienced with their water moderated reactors; and Canada's heavy-water reactor is expected to have very low fuel costs, although it will have a high capital cost.

These types of reactors, by the end of the century, would be using 100,000 tons of uranium per annum, on reasonable assumptions as to the rate of development of nuclear stations. To avoid a rise in the cost of raw materials, more of the uranium-238 must be used; the experimental fast reactor at Dounreay has operated well enough now to justify a design study for a 1,000-MW reactor to be ready for the late 1970's; this might herald a new generation of stations to use up the plutonium from the slow-neutron reactors and the fast reactor might breed 1·5 times the amount of fuel it uses.

Fusion reactions still remain as a possibility and as a hope for the future; stability of the confined plasma at low pressures has been achieved but not at high densities. It would be idle at present to predict whether these schemes will be successful. T. E. Allibone

NUMERICAL METHODS IN SUBSONIC FLUID DYNAMICS

A SYMPOSIUM on "Numerical Methods in Subsonic Fluid Dynamics" was held at the National Physical Laboratory during September 27–29, 1965, the local organization being shared jointly by the Aerodynamics and Mathematics Divisions. More than one hundred and thirty visitors from industry, universities, technical colleges and Government establishments, including several from Europe and the United States, participated in the programme, which was divided into four sessions, each consisting of two general survey lectures followed by three parallel discussion groups *A*, *B* and *C*. The subjects of the discussion groups were arranged so that *A* was a follow-up of the general lectures of the session, *B* was an independent subject introduced by a scheduled talk, and *C* was a more specialized subject determined by popular demand from the participants themselves.

The original initiative for the meeting came from the Aeronautical Research Council, which had some two years ago anticipated the need for a comprehensive examination of the role of the computer in fluid dynamics by setting up a Computer Panel. Quite early on, it became clear to the Panel that the full potentialities of the modern 'third generation' computer were not being adequately explored; consequently this symposium was arranged with the objects of elucidating some of the problems involved in the application of computers to fluid motions, and of stimulating interest in the exploration of new approaches to the subject which might be brought within the realms of possibility by the latest computing facilities.

The participants were welcomed by Dr. R. C. Pankhurst, representing the Acting Director of the Laboratory. Prof. B. Thwaites of Southampton University, chairman of the Computer Panel, then gave an introductory talk, in which he outlined the main problems facing the symposium. He suggested that scientists now needed to re-examine their motivations in research—were they interested mainly in mathematical analysis (which, in fluid dynamics, is very difficult) or in numerically expressed results? The answer might be different for the designer and for the 'pure' scientist. In either case, however, it was necessary to take into account the possibility of currently outstanding problems being solved, even within the next ten or twenty years, at the touch of a button; for this should surely influence the directions which work will take in the intervening period. Thus he emphasized the importance of directing the discussions towards what will be done in the future.

This was followed by a talk on "The Relevance of Numerical Analysis" by Prof. L. Fox of the University of Oxford. He concerned himself mainly with the finite-difference solution of the relevant types of partial differential equation, paying particular attention to the solu-

tion by iterative methods of the associated difference equations. He also discussed the problem of truncation error, in particular the treatment of singularities, but emphasized the outstanding gap remaining between the practical problems one wanted to solve and the problems for which rigorous error estimates could be given. Questions of stability arise with initial-value problems, and these he discussed with reference to parabolic equations. Among other topics mentioned were the effect of non-linearities and problems with free boundaries.

The participants then split up into the three parallel discussion groups, *A*, *B* and *C*. In this session, group *A* was on "Numerical Analysis" and was chaired by Dr. E. T. Goodwin (National Physical Laboratory); the discussion ranged over a wide variety of questions raised by Prof. Fox's talk. Group *B* was concerned with "Meteorological Forecasting by Computer" and was chaired by Prof. P. A. Sheppard (Imperial College of Science and Technology); it was introduced by Mr. E. Knighting of the Meteorological Office. Mr. Knighting outlined the three main computing tasks involved: selection of reliable data from those received from the international recording stations, conversion of this for use on a regular square grid, and, finally, integration of the relevant equations. With regard to the latter he discussed methods of integrating simple vorticity equations with the emphasis on simplicity and time-saving since forecasting must be carried out in real time. A lively and worth-while discussion followed.

In the other parallel discussion group (*C*) a relatively small number had assembled to discuss "Magnetohydrodynamics" under the chairmanship of Mr. D. S. Butler (University of Strathclyde). Several problems were mentioned that clearly needed the use of a large computer, but it appeared that in some cases these were being tackled without sufficient consideration of the physical concepts involved; in particular, problems were being tackled which seemed to be far too complex to offer any hope for a successful conclusion, and it was remarked that the computer seemed to be inducing people to run away from reality. A lively discussion followed on how computers could best be used, and the view that it would be profitable at this stage to try to compute simple 'component flows' first was widely supported.

The morning session of the following day began with a lecture by Mr. A. M. O. Smith of Douglas Aircraft on "Integral Equation Methods for Potential Problems", in which he demonstrated that, with the advent of large computers, these methods, based on source distributions over the surface of the body, had become a practical means of computation for general non-lifting three-dimensional problems. The numerical procedure was basically quite simple and consisted in reducing the problem to an algebraic one by dividing the body up into a large number of small elements and assuming the source density constant over each element. The most complicated problem attempted to date was the determination of the flow over a swept wing having two jet-engine nacelles complete with pylons and internal ducts. Though this problem was really beyond the capacity of the present computing equipment, the calculations and test data were in better than merely qualitative agreement.

Mr. J. A. Bagley of the Royal Aircraft Establishment followed with a survey lecture on "Subsonic Wing Design". He traced the development of the traditional methods of wing design and discussed the difficulties which have to be faced in improving them. He made it abundantly clear that it will be quite insufficient merely to improve methods of calculating the inviscid incompressible flow, but pointed out that even for the much simpler two-dimensional case the problem of calculating the viscous compressible flow seemed to be some way from being solved.

In the following discussion groups, *A* was concerned with "Inviscid Flow" (chairman: Prof. G. N. Ward, University of Sussex), *B* with "Programming Languages and Programme Interchange" (chairman: Prof. S. Gill, Imperial College), and *C* with "Hydrodynamic Stability" (chairman: Mr. M. Gaster, National Physical Laboratory). In the *A* group the topics discussed were centred around wing theory; in particular there were discussions on recent methods for lifting surfaces, and on the non-convergence of the circulation when the displacement effect of the boundary-layer is taken into account. Mr. P. M. Woodward of the Royal Radar Establishment introduced discussion *B* with a brief historical review of programming and an analysis of the role of the specialist systems-programmer. He emphasized the need for mathematicians and numerical analysts to become literate in Algol. A lively discussion ensued which tended to underline the difficulties of thinking in terms of using a nebulous machine to which one might have access in the future when results were needed to-morrow. In group *C* the main topic discussed was the application of modern computer techniques for eigenvalue problems to linear stability theory, particularly in regard to boundary-layer stability.

The first half of the afternoon session consisted of a double lecture on "Boundary-layer Problems" shared by Prof. K. Stewartson of University College, London, and Dr. K. W. Mangler of the Royal Aircraft Establishment. This was confined solely to laminar boundary-layers, for which a determinate system of equations exists. Prof. Stewartson gave detailed consideration to the nature of the initial-/boundary-value problems which arise for these equations and to the simpler numerical techniques for their solution. He observed that the first real difficulty arises near separation, where in general the wall friction vanishes with a singularity, and mentioned techniques which could deal satisfactorily with this. He emphasized the importance of setting oneself a well-posed problem for which existence or uniqueness could be proved, and, looking to the future, briefly discussed this question with regard to the treatment of backflow on the basis of the boundary-layer equations. Dr. Mangler carried the story further, dealing with the extra complications encountered with compressible flow and quasi-three-dimensional flow on a conical surface. He also described in greater detail the various methods that had been programmed for specific problems and discussed a technique for adjusting the external pressure distribution so as to obtain a regular solution at separation.

The subjects for the discussion groups after tea were as follows: *A*—"Boundary-layer Problems" (chairman, Prof. L. Howarth, University of Bristol; *B*—"Free-boundary Problems" (chairman, Prof. M. B. Glauert, University of East Anglia); *C*—"Numerical Analysis" (chairman, Mr. C. W. Clenshaw, National Physical Laboratory). In group *A* Mr. G. S. Raetz (Northrop Aircraft) gave an account of his finite-difference method for solving the three-dimensional boundary-layer equations. This was followed by a general discussion of points arising out of the previous lecture. Mr. J. H. B. Smith of the Royal Aircraft Establishment introduced discussion *B*. After indicating the variety of situations in which free boundaries occur (such as at the surfaces of oceans, or of air bubbles rising through water or of the oil in journal bearings, in the design of wing shapes for a given pressure distribution, at the trailing vortex sheet behind a wing, at shock waves in locally supersonic flow, and at the surface of a sail distorted by wind forces), he drew a distinction between those problems which could be transformed into or approximated by a fixed boundary problem before numerical treatment and those which could not. He then described briefly a number of proper free-boundary problems and discussed in detail one in particular which had recently been solved successfully, namely, the determination of the strength and shape of spiral vortex sheets in a model of a flow with separation from a highly swept leading edge. In group *C* the discussion on "Numerical Analysis" was continued; more detailed consideration

was given to the treatment of singularities, and a general debate ensued on the kind of problems that numerical analysts ought to be examining.

The final session on the morning of September 29 was devoted largely to what must be the most challenging problem to fluid dynamicists, namely, the solution of the full Navier–Stokes equations. In the first lecture, Dr. S. C. R. Dennis of the University of Sheffield surveyed the progress to date in the field of steady flow, and discussed in considerable detail the difficulties that arise in tackling the classical external problem of two-dimensional flow past a circular cylinder. In particular, he considered suggestions that might overcome the major difficulty of the flow in the wake, which is especially troublesome at higher Reynolds numbers.

In the next lecture, on "Unsteady Flow", Dr. J. E. Fromm of the Los Alamos Scientific Laboratory demonstrated the feasibility of using a large digital computer to simulate the complex asymmetric fluid motions which really do occur in practical flow régimes, where the steady flow is unstable. He first gave a description of a finite difference technique for obtaining solutions of the two-dimensional unsteady Navier–Stokes equations which had been developed with due regard for stability and conservation properties. He then showed a series of impressive ciné films of the computer output for several flow problems. In order to make best use of the finite computer storage, only restricted flow fields were considered, namely, those between parallel plates. In the first problem the plates were moved so as to accelerate the fluid past a rectangular body; in the second the same configuration was considered, but the uncoupled temperature equation was solved as well, so as to simulate forced heat convection from the body. In the third problem, the Benard problem of thermal convection between fixed plates was considered; here, of course, the coupling between the temperature and momentum equations had to be taken into account. The filmed computer output showed the development in time of the streak-line patterns, the general features of which resembled the corresponding patterns produced in experiments to a remarkable degree. Shown also were the changing vorticity line patterns (or for the convection problems isotherm patterns). It should be noted that if the data given were exactly symmetric, the symmetry was preserved in the solutions. The asymmetry had to be stimulated by introducing small perturbations; these were damped out for small Reynolds numbers, but amplified into typical eddying motions for larger Reynolds numbers. In the discussion group *A* that followed (chaired by Prof. I. Proudman, University of Essex), it was pointed out that external flow problems were substantially more difficult to deal with numerically than internal ones, and Dr. Dennis suggested that a combination with analytical treatment of the far field might be the answer; however, it would be very interesting to see Dr. Fromm's method applied directly to external flow problems. Another restriction that had been imposed because of limited storage capacity was a periodic boundary condition in the general flow direction; it would be of considerable interest to remove this so that any ultimate quasi-steady patterns for large times could be studied.

The other two discussion groups on this last morning were: *B*, "Internal Aerodynamics" (chairman, Prof. J. H. Horlock, University of Liverpool) and *C*, "Turbulent Boundary-layers" (chairman, Prof. A. D. Young, Queen Mary College). Mr. R. Hetherington of Rolls-Royce, Ltd., gave an introductory talk in discussion group *B*, in which he discussed the two main two-dimensional problems that were used to model the essentially three-dimensional process of flow through the complex of rotating and stationary blades that comprise a turbomachine. These are concerned mainly with curved axial flow and cascade flow, respectively. Presently available methods were briefly described and the extent to which computers were being used was surveyed. Although new methods were clearly needed to deal with the non-linear compressible and viscous effects, it was concluded that much could still be gained by developing existing methods with the aid of the modern computer.

The subject of turbulent boundary layers is, of course, of the utmost practical importance, but, up to the present, methods for calculating them have been developed on a purely empirical basis. Discussion group *C* did, however, indicate that some substantial progress towards removing the empiricism from the direct relationship between shear stress and mean velocity profile to a less critical level is at last being made through analysis of the turbulent energy equation. This method leads (in two-dimensional flow) to a third-order hyperbolic system of partial differential equations, for which a characteristics method has been devised for solution on the computer. The computer can also be used to develop the traditional approach by exploring quickly and accurately the consequences of the various empirical assumptions regarding the relationship between shear stress and mean velocity profile. Here the differential equations are of similar type to the laminar boundary-layer equations, so with appropriate modifications the numerical techniques developed for the latter may be carried over.

The attention of the Computer Panel had recently been brought to work done at the National Physical Laboratory on the optimization of hull form parameters based on a statistical analysis of results from model tests of trawlers. The possibility of applying similar techniques to aircraft was thought worthy of investigation, so an additional discussion group was arranged to consider this after lunch on the last day. Mr. J. G. Hayes of the National Physical Laboratory described the technique in an introductory talk based on his paper on "Optimum Hull Form Parameters" read at the symposium on "Numerical Methods Applied to Shipbuilding" held at Bergen in 1963. Mr. D. J. Doust of the National Physical Laboratory added details of the significant improvements obtained in trawler design, and a short discussion followed under the chairmanship of Mr. J. G. L. Michel (National Physical Laboratory).

No proceedings are being published, but some copies of the programme and abstracts are still available and may be obtained from Mr. P. G. Williams, Mathematics Division, National Physical Laboratory, Teddington, Middlesex, under reference *Ma.* 8/35/02.

P. G. Williams

BIOLOGICAL SCIENCES IN THE FUTURE UNIVERSITY OF BATH

On October 14, 1965, the first and preliminary-phase building of the future University of Bath was officially opened by the Mayor of Bath. Designed by the University architects, Robert Matthew, Johnson-Marshall and Partners, this building occupies a corner of the 197-acre site set aside by the City of Bath for the development of the new University. Constructed on the C.L.A.S.P. system, the structure was virtually completed in less than ten months, between the turning of the first sod in September 1964 to the handing over by the contractors in July 1965.

The two-storey structure forms a quadrangle, surrounding a central paved courtyard, the inner sides consisting mainly of staff offices and workrooms and the outer sides containing teaching laboratories, lecture and seminar rooms. Included in the specialist accommodation are

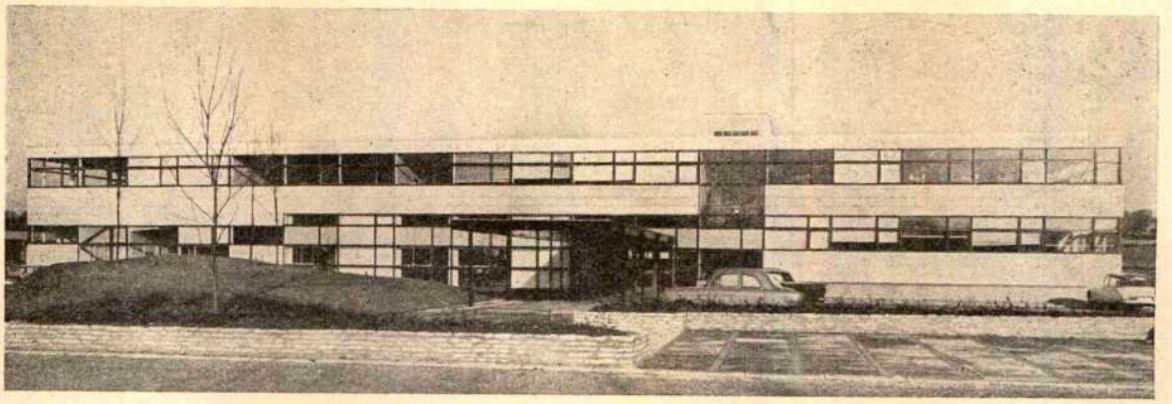


Fig. 1. The first and preliminary-phase building of the future University of Bath

laboratories for biochemistry, microbiology, crop protection, horticultural science, plant and animal physiology, electron microscopy and a variety of instrumental techniques. In addition to housing the School of Biological Sciences, about one-quarter of the total teaching space of 20,000 sq. ft. is being used by the School of Humanities and Social Sciences. The building also provides the essential ancillary accommodation, including library, refectory and staff and student common rooms. The total biological accommodation provides for an annual intake of about sixty students, giving a total for the four years of the first degree sandwich courses of about 240, divided almost equally between the existing course in applied biology and new first degree courses in applied biochemistry and horticulture starting in the present session. The development plan for the University provides for a further instalment of about 12,000 sq. ft. of biology accommodation in the next phase of the building programme, scheduled for completion in 1967.

The history of applied biology in the University of Bath may be traced back to 1956 when the Bristol College of Technology was designated as one of the original Colleges of Advanced Technology. At that time, in the newly-styled Bristol College of Science and Technology a four-year sandwich course in applied biology was started at the college site in Ashley Down, Bristol, under the aegis of the National Council for Technological Awards and leading to the Diploma in Technology. This course, which was designed to train biologists for employment in industry, research and development, provided for three years of broadly based biological training, followed by a fourth-year specialist option in either pharmacology, microbiology, plant physiology, plant pathology or entomology. Each year of the course consisted of about equal periods in college and in industrial or research establishments. The course has since been modified in the light of experience, by an extension of the period of specialized study to two years, with the fourth year spent entirely in college, and by the combination of entomology and plant pathology in a combined course in crop protection.

The establishment of a School of Biological Sciences coincided with the opening of the new building for the autumn term. The title formally expresses the intention to preserve and extend the integration of biological education and research in those fields of biology with which the School is concerned. Both for educational reasons, and to preserve the greatest possible flexibility in students' choice of their final specialist training, it is hoped that this integration may eventually be extended by providing a common first-year course in the groundwork of biology to be followed by all new entrants to the School. For administrative convenience the teaching and research staff are divided into working groups, corresponding so far as possible with the main areas of interest within the School. Initially these groups are biochemistry, microbiology, comparative physiology, horticulture and plant physiology. The present teaching staff numbers twenty-two and there are nine research students. The head of the School and first professor of biology is Dr. L. Broadbent, at present honorary secretary of the Institute of Biology and formerly head of the Plant Pathology Division of the Glasshouse Crops Research Institute. M. W. HARDISTY

POLYMERIZATION IN A GLOW DISCHARGE

By DR. T. WILLIAMS and M. W. HAYES
British Iron and Steel Research Association, London, S.W.1

GLOW-DISCHARGE polymerization has been known for many years; for example, Linder and Davies[1] described the gases and solids formed by some hydrocarbon vapours in a glow discharge. In a more recent article, Brick and Knox[2] have described the major features and variables associated with this process. Other articles have been published describing the chemical, physical[3] and electrical[4] properties of glow-discharge polymers.

In glow-discharge polymerization, organic vapours at a pressure of about 1–5 torr are introduced into a chamber containing two parallel electrodes. An a.c. voltage is applied across the electrodes so as to initiate, and then sustain, a uniform glow discharge in the intervening space. High-energy ions and electrons are produced in the glow and these bombard both the electrode surfaces and the molecules in the gas phase. These interactions initiate complex chemical reactions which result in the formation of thin (up to about 5μ) uniform and continuous coatings on the electrode surfaces.

A schematic diagram of the apparatus used in the glow discharge polymerization experiments is shown in Fig. 1. The electrodes were made of mild steel (approximately 5 cm by 10 cm) and held in position by non-conductng supports. They were normally placed about 2 cm apart. The apparatus also had provisions for cooling one electrode down to a minimum temperature of −30° C.

The electrical equipment consisted of an oscillator and 1 kW audio amplifier. With frequencies of about 10 kc/s the glow was initiated at about 800 V and sustained at approximately 400 V. Most of the present work was

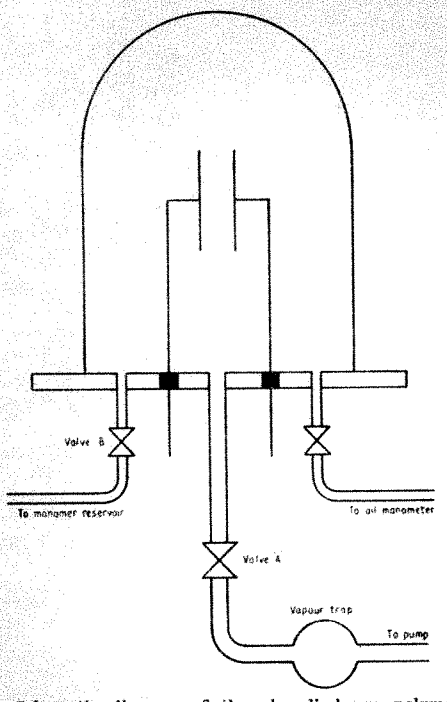


Fig. 1. Schematic diagram of the glow-discharge polymerization apparatus

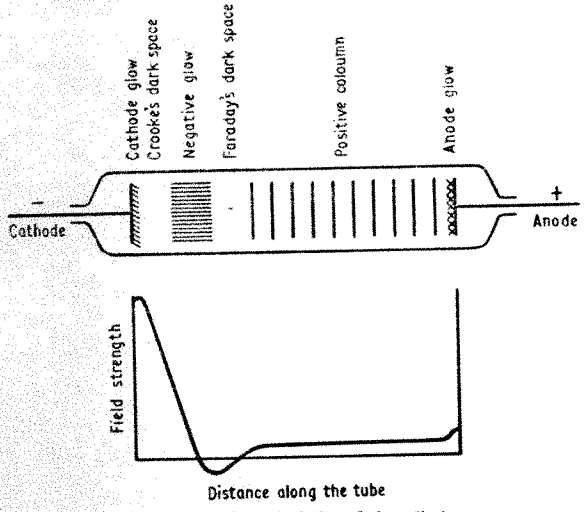


Fig. 2. Some characteristics of glow discharge

carried out with discharge currents varying between 1 and 5 m.amp/cm^2.

Two experimental procedures were adopted: in the first the bell-jar was pumped down and purged continuously with monomer vapour for about 15 min. Valve *A* was then closed and monomer vapour allowed to build up to the required pressure. Finally, valve *B* was closed. In the second procedure—where there was a continuous flow of vapour through the apparatus—valves *A* and *B* were adjusted manually to give the desired pressure.

With an a.c. field, the two electrodes were experimentally indistinguishable. The weight of polymer on each electrode was determined and an average of the two readings recorded. Film thickness was calculated from the weight, area of electrode and the density of the polymer; the latter was taken as 1·0. Results obtained in this manner compared well with those calculated from film capacity measurements, where the dielectric constant was taken as 3·0. All results were reproducible to within ± 5 per cent.

Some essential features of glow discharge. The essential features of a glow discharge have been described elsewhere[2,5]. A diagram of a d.c. discharge conducted in a gas at a pressure of about 1–5 torr is shown in Fig. 2. Electrons originating near the cathode experience the electrostatic repulsion of the cathode and move into the Crooke's dark space which is the region of the greatest potential drop. In this region the electrons are accelerated to their maximum energy, which depends on the potential drop and the pressure of the discharge. The electrons emerge from the Crooke's dark space and enter the negative glow, where they bombard the gas molecules, forming a wide range of species which include ions and activated molecules. Positive ions formed in the negative glow move into the Crooke's dark space and are accelerated by the high field in this region; these ions then strike the cathode, liberating more electrons which follow the sequence already described.

The electrons leaving the negative glow have lost most of their energy in collisions with gas molecules. What happens to the electrons afterwards is not important for this discussion, and the rest of the discharge may be regarded as diffusion of electrons towards the anode, where they are neutralized.

When alternating current is used instead of direct current, then, again for the purpose of this discussion, each electrode may be regarded as acting alternately as the cathode.

The description already given is general for all glow discharge. Most of the work from which the discharge mechanism was established was carried out with such gases as hydrogen, nitrogen and the noble gases. In the present investigations, all the work is concerned with organic vapours which react chemically in the presence of a glow forming polymer coatings on the electrode surfaces. Some of the complications introduced by such a behaviour are discussed below.

The equivalent circuit for the glow-discharge polymerization process. Fig. 3 represents an a.c. glow discharge occurring in an organic vapour. Fig. 4*a* is a simplified equivalent circuit for the process after the formation of polymer films on the electrode surfaces. Let:

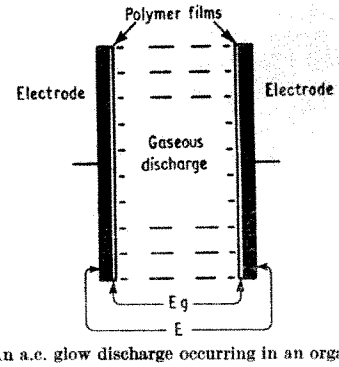


Fig. 3. An a.c. glow discharge occurring in an organic vapour

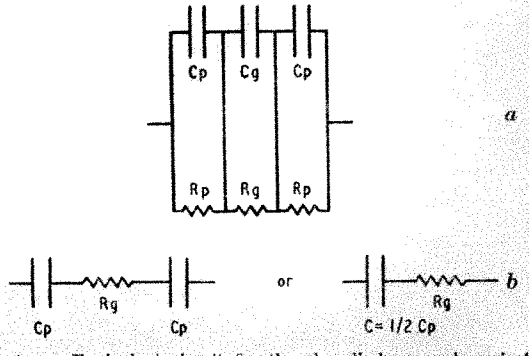


Fig. 4. *a*, Equivalent circuit for the glow-discharge polymerization process. *b*, Simplified equivalent circuit for the glow-discharge polymerization process

R_p be the resistance of the polymer film in ohms (Ω);
C_p, the capacitance of the polymer film in farads (F);
R_g, the resistance of the gas in Ω;
C_g, the capacitance of the gas in F;
X_p, the reactance of the polymer film in Ω;
X_g, the reactance of the gas in Ω.

With frequencies at least up to 10 kc/s the circuit shown in Fig. 4*a* may be further simplified (see Fig. 4*b*) because for a thin polymer film the resistance $R_p \gg$ reactance X_p and also for the gas, reactance $X_g \gg$ resistance R_g.

Since the films deposited on the two electrodes are identical in thickness and properties, the effective capacitance of the circuit is:

$$C = 1/2\ C_p \quad (1)$$

The reactance of each polymer film is:

$$X_p = 1/\omega C_p \quad (2)$$

where $\omega = 2\pi f$ and f is the frequency. The effective reactance of the circuit is:

$$X = 1/\omega C \quad (3)$$

Now the capacitance of a polymer film is given by:

$$C_p = \varepsilon_0 \varepsilon_r\ A/d \quad (4)$$

where ε_0 is the permittivity of vacuum ($8{\cdot}854 \times 10^{-12}$ F/m); ε_r, the dielectric constant (or relative permittivity) of the polymer; A, the area in square metres; and d, the film thickness in metres. The impedance of the circuit (Z) is expressed by equation (5) (where j is $\sqrt{-1}$):

$$Z = R_g + 1/j\omega C \quad (5)$$

The magnitude of Z can be calculated from the expression:

$$|Z| = \sqrt{R^2_g + (1/\omega C)^2} \quad (6)$$

The voltage drop across each polymer film, E_p, is given by:

$$E_p = I\ X_p \quad (7)$$

where I is the current in the circuit.

The voltage drop across the whole circuit may be calculated from the following equation:

$$E = I\ |Z| \quad (8)$$

$$= I\ \sqrt{R^2_g + (1/C\omega)^2} \quad (9)$$

If the field strength across a polymer film exceeds the dielectric strength of the polymer, then electrical breakdown of the film occurs. With a d.c. discharge ω is zero, and therefore from equation (7) the voltage drop across any film covering the electrode must be infinite. In practice this means that no continuous films can be formed with a d.c. discharge.

According to equations (2), (4) and (7) the voltage across the film depends on the current density and frequency. At a given frequency continuous coatings are formed when the current density is such that the voltage across the films is not greater than the electrical breakdown of the polymer. Table 1 shows the variation of voltage across a film with current density and frequency. In the megacycle range the field strength across the film is low compared with breakdown voltage of polymers even at very high current densities ($\sim$ 50 m.amp/cm²).

The effect of film thickness on the discharge voltage is very small under normal operating conditions. If the electrode area is taken as 1 cm², current as 10 m.amp, frequency as 10 kc/s and the dielectric constant of the polymer as 3·0, then the rise in discharge voltage due to the build up of a micron film on each electrode may be calculated from equations (4) and (9).

Table 1

Frequency	Current (m.amp/cm²)	Field strength across film (V/μ)
10 kc/s	1·0	6·0
10 kc/s	5·0	30 0
10 kc/s	10 0	60·0
100 kc/s	5·0	3·0
1 Mc/s	5·0	0·3
1 Mc/s	50·0	3·0
10 Mc/s	50·0	0·3

Initially, when there is no polymer on the electrode the voltage across the gas is equal to the voltage across the whole discharge, that is:

$$E_g = E \text{ at } d = 0$$

If $E = E_g = 500$ V, then the resistance of the gas is 50,000 Ω.

The effective capacitance of the discharge calculated from equations (3) and (4) is $1{\cdot}3 \times 10^{-9}$ F and the total reactance of the discharge is:

$$X = 1/\omega C$$
$$= 12{,}000\ \Omega.$$

Substituting these figures in equation (9) the voltage across the discharge, when the electrodes are coated with a 1μ film, may be calculated; this voltage is 514, which is only a 2·8 per cent increase on the initial voltage when the electrodes were uncoated. At 1 Mc/s the voltage change is negligible even up to film thickness of 10μ.

Clearly, if the voltage is kept constant there is very little change in current as the film thickness increases up to 1μ at 10 kc/s.

Polymerization and polymerization initiation. Polymerization reactions in a glow discharge may occur either in the gas phase or at the electrode surface. Since the rate of polymerization is closely related to the degree of monomer adsorption (see later), it is probable that polymerization occurs at the electrode surface; the electrode surface being (except in the initial stages) the polymer which is being deposited on the electrode. Also the rate of polymerization in a glow discharge is independent of pressure above a certain minimum (see Fig. 5), which again indicates a surface reaction: a gas-phase reaction is generally proportional to pressure.

The formation of active species, which initiate polymerization reactions, may also occur either in the gas phase or on the electrode surface. It is well known that electron bombardment of organic vapours in the negative glow of a discharge produces a wide range of species, many of which could initiate polymerization reactions by condensing on the electrode surface and reacting with the unactivated adsorbed molecules. However, the absence of polymer on other parts of the apparatus close to the discharge suggests that this type of reaction plays no significant part in the overall polymer formation. Also the fact that polymer films cannot readily be formed by passing vapours through a discharge and impinging the emergent gases on to a suitable surface tends to confirm the foregoing suggestion.

Bamford *et al.*[6] showed that many polymers, when submitted to the action of a high-frequency electric discharge (maintained in oxygen, nitrogen, hydrogen and argon), produce radicals characteristic of the polymer in a surface layer less than 1μ thick. These discharge-induced surface radicals are similar to those obtained by irradiation with X-rays or high-energy electrons and are capable

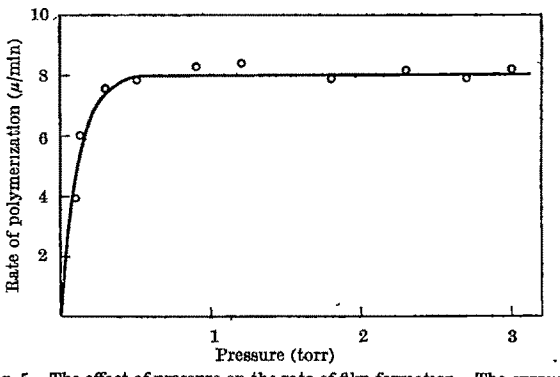


Fig. 5. The effect of pressure on the rate of film formation. The current density is 3·3 m.amp/cm²

of initiating polymerization reactions when exposed to the vapours of vinyl monomers.

The foregoing evidence suggests that active species are formed on the electrode surface rather than in the gas phase, probably as a result of ion or electron bombardment. With direct current, practically no polymerization occurs at the anode which indicates that the direct bombardment of electrodes by electrons plays no important part in polymer formation. Probably the sequence of events leading to polymer formation is as follows. Ions which are produced in the negative glow by electron bombardment of the vapour molecules are attracted towards the polymer coated electrodes. The primary step when an ion strikes the polymer surface is probably the same as in the case of high-energy radiation, that is, ionization or excitation of the polymer molecules followed by fragmentation resulting in active species which can propagate the polymerization reaction by addition of unactivated adsorbed molecules.

Adsorbed monomer molecules can also be activated by ions in a similar manner and this must happen when the first layer of polymer is formed.

Rate of polymerization and adsorption. The adsorption of a monomer at constant temperature on an electrode may be expressed by the relation:

$$x/m = \int (p/p_0)T$$

where x/m is the amount adsorbed and p/p_0 the relative vapour pressure, that is, the vapour pressure after adsorption divided by the saturated vapour pressure at the operating temperature.

In Table 2 the rate of polymerization of various monomers, determined at room temperature and pressures varying between 1 and 3 torr, are correlated with the amount of monomer absorbed on the electrode surface (p/p_0). Monomers with vapour pressures less than 3 torr at room temperature are not included for obvious reasons.

Clearly the amount of monomer adsorption on the surface of the electrode is extremely important in determining the rate of polymerization of various monomers in a glow discharge. Other factors must affect this rate, but presumably to a much lesser degree than adsorption. The results are not accurate enough to attempt a more quantitative correlation of these two parameters and also there is the added difficulty of taking into consideration the effect of other factors.

Some polar molecules with high dipole moments, such as acrylonitrile and vinylidene chloride, polymerize very rapidly even at low values of p/p_0. The explanation for the irregularity of these monomers is obvious and requires no comment.

The rate of polymerization of a particular monomer at room temperature and a fixed current density is, as already explained, dependent on the number of molecules adsorbed on the electrode. At low pressure ($p/p_0 \rightarrow 0$), this number is small and if there are enough ions striking the surface to allow every adsorbed molecule to react, then the reaction is proportional to the number of adsorbed molecules (that is, p, p_0 being constant). Above a certain saturation pressure the number of adsorbed molecules becomes so large that the number of active species formed is now dependent on the number of ions striking the surface. Under these conditions, the rate of polymerization is independent of pressure and is determined only by the current density. The effect of pressure on the rate of polymerization of styrene is shown in Fig. 5; the shape of the graph is somewhat similar to a normal adsorption isotherm as expected. The actual value of the saturation pressure is determined by the number of ions striking the surface, that is, by the current density.

Table 2. Variation in the Rate of Polymerization of Various Monomers with Adsorption

Rate of polymerization	Monomer	Approximate value of p/p_0
High	Styrene	0·2
	α-Methyl styrene	0·2
	Dicyclopentadiene	0 2
	Acrylic acid	0·2
Moderate to slow	Allyl alcohol	0·05
	Ethyl acrylate	0·03
	Methyl methacrylate	0·03
	Methyl acrylate	0 01
Slow to zero	Vinyl acetate	< 0·01
	Isoprene	0·002
	Isobutene	0·001
	Ethylene	0 001

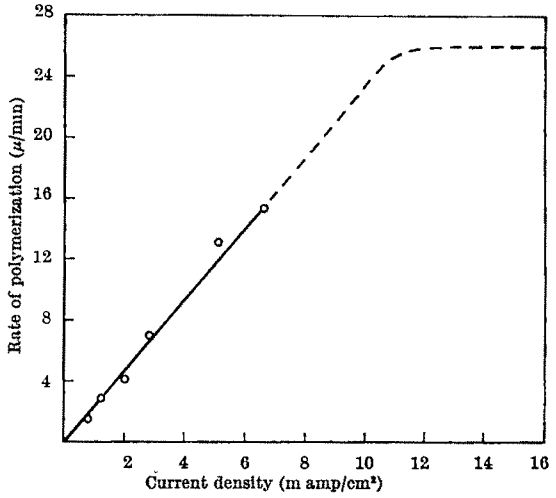


Fig 6. The effect of current density on the rate of film formation. The pressure is 2–3 torr

In Fig. 6 the rate of polymerization of styrene is plotted against current density; pressure is constant and above saturation pressure for all the current densities investigated. Under these conditions the rate of polymerization, as already explained, is directly proportional to the current density and may be expressed by the equation[7]:

$$m = \alpha I t$$

where m is the mass of polymer deposited per unit area, α a constant which varies with degree of adsorption of the monomer, I the current density and t the time of deposition.

If the current is further increased, a point is reached at which rate of polymerization is constant and independent of current density as shown in a broken line in Fig. 6. This flattening off of the rate-current density graph may be readily observed with styrene at lower pressures ($\sim$ 0·5 torr), where the saturation pressure is attained at a relatively low current density.

Another equation expressing the degree of adsorption of a monomer on an electrode is:

$$\theta = 1/[1 + (n/kp)]$$

where θ is the fraction of electrode covered with adsorbed molecules, k is a constant, p the pressure, and n the number of molecules evaporating off the electrode. At constant pressure the amount of adsorbed monomer clearly depends on n and hence varies with the temperature of the electrode. Therefore, monomers which polymerize slowly at room temperature should polymerize much more readily at lower electrode temperature. This was verified experimentally; for example, vinyl acetate, which polymerizes very slowly at room temperature, polymerizes very rapidly on an electrode at a temperature of $-30°$ C to give clear films of polyvinyl acetate.

All 'difficult' monomers would probably polymerize quite readily on electrodes maintained at a suitable temperature. For example, ethylene, which polymerizes very slowly at room temperature, would probably react quite rapidly on an electrode maintained at a temperature of about $-200°$ C.

Properties of glow-discharge polymers. Let the ratio:

$$\frac{\text{Number of adsorbed molecules}}{\text{Number of ions striking the surface}} = \varphi$$

At very low φ (because of a low p/p_0 or an unfavourable electrode temperature) the rate of polymerization is slow

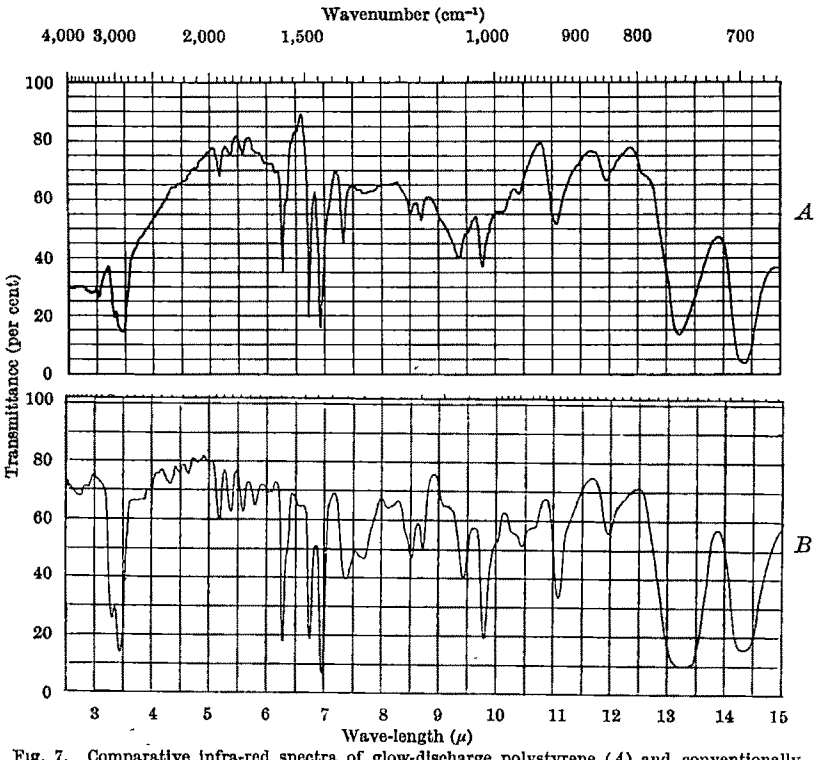


Fig. 7. Comparative infra-red spectra of glow-discharge polystyrene (*A*) and conventionally produced polystyrene (*B*)

and the films are coloured, highly degraded, insoluble and loosely adherent to the electrode. Under these conditions the number of ions striking the polymer surface must be far in excess of that required to allow every adsorbed molecule to react and thus a certain amount of film damage must occur. In the extreme with no adsorbed molecules the polymer is subjected to a continuous ion bombardment and must experience some radiation damage. However, a glow-discharge film subjected to an argon discharge for a prolonged period showed no visible signs of degradation, which suggests that ion bombardment is not primarily responsible for producing coloured, highly degraded films.

When φ is very large the number of active sites formed by ion bombardment is not sufficient to allow every adsorbed molecule to react. Under these conditions unreacted molecules become incorporated into the growing polymer, making it slightly tacky. However, on standing, these polymers slowly become hard due to the loss of trapped monomer; this latter process may be accelerated by moderate heating.

Clearly, for the best results the value of φ must be carefully selected. However, even under optimum conditions contamination of the polymer, as already indicated, proceeds by another mechanism.

Much information has been given in the literature concerning the species formed by hydrocarbon vapours in a glow discharge[8]. Electron bombardment of monomer molecules in the negative glow produces species ranging from carbon ions (C^+) to activated monomer. These species find their way to the electrode surface and either co-polymerize or become incorporated in the polymer. Co-polymerization of these species must produce a mixed type of polymer, and the differences between the infra-red spectra of glow-discharge polystyrene (Fig. 7) and ordinary polystyrene at 7·7, 7·8, 10·4, 13 and 15 microns and the significant background absorption of the former support this view[7]. Experimental evidence suggests, as expected, that the value of φ determines the degree of polymer contamination; at low φ the percentage contamination is high but progressively decreases with increasing φ.

Contamination of polymers by this mechanism may be minimized by lowering the concentration of monomer in the gas phase (that is, lowering monomer pressure) while still maintaining the concentration of adsorbed molecules at the desired level. This may be achieved by working in an atmosphere of monomer and inert gas; the monomer being present at very low partial pressure and the inert gas being added to keep the optimum glow-discharge pressure (1–5 torr). The degree of adsorption required may be achieved by selecting a suitable electrode temperature.

High monomer flow rate through the discharge is also beneficial in reducing contamination; this helps to reduce the amount of breakdown products from the discharge reaching the electrode surface.

Polymer films produced under optimum conditions are of excellent appearance, and are even and clear. The molecular weights of these polymers have not been determined since they are completely insoluble in most solvents. This suggests that all glow-discharge polymers are formed in a highly cross-linked state and that linear polymers cannot be formed by this technique. This is really to be expected if polymerization is initiated by activation of the polymer.

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ALTERNATING CURRENT RESISTIVITY PROFILES THROUGH ANODIC OXIDE FILMS ON ALUMINIUM AND ITS ALLOYS

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THE elegant method of Heine and Pryor[1], namely measurement of the impedance of a barrier-type anodic oxide film during uniform thinning in a passivating alkaline chromate solution, has been used to obtain the a.c. resistivity profiles through such films formed on aluminium and its alloys in various electrolytes.

Specimens of annealed 99·99 per cent or 99·9999 per cent aluminium or of Al–Mg, Al–Zn or Al–Cu as single-phase solid solutions were etched for 15 min in 0·5 N sodium hydroxide solution at 20° C followed by a 1 sec dip in 50 per cent nitric acid at 90° C and washed. They were anodized at various constant current densities

(generally 10 m.amp/cm² or 100 μamp/cm²) in a flowing electrolyte at 25° C. Measurement of the balancing series capacitance and dissipation factor on specimens 0·2 cm² and 0·02 cm² in area during thinning in fresh 1 M sodium-chromate solution at 25° C (*p*H adjusted to 7·2), using a General Radio capacitance measuring assembly type 1610-*B*, permitted an area-independent calculation of the average resistivity of the film (also corrected for double-layer resistance[1]) at various stages. The reproducibility was within ± 5 per cent for all materials and for aluminium was within ± 3 per cent. The electrode potential during thinning was sufficiently high for the capacitance to give an accurate measure of film thickness during thinning. Electron microscopy with replicas and stripped films revealed uniform thinning on a sub-microscopic scale and a flaw density which remained constant during thinning and was generally too low to affect impedance measurements.

Fig. 1*a* and *b* show the average a.c. resistivities at 1 kc/s and 100 kc/s, respectively, obtained during thinning of 240 Å films formed in 20 per cent w/v $NH_4B_5O_8.4H_2O$ in ethylene glycol. The resistivity value plotted at each thickness represents the average value for the film of that thickness remaining on the specimen, not the

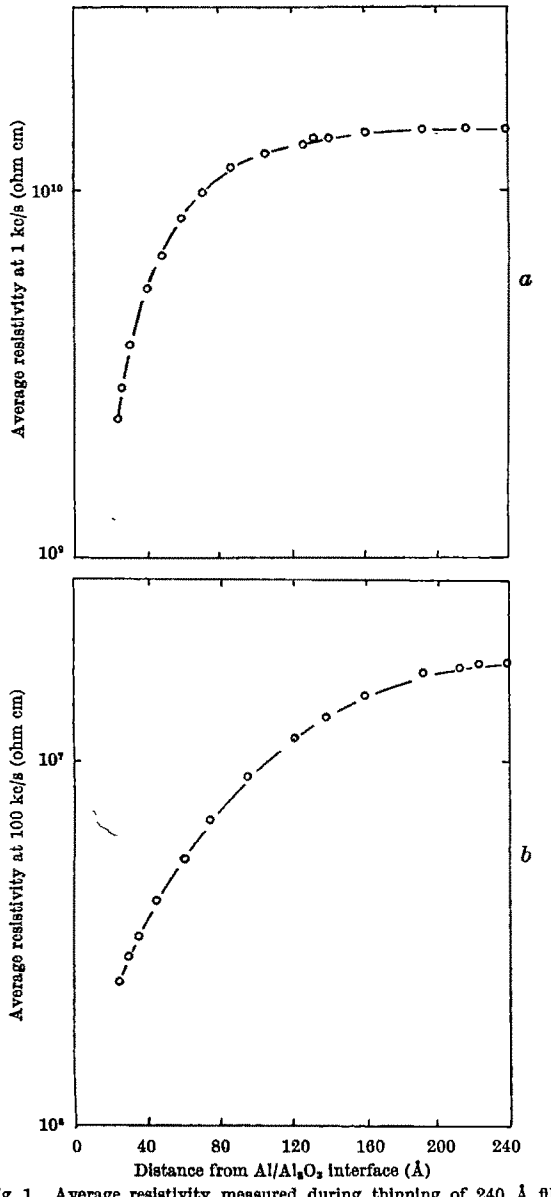


Fig. 1. Average resistivity measured during thinning of 240 Å film formed in ammonium borate in ethylene glycol. *a*, 1 kc/s; *b*, 100 kc/s

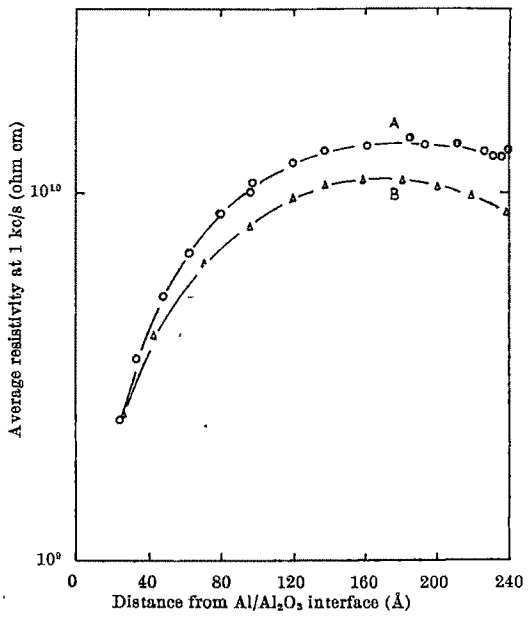


Fig. 2. Average resistivity at 1 kc/s measured during thinning of 240 Å films formed in 3 per cent w/v ammonium tartrate solution (*p*H 7) at: *A*, 10 m amp/cm², *B*, 100 μamp/cm²

resistivity of a small element of oxide in that location[1]. Films produced in this largely non-aqueous solution form with almost 100 per cent current efficiency in the range 1 μamp/cm²–10 m.amp/cm², contain a little boron but no water or glycol[2], and represent the simplest and most perfect films formed. The resistivity profiles depicted are independent of the current density of formation in the foregoing range and also of whether the current is terminated abruptly or allowed to decay slowly. They do not depend on the borate content of the electrolyte (5–25 per cent w/v salt) and therefore on the boron content of the film. There is no region towards the outside of the film where partial hydration has occurred[1]. When the results in Fig. 1 are plotted as balancing parallel resistances, R_p (obtained by multiplying the resistivity by the film thickness at each thickness), against thickness, a 60–80 Å-thick region of low resistance adjacent to the metal is evident at both frequencies. The Heine–Pryor theory predicts that this reflects primarily electronic relaxation processes, due to partially trapped electrons associated with O^2 ion vacancies or possibly Al^{3+} interstitial ions in the *n*-type oxide there. Furthermore, if R_p is plotted against the square of the thickness for this inner region, a straight line through the origin is obtained, indicating that the number of these *n*-type defects varies with the inverse distance from the metal interface[3]. This must represent a steady-state situation persisting for many hours and is not directly related to the conditions of film formation. Thus, Young has shown for tantalum that about 70 per cent of Frenkel defects rapidly disappear when the forming current is switched off[4]. The outer region of film where R_p increases linearly with thickness is said to be normal *n*-type oxide[1].

The resistivity profiles for films formed in aqueous electrolytes depend on the current density (and also possibly the time) of formation, and on the *p*H and anion, but are independent of small amounts of impurities in metal or electrolyte. Fig. 2*A* and *B* give the results at 1 kc/s for 240 Å films formed in 3 per cent w/v ammonium tartrate solution (*p*H adjusted to 7) at 10 m.amp/cm² and 100 μamp/cm² respectively, the values for 100 kc/s being identical with those in Fig. 1*b*. The results at 10 m.amp/cm² are very similar to those of Heine and Pryor, showing a partially hydrated region of low resistivity towards the outside and a very thin region of high resistivity at the outer interface. At 100 μamp/cm² this final region is not observed, possibly because of the more pronounced major

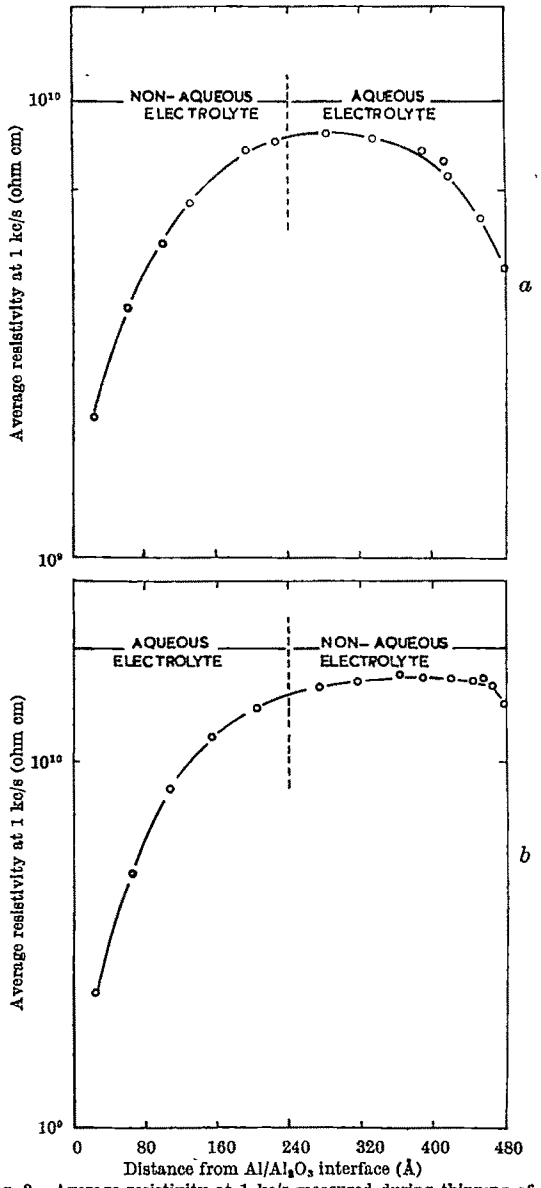


Fig. 3. Average resistivity at 1 kc/s measured during thinning of 480 Å duplex films formed at 100 μamp/cm² as follows. *a*, 240 Å in non-aqueous borate, 240-Å in tartrate; *b*, 240 Å in tartrate, 240 Å in non-aqueous borate

hydrated region of low resistivity. It has been suggested that the presence of the low-resistivity region at 1 kc/s but not at 100 kc/s is due to conductivity contributions from dipoles with relaxation times rather greater than 10^{-5} sec, possibly consisting of OH^- ions and their associated electrons replacing O^{2-} ions in the film[1]. There is some evidence from the curves that the inner regions of the film are also slightly hydrated. No pores could be detected in these films by electron microscopy. Change of tartrate *p*H in either direction from 7 increases the degree of hydration in the outer regions but the effect is greater at *p*H 9 than at *p*H 5. This possibly suggests that direct OH^- movement with the field is an easier and more effective process than indirect OH^- movement by way of a proton-portering mechanism, or than simple H^+ entry against the field[5,6], under comparable conditions. Both processes would tend to leave OH^- ions in the film. For *p*H values 6–8, the outer region of the resistivity curve is much lower for 100 μamp/cm² than for 10 m.amp/cm², but at *p*H 5 or 9 there is little difference and it is low in both cases. This suggests that where H^+ or OH^- ion activities are large their entry into the film by the appropriate mechanism is little affected by forming rate. The outer surfaces of films formed at *p*H 5 and 9 showed a few discrete pores and a general sponginess respectively. These phenomena, together with the likelihood of invisible pores in films formed at 100 μamp/cm², are natural extensions of the hydration processes described. For electrolytes at *p*H 7, the degree of hydration, generally but not always more noticeable at 100 μamp/cm², increases in the order borate, phosphate, tartrate and citrate. This is to be expected because it is well known that phosphate and to a lesser extent borate inhibit hydration of alumina at high temperatures. Presumably the main action of the anion is by adsorption on the surface but it may also compete with OH^- for entry and in some cases may supply the oxygen ions from which the film is composed. Electron microscopy showed that films formed in citrate are porous or heavily flawed, which means that the results of marker experiments of other workers using this electrolyte require re-appraisal. Addition of sodium chloride (up to 0·01 M) to tartrate forming electrolyte appears to give gross impurity substitution dipole effects of a type previously produced another way[7,8] and may also promote OH^- entry.

Fig. 3*a* and *b* show the resistivity profiles at 1 kc/s for duplex 480 Å films formed as follows at 100 μamp/cm²: *a*, 240 Å in non-aqueous borate, 240 Å in tartrate, *p*H 7. *b*, reverse order of *a*. The general conclusion is that oxide characteristic of the second electrolyte lies at the outside of the film, clearly favouring considerable cation movement. It is likely that this is the case for the non-aqueous borate but some OH^- movement (and possibly H^+ entry against the field) contributing to mass and current transport is indicated for tartrate. Thus, slight hydration of the inner borate-formed films occurs when tartrate anodization is carried out second. When the borate is anodized second the outer hydrated region of the initial tartrate-formed film must presumably be dispersed through the film, but there is some evidence of the most seriously affected region residing at the outside of the duplex film. Perhaps electrolyte can enter this very thin hydrated or quasi-porous region so that it does not take part in film growth. Detailed study of the results, to be reported elsewhere, also favours some O^{2-} ion movement.

Single-phase Al–Mg, Al–Zn and Al–Cu alloys, containing respectively less than 5 per cent magnesium, 2 per cent zinc and 4 per cent copper, give films with 100 per cent current efficiency in non-aqueous borate, but resistivity profiles show a reduction in resistivity through the film at 1 kc/s but not at 100 kc/s. This is presumably not due to OH^- entry but to Mg^{2+}, Zn^{2+} or Cu^{2+} ions (which have been shown by electron-probe microanalysis to enter films in their alloy proportions) in the film. According to the Heine–Pryor theory this would again seem to indicate dipoles which lose their effectiveness at rather less than 100 kc/s but alternative explanations are being considered. The effect is independent of current density. This is not so for films formed in the aqueous tartrate, where the effect is superimposed on that produced by OH^- ions in the film. The curves for films on Al–Zn are lower than those for Al–Cu or Al–Mg under these conditions.

Details of this investigation will be published later, together with a more critical appraisal of the theory and interpretation.

We thank Prof. T. K. Ross for providing facilities and for encouraging this work and the Science Research Council for a maintenance grant to one of us (A. J. B.).

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PALAEOMAGNETISM AND POTASSIUM-ARGON AGES OF SOME VOLCANIC ROCKS FROM THE GALAPAGOS ISLANDS

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SCIENTISTS of the Galapagos International Scientific Project visited the Galapagos Islands in January and February of 1964. As part of this project, rock samples were collected from 45 lava flows on thirteen of the islands. Our objective was to learn something about the ages of the rocks of the Galapagos Islands using potassium–argon dating and palaeomagnetic stratigraphy. The palaeomagnetic method for correlating young rock strata is based on the fact that the Earth's magnetic field has two stable dipolar states, a normal one during which the global field direction is toward the north, as at present, and a reversed state during which the field is directed toward the south. Lava flows contain ferromagnetic minerals which, like tiny magnets, become polarized when the flow cools. Thus, lava flows have a magnetic memory and record whether the geomagnetic field was normal or reversed at the time the flows originally cooled. They therefore may be grouped by age in sequences of normal or reversed polarity.

Locations of sampling sites and polarities of remanent magnetization are shown in Fig. 1. From six to eight oriented core samples were collected at each site, using a water-cooled diamond drill. Standard techniques were used for orienting the cores and measuring their remanent magnetization; these permit the direction of magnetization of the flow to be determined with an accuracy of 3°–4° (ref. 1). The magnetic stability of one core from each flow was tested in alternating fields up to 800 oersteds and was found to be of the type acquired by lava flows as a result of cooling in the Earth's magnetic field.

From previous investigations of volcanic rocks from other parts of the world, the following potassium–argon time scale has been found for the changes in polarity of the Earth's magnetic field[2–4]: 0·0 million years; Brunhes normal polarity epoch; 0·85 ± 0·15 million years; Matuyama reversed polarity epoch; 2·4 ± 0·1 million years; Gauss normal polarity epoch; 3·35 ± 0·1 million years; Gilbert reversed polarity epoch; where the (±) figures are subjective estimates of the range of uncertainty in epoch boundaries. In addition, there have been much more brief polarity periods which are important for purposes of detailed stratigraphic correlation but which will not concern us here[2]. Our correlation of lava flows from the Galapagos Islands will be based on their relation to the foregoing polarity time scale.

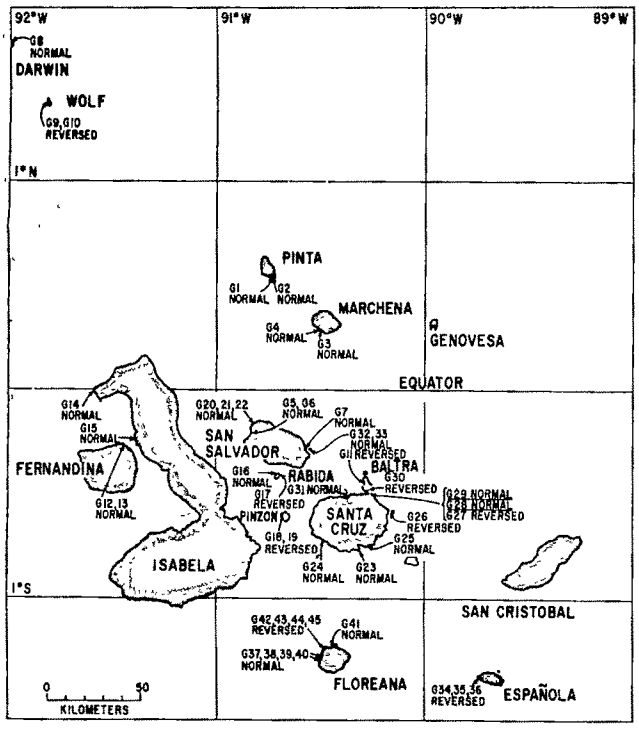


Fig. 1. Sampling sites and magnetic polarities

An ambiguity arises in correlating rock strata by polarity because the geomagnetic field has only two stable states. Thus a lava flow of normal polarity may have formed either during the Brunhes normal polarity epoch or during an earlier normal epoch. For young rocks, this ambiguity may be reduced by relating polarities to geomorphological indicators of age, such as the amount of erosion, the extent of faulting, and the development of vegetation cover. In Alaska, for example, we have found that primary volcanic features such as volcanic craters and flow surfaces are still recognizable in rocks as old as the beginning of the Brunhes normal epoch, but are almost completely obliterated in rocks formed during the Gauss normal epoch. In the Galapagos Islands, which are both arid and free from frost, such erosional differences are less pronounced.

All the sampled lava flows, which, from their state of preservation, appeared to be extremely young, are normally magnetized. A few of the reversely magnetized flows also are surprisingly young in appearance. The reversed flows on Pinzón, for example, occur on the walls of a crater that still retains its original form. On geomorphological grounds, however, most of the reversed flows appear to be older than the normal group. Those on Wolf, Baltra, northern Santa Cruz and Española Islands are exposed along high sea cliffs, some of which are faulted. The reversed flows on Floreana are from low volcanoes that have been partly destroyed by wave action where they face the sea.

We have used potassium–argon dating in conjunction with palaeomagnetic stratigraphy to provide additional information about the age of the rocks of the Galapagos Islands and to help in placing some of these lava flows in the correct polarity epochs. This dating method, which utilizes the constant decay of radioactive potassium-40 to argon-40, is the only known radiometric technique that can be applied to volcanic rocks that crystallized in the time interval of the polarity epochs outlined here.

The age analyses of this investigation were carried out on holocrystalline whole-rock samples of basalt. Typically, analyses on basaltic lavas that contain glass or alteration products do not give reliable age data. Therefore, the ten rocks for this investigation were selected only after thin sections had been analysed petrographically to ascertain that they were suitable for dating by the potassium–argon method. The argon analyses were carried out by standard isotope dilution techniques and the potassium determinations by flame photometry. The data from these analyses and the resulting calculated ages are given in Table 1.

The uncertainties in most of the age calculations are greater than is usual for rocks of this age because some of

Table 1. Analytical Data for K–Ar Age Determinations
All determinations on whole-rock basalt samples

Site	Polarity	Per cent K_2O			Ar analyses			Age* (10^6 years)
		(1)	(2)	Avg.	Weight (g)	$\frac{^{40}Ar_{rad} \times 100}{^{40}Ar_{total}}$	$^{40}Ar_{rad}$ (10^{-13} moles)	
G4	Normal	0·219	0·223	0·221	9·792	3·16	0·96	0·30 ± 0·10
G11	Reversed	0·565	0·565	0·565	11·977	6·84	13·73	1·37 ± 0·16
G14	Normal	0·761	—	0·761	9·802	3·52	0·99	0·090 ± 0·035
G17	Reversed	0·519	0·520	0·520	9·287	Nil	Nil	< 1·32
G21	Normal	0·674	0·677	0·676	8·945	1·26	1·92	0·22 ± 0·15
G23	Normal	0·424	0·426	0·425	10·726	1·48	1·86	0·28 ± 0·30
G27	Reversed	0·567	0·567	0·567	11·227	4·30	13·81	1·47 ± 0·40
G28	Normal	0·315	0·319	0·317	9·157	4·01	3·15	0·74 ± 0·22
G32	Normal	0·098	0·099	0·098	10·311	Nil	Nil	< 4·2
G43	Reversed	0·848	0·857	0·852	10·851	1·41	9·81	0·72 ± 0·40

* Calculated using $\lambda_\varepsilon = 0{\cdot}585 \times 10^{-10}$ yr.$^{-1}$, $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr^{-1}, and $^{40}K/K = 1{\cdot}19 \times 10^{-4}$ moles/mole. The ± figures are the estimated standard deviation of precision.

the Galapagos rocks are abnormally high in contaminating atmospheric argon and most of the lavas have a low potassium content. However, for eight of the samples the dates are sufficiently precise to make it possible to assign the flows to polarity epochs. Even the results from the two flows with no detectable radiogenic argon are useful, because a maximum possible age can be found from the minimum amount of radiogenic argon that could have been detected in the presence of the observed atmospheric argon. In particular, the 1·32 million-year maximum age for the reversed lava from site *G17* precludes this flow from being assigned to any reversed polarity epoch other than the Matuyama.

Stratigraphic correlation of the flows collected on the different islands is given in Table 2. Flows not directly dated are correlated with other dated flows on the basis of geomorphologic and stratigraphic relations and on the basis of magnetic polarity.

Of greatest interest from the point of view of the history of life in the Galapagos is the time that each island emerged from the sea. Three considerations make it necessary to be cautious of interpreting the present results as being indicative of the sequence in which the islands emerged. On some islands, an older core may be completely masked by a shield of young lava, so that the time of emergence of the island cannot be determined geologically. Secondly, we were unable to sample the oldest exposures on some of the islands in the time available. Finally, some of the rocks that we sampled may have formed beneath the sea much earlier than the appearance of the first land.

However, even with these limitations of the present results in mind, several generalizations are apparent. The first is that most of the volcanic terrain exposed above sea-level on the Galapagos formed during a remarkably short interval of several million years. This follows from the observation that most of the lava flows were extruded during the Brunhes and Matuyama polarity epochs, which span the time from the present back to 2·4 million years.

The present results also have a bearing on Wilson's theory[5] that islands form over the mid-oceanic ridges,

Table 2. Assignment of Sampling Sites to Polarity Epochs

Polarity epoch	Island [sampling site*]
Brunhes normal polarity epoch	Isabela [*G14**, *G15*] Fernandina [*G12*, *G13*] Pinta [*G1*, *G2*] Marchena [*G3*, *G4**] San Salvador [*G5*, *G6*, *G7*, *G20*, *G21**, *G22*, *G32**, *G33*] Rabida [*G16*] Santa Cruz [*G23**, *G24*, *G25*, *G28**, *G29*, *G31*] Floreana [*G37*, *G38*, *G39*, *G40*, *G41*] Darwin [*G8* ?]
Matuyama reversed polarity epoch	Rabida [*G17**] Pinzón [*G18*, *G19*] Baltra [*G11**, *G30*] Santa Cruz [*G27**] Floreana [*G42*, *G43**, *G44*, *G45*] Española [*G34* ?, *G35* ?, *G36* ?] Wolf [*G9* ?, *G10* ?]
Older	Santa Cruz [*G26*]

* Potassium–argon date listed in Table 1.
? Assignment to epoch uncertain.

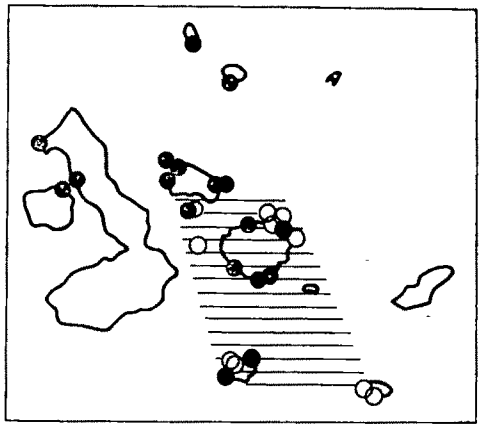


Fig. 2. Age relations among the 45 sampled flows. Shaded area contains all flows older than the Brunhes normal polarity epoch except for those on Wolf Island (off the diagram to the north-west). Solid circles are flows of the Brunhes normal polarity epoch and open circles are older flows. Adjacent flows of the same polarity are shown with one symbol

which are assumed to be topographic expressions of the upwelling limbs of convection cells in the Earth's mantle. Volcanic islands are assumed by Wilson to be active as they form over a ridge, then to become extinct as they are carried away from the ridge. In an archipelago formed this way, lava flows spanning the entire time of volcanic activity will not be found superimposed on one island. Rather, lava flows of increasingly older age will be found on islands progressively farther from the ridge. Immediately north-west of the Galapagos, there is the Cocos Ridge, which is an offshoot of the immense north–south East Pacific Rise. Thus the inferred direction of movement of the Galapagos would be toward South America, and the oldest islands would be at the south-east end of the chain. In Fig. 2, flows older than the Brunhes normal polarity epoch are indicated as open circles, and these may be seen to lie within the shaded area, which, in a general way, is at the south-east part of the archipelago as predicted. However, rocks of this older group also occur on Wolf Island, which is at the extreme north-west end of the chain. Moreover, the oldest rocks occur on the northern coast of Santa Cruz, which is near the middle of the chain. A further difficulty is that both young and old lava flows are found on many islands, such as Santa Cruz, where the oldest flows of the Galapagos and some of the youngest are closely juxtaposed. The age relations thus appear to form a more complicated pattern than that anticipated by Wilson's theory of oceanic island formation.

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RELEASE OF TRITIATED BRETYLIUM BY SYMPATHETIC NERVE STIMULATION

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BRETYLIUM is a sympathetic blocking agent which has been used in the treatment of hypertension[1]. This drug does not interfere with conduction of the nerve impulse by the sympathetic nerve fibre[2], nor does it prevent the action of administered norepinephrine[3]. Sympathetic blockade after bretylium treatment appears to be a consequence of failure of the sympathetic nerve ending to release norepinephrine in response to the nerve impulse.

It has been suggested that replacement of norepinephrine by an inactive counterfeit transmitter molecule may explain the partial sympathetic blockade seen after α-methyldopa[4] and metaraminol[5], and the tachyphylaxis in response to some sympathomimetic amines[6,7] and chronic administration of monoamine oxidase inhibitors[6,7].

Since bretylium accumulates in sympathetic nerves[8], releases norepinephrine[9], and interferes with subsequent release of norepinephrine by nerve stimulation[9], it was suggested that this drug may play a part similar to false neurochemical transmitters[7]. In the experiments to be described, the release of tritiated bretylium, administered *in vivo*, from the isolated perfused cat heart and spleen was examined during sympathetic nerve stimulation.

Bretylium was tritiated by catalytic exchange (New England Nuclear Corp., Boston, Mass.) and purified by paper chromatography.

Eight cats received 80 μc./kg bretylium-^{3}H intramuscularly. 20 h later the splenic circulation was isolated using a modification of the technique of Brown and Gillespie[10] previously described[6]. The organ was perfused with Krebs–Ringer solution saturated with 95 per cent oxygen–5 per cent carbon dioxide (containing 100 mg heparin plus 1 g glucose/l.) and the splenic nerve was stimulated using a Grass Model 4 stimulator, with impulses of 1 msec duration and supramaximal voltage. Fluid in the spleen was expressed by nerve stimulation with 100 impulses (10 impulses/sec) 20 and 25 min after the start of perfusion. After 30 min, samples of splenic vein effluent were collected during consecutive 2-min intervals. Following two control intervals, the splenic nerve was stimulated with 300 impulses, at frequencies of 10–100 i.p.s.

Splenic contraction without nerve stimulation was induced by injection of 10^{-7} g *l*-norepinephrine into the splenic artery (at *NA*, Fig. 1). To block splenic contraction, 5 mg phenoxybenzamine hydrochloride was infused into the splenic artery; 10 min later, the stimulations were repeated.

Stimulation of the splenic nerve in the isolated perfused cat spleen resulted in increased amounts of bretylium-^{3}H in the effluent perfusate (Fig. 1). Infusion of norepinephrine, which caused a greater increase in splenic arterial pressure than did nerve stimulation, resulted in no release of bretylium-^{3}H into the perfusate. Release of the labelled amine by nerve stimulation persisted even though splenic contraction was blocked by phenoxybenzamine.

In other experiments, two cats, weighing 2–4 kg, received 80 μc./kg bretylium-^{3}H (20 mc./mg) intramuscularly. 14 h later they were anaesthetized with 'Nembutal' (30 mg/kg intraperitoneally), a tracheotomy performed, and artificial respiration instituted using a Harvard respirator. The right sympathetic chain was isolated just above the clavicle and the chest opened. The right stellate ganglion, upper thoracic sympathetic ganglia, cardiac nerves and heart were removed *en bloc*. The heart was perfused by the Langendorff technique with Krebs–Henseleit solution saturated with 95 per cent oxygen–5 per cent carbon dioxide and containing 1 g glucose/l. To ensure adequate drainage, both ventricles were vented with stab wounds. Cardiac rate and left ventricular contractile force were recorded on a Grass 5-*D* polygraph, with *FT*-03*B* force transducer. The stellate ganglion was stimulated with rectangular impulses of supramaximal voltage, 1-msec duration, using a Grass model 4 stimulator. Ten minutes after the start of perfusion, the preparation

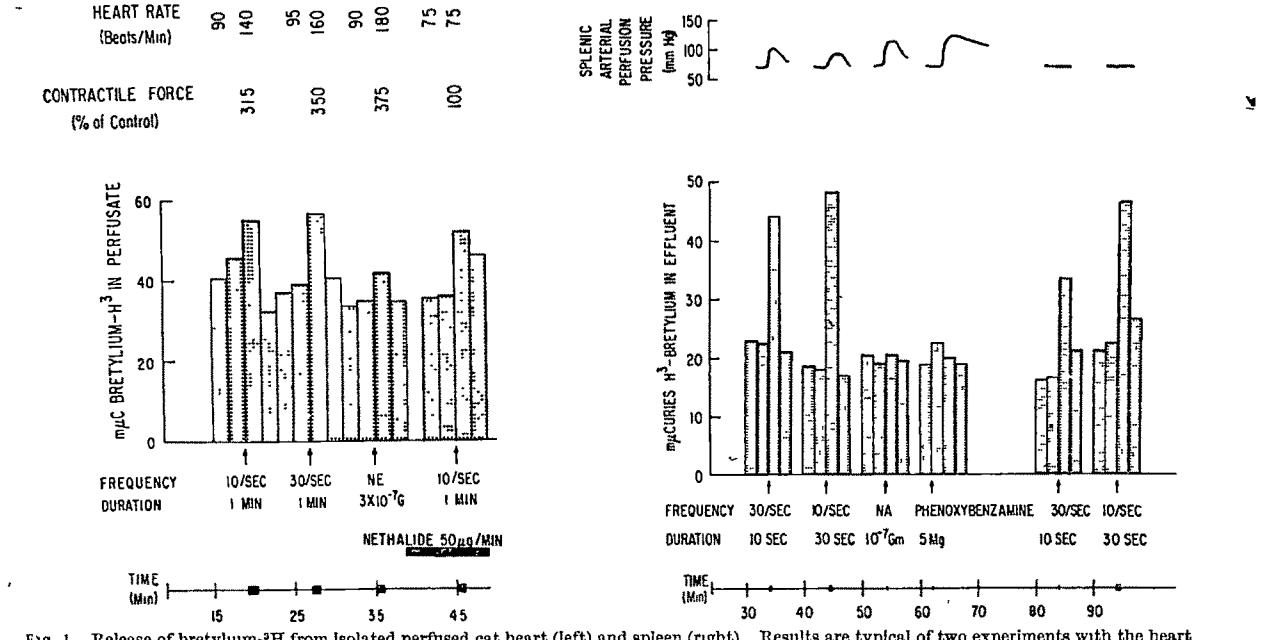


Fig. 1 Release of bretylium-^{3}H from isolated perfused cat heart (left) and spleen (right). Results are typical of two experiments with the heart and eight with the spleen

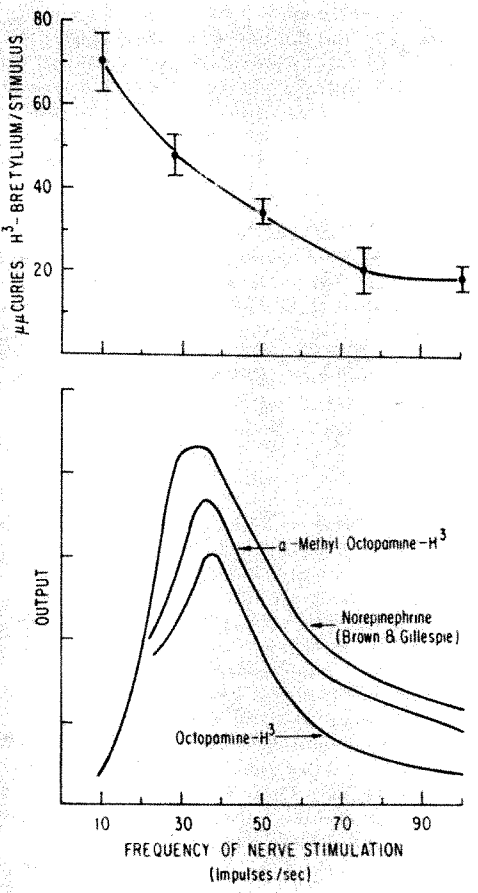


Fig. 2. Output of bretylium-^{3}H (top) at various frequencies of splenic nerve stimulation is compared with curves obtained for norepinephrine and other adrenergic false transmitters previously investigated

was tested by stimulating with 150 impulses during a 15-sec interval. Five minutes later, collection of the perfusate during 2-min consecutive intervals was begun. After two control periods, the ganglion was twice stimulated for 1 min at a frequency of 10 or 30 i.p.s. Norepinephrine 3×10^{-7} g was infused into the aortic cannula 8 min later. Stimulation was repeated during an infusion of nethalide (50 μg/min).

The effluent perfusate was centrifuged to sediment any red blood cells and bretylium was absorbed on 'Dowex 50' (NH_4^+) at 6·5 *p*H, and afterwards eluted with 6 ml. 3 N hydrogen chloride in ethanol. A portion of the eluate was assayed in a liquid scintillation spectrometer.

During sympathetic nerve stimulation an increased amount of bretylium-^{3}H was released from the isolated perfused cat heart (Fig. 1). A marked increase in cardiac rate and contractile force was noted during nerve stimulation (Fig. 1). Norepinephrine, which caused a similar increase in cardiac rate and contractile force, failed to release bretylium-^{3}H. When the inotropic and chronotropic effects of sympathetic nerve stimulation were blocked by the infusion of nethalide, a potent adrenergic-β-blocking agent, bretylium-^{3}H was still released by nerve stimulation. The spontaneous release of bretylium-^{3}H was unaltered by the adrenolytic drug.

The relationship of frequency of nerve stimulation to release of bretylium is shown in Fig. 2. The output of the labelled amine at stimulus rates of 10 i.p.s. is greater than that at 30 i.p.s. Phenoxybenzamine failed to elevate the rate of spontaneous release of bretylium (Fig. 1). Adrenergic blocking agents increase the resting output of norepinephrine[10] and false transmitters in heart[11] and spleen[6]. Furthermore, maximal release of norepinephrine[10] and false transmitters[6] is seen at frequencies of about 30 i.p.s. (Fig. 2). Bretylium does not behave in this manner (Figs. 1 and 2). Neither nethalide nor phenoxybenzamine elevated bretylium-^{3}H output at low frequencies of nerve stimulation. While norepinephrine and other typical adrenergic transmitters are released by acetylcholine, as well as by nerve stimulation, bretylium-^{3}H is not released by acetylcholine[11]. Bretylium is not a typical adrenergic false neurochemical transmitter, and probably does not replace norepinephrine. These findings suggest that bretylium acts at a stage between the nerve impulse and release of the adrenergic transmitter, possibly replacing an intermediate substance necessary for norepinephrine release.

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In vitro MALIGNANT TRANSFORMATION OF CELLS BY FRIEND VIRUS

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PROPAGATION of Friend virus in mouse embryo cell culture was previously demonstrated in this laboratory[1,2].

This article concerns cellular morphological changes associated with the neoplastic property induced in early passages of the virus-infected mouse embryo cells and general characteristics of the resulting transformed cell line which has been maintained in this laboratory for 18 months.

The Friend virus used in this work was originally obtained from Dr. Charlotte Friend and was in its 48th serial passage in *HA/ICR* Swiss mice in this laboratory[3]. The titre of a 10 per cent infected spleen filtrate was approximately $10^{4.6}$ $I.D._{50}$/ml. when inoculated intraperitoneally into mice. This inoculum was free of polyoma virus so far as the incidence of multiple tumours and the antibody response against polyoma virus in injected mice were tested.

The test animals were *HA/ICR* Swiss new-born, 5–6-day-old suckling and 4–6-week-old mice obtained from the Roswell Park Memorial Institute breeding colonies.

The cell cultures used for inoculating Friend virus were primary cultures of *HA/ICR* Swiss mouse embryo tissues. Eight-ounce bottle cultures were prepared using Eagle's medium containing 10 per cent heat-inactivated calf serum as growth medium. Eagle's medium with 2·5 per cent heat-inactivated horse serum was used as the maintenance medium. These media were also used for

serial subcultivation of the virus-infected cells during the first 9 months, then substituted by Eagle's medium containing 10 per cent heat-inactivated foetal calf serum. Coverslip cultures were prepared in Leighton tubes for immunofluorescent staining and Wright–Giemsa staining. The indirect staining method was used in the immunofluorescent investigations[1,2].

Cell suspensions and cell-free supernates were injected intraperitoneally into mice to test leukaemogenic activity. Approximately 5×10^5 trypsinized cell suspensions were injected intraperitoneally or subcutaneously into suckling and young Swiss mice. Culture media were centrifuged and the supernates were injected intraperitoneally into new-born and young mice. To test intracellular leukaemogenic activity, cells were trypsinized and resuspended in small amounts of maintenance medium. They were disrupted by homogenizing and by freezing and thawing. After centrifugation the supernates were injected intraperitoneally into new-born and young mice. Criteria of Friend disease were based on splenic enlargement and histological and haematological examinations of the test mice.

To test the neoplastic property of the transformed cells, 1×10^5–5×10^5 cells were injected into new-born Swiss mice intraperitoneally or subcutaneously in the back.

For pathological investigations, specimens were fixed in 10 per cent formalin and stained with haematoxylin and eosin.

Three monolayer bottle cultures were inoculated with a 10 per cent infected spleen-cell-free filtrate and incubated 37° C for 3 h. The cells were then washed three times with Eagle's medium and held in a stationary position at 37° C following addition of maintenance medium. As controls, two out of eight bottles were inoculated with normal spleen filtrate, two with heat-inactivated infected spleen filtrate, two with neutralized infected spleen filtrate and the two remaining were not inoculated. Medium change was carried out every other day. After 25 days of incubation, each culture was transferred to two bottles using 0·2 per cent trypsin. Succeeding several transfers were then carried out every 4–10 days.

When leukaemogenic activity of the culture fluids of the virus-infected cells was tested on the sixteenth day, fifteen of sixteen mice (93·7 per cent) showed massive spleen involvement and polycythaemia, that is, typical Friend disease. Approximately 40 per cent of monolayer cells of the infected coverslip cultures were stained specifically by immunofluorescent staining.

More fluorescent cells were observed after transfer of the virus-infected cultures. When cell-grown coverslips were tested at the third passage, most cells were strongly fluorescent. The growth rate of these infected cells was considerably higher than that of controls.

In the fourth to sixth passages, after 1·5–2 months of the virus inoculation, proliferative foci of epithelioid cells with multi-nucleated giant cells were noted among fibroblastic monolayers. There was a tendency for the epithelioid cells to grow on top of one another, forming whitish and thickened plaques which were macroscopically visible. In the proliferative foci, only occasional fluorescence was observed in multi-nucleated giant cells. No significant degenerating process was observed in connexion with the appearance of the proliferative foci. These foci rapidly took the place of fibroblastic monolayers in two to three transfers. In the seventh passage, in which transformed foci were frequently observed, leukaemogenic activity of culture fluids was very weak. Only three of sixteen mice (18·7 per cent) showed splenic enlargement. In the eighth passage, there was no more leukaemogenic activity in cultured fluids. These cultures have been serially transferred once a week. This line (*TSME*-1) is at present in its 77th passage. The cell type is still epithelioid, but spindle-shaped cells are also present. Multi-nucleated giant cells are frequently observed at each passage-level. Confluent sheets are rapidly obtained with a tendency to overgrow one another, forming multi-layered areas. Rapid acidification of the culture medium is also noted. Control cultures were treated in the same way as infected cultures; however, all cultures degenerated after a few serial transfers. Fig. 1 shows 57th-passage *TSME*-1 cells in comparison with normal secondary mouse embryo cells. Epithelioid cells are predominant, but spindle-shaped cells are also present. Many multi-nucleated giant cells are observed. The nuclei are pleomorphic and the nuclear/cytoplasmic ratio is increased. Nucleoli increase in size and number and show a rounded outline. In contrast to the transformed cells, the cell type of normal mouse embryo cultures is primarily fibroblastic.

There have been no significant intracellular and extracellular leukaemogenic activities and no significant amount of Friend viral antigen in *TSME*-1 cell cultures since the transformed cell line was established. None of the 390 (0 per cent) injected with culture fluids and only 3 of 214 mice (1·4 per cent) injected with supernates after disrupting cells showed splenic enlargement. However, by injecting cell suspensions, 48 of 210 mice (22·9 per cent) have shown massive splenic enlargement although only occasional fluorescence has been observed in transformed cell culture by immunofluorescent staining.

To test the neoplastic property of the transformed cells, approximately 5×10^5 cells at the tenth passage were first inoculated into the subcutaneous tissue of the back of eleven new-born and intraperitoneally into twelve new-born Swiss mice respectively. By the ninetieth day, tumours developed in three of eleven (27·3 per cent) and in ten of twelve (83·3 per cent) respectively, and were first evident at 3–4 weeks. There was essentially no difference in growth characteristics between tumours induced with cells of different passage levels. In the latest experiment, approximately 5×10^5 *TSME*-1 cells in the 61st passage

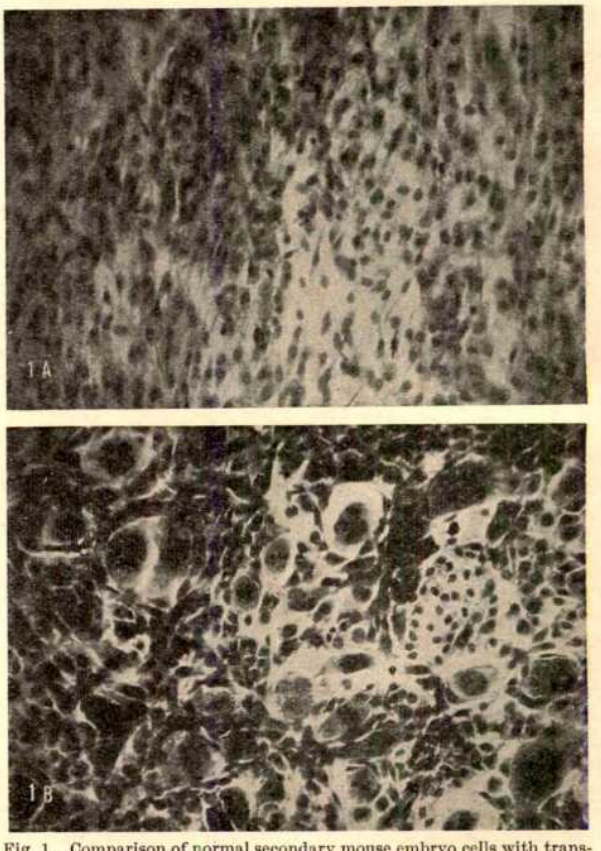


Fig. 1. Comparison of normal secondary mouse embryo cells with transformed cells at the fifty-seventh passage level. Wright–Giemsa stain (×*c*. 50). (*A*) Control cells, primarily fibroblastic in morphology; (*B*) transformed culture, *TSME*-1. Note epithelioid morphology, multi-nucleated giant cells, and spindle-shaped cells

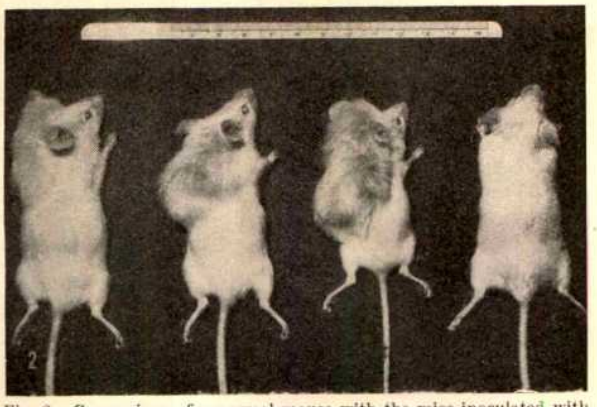


Fig. 2. Comparison of a normal mouse with the mice inoculated with sixty-first passage *TSME*-1 cells subcutaneously in the scapular region 30 days ago when new-born. Note big tumours in the back of 3 mice

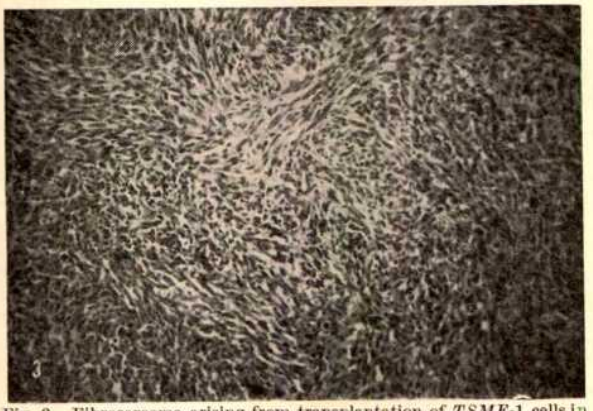


Fig. 3. Fibrosarcoma arising from transplantation of *TSME*-1 cells in the sixty-first passage. Haematoxylin and eosin stain. (× *c.* 50)

were inoculated into 89 new-born Swiss mice. By the sixtieth day, tumours developed in 14 of 44 mice (31·8 per cent) inoculated into the subcutaneous tissue of the back and 38 of 45 mice (84·4 per cent) inoculated intraperitoneally respectively, and were first evident at 1–2 weeks, attaining a diameter of 3–5 cm by the fourth–fifth week. They remained fairly localized. There was no gross evidence of metastasis. Fig. 2 shows a normal mouse and the mice which were inoculated subcutaneously in the scapular region with 5×10^5 cells at the 61st passage 30 days when new-born, followed by the development of large tumours. As illustrated in Fig. 3, these tumours are fibrosarcomatous. No tumours arose over a 5-month period of observation when 1×10^6–5×10^6 normal mouse embryo cells were inoculated subcutaneously or intraperitoneally into new-born mice. Also, no tumours arose by injecting Friend virus itself or cell-free materials of the transformed cultures into mice.

In the tumour-bearing mice, splenomegaly was frequently observed and large numbers of proliferating reticulum cells and erythroblasts were evident even in spleens which grossly were normal. High peripheral blood leucocyte counts with blast cells were usually found in the animals. Fig. 4 shows a normal mouse and the mouse which was inoculated intraperitoneally when new-born with 1×10^5 52nd passage *TSME*-1 cells at 60 days with the development of large abdominal tumours and an enlarged spleen. In contrast to the typical Friend disease, all the mice with leukaemia induced by inoculating the transformed cells were markedly anaemic regardless of the presence of tumours.

These findings indicate that Friend virus can induce a malignant transformation of normal cells *in vitro* as do certain oncogenic viruses[4]. In the transformed culture induced by RNA containing avian myeloblastosis virus, transformed cells were well differentiated and produced infectious virus progeny[5]. In the case of the RNA containing Rous sarcoma virus, transformed cells generally produce infectious virus progeny. Since the Rous sarcoma virus is defective, they totally fail to produce infectious virus under certain conditions. However, the presence of the viral RNA in the transformed cells can be demonstrated by infecting them with a related helper virus[6]. These transformed cells are characteristic of rounded or fusiform cells. Exceptionally, a transformation characteristic of polygonal cells with multi-nucleated giant cells has been reported[7].

In contrast to this, general characteristics of the Friend murine leukaemia virus-induced transformed culture resemble those of DNA containing SV_{40} (refs. 8 and 9). Friend virus proliferated continually in early passages of the virus-infected cells and rapidly became more difficult to detect in transformed cultures. Virus could only be recovered when transformed cells were inoculated directly into animals. There was loss of contact inhibition with a tendency for cells to 'pile up' in multiple layers. The cell type was epithelioid but spindle-shaped cells were also present. Multi-nucleated giant cells were frequently observed. Cultures acidified rapidly. An inverse relation between ease of viral propagation and ease of induction of cellular transformation *in vitro* was generally observed with polyoma virus and SV_{40} (refs. 10 and 11). However, it is characteristic of the system described here that transformed foci occurred rapidly in specifically fluorescent non-degenerating monolayers.

In the *TSME*-1 cell line, no significant amount of viral antigen and no significant intracellular and extracellular leukaemogenic activities have been detected; however, the results of the inoculation experiment of transformed cells may suggest that the cell line may be carrying the genetic information of Friend virus. Friend virus may be synthesized and released in a few cells among a large non-producing population, or the virus may be synthesized and released so slowly that it may be difficult to detect it by animal titration and immunofluorescent staining. It can also be assumed that the viral information can be carried as viral RNA in this transformed cell line. Such a small amount of virus or viral RNA in transformed cells will be activated by inoculating the cells into animals. Sufficient evidence to permit a conclusion is not available.

Friend disease induced by injecting the virus-infected tissue culture fluids into mice was characterized by a typical biphasic response—uncontrolled proliferation of reticulum cells followed by erythroblastosis and polycythaemia[12]. This was also the case with Friend disease induced in mice by injecting culture fluids in early passages of the virus-infected cells in which transformed cells were not yet observed. In contrast to this, mice with leukaemia induced by inoculating with transformed cells were markedly anaemic. This may suggest the presence of a variant of Friend virus in transformed cells. Previous work in this laboratory suggested that Friend disease may involve two agents—one responsible for the reticulum cell

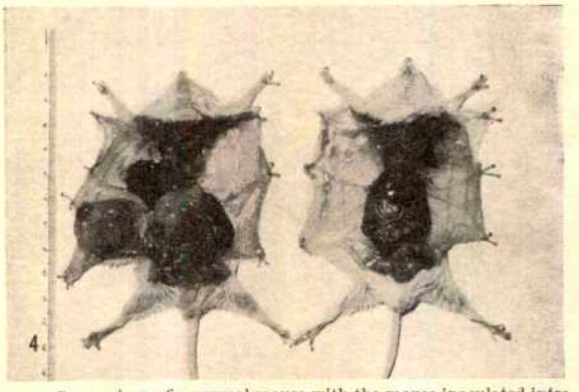


Fig. 4. Comparison of a normal mouse with the mouse inoculated intraperitoneally with fifty-second passage *TSME*-1 cells 60 days previously when new-born. Note big abdominal tumours and an enlarged spleen in the mouse on the left

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disease and the other producing erythropoietic response (erythroblastosis and polycythaemia[13]). If this were the case, the former agent may be favourably selected in transformed cells.

Tumours arose after transplantation of cells at the tenth passage of the *TSME*-1 cell line at which transformation was established. This indicates a correlation of transformation by Friend virus *in vitro* with *in vivo* oncogenicity for the same species. The high tumour incidence (85–90 per cent) resulting from intraperitoneal transplantation of transformed cells into new-born *HA/ICR* Swiss mice is striking. Moreover, it has been consistently possible to re-isolate Friend virus from enlarged spleens of tumour-bearing mice. These splenic filtrates produced typical Friend disease but without polycythaemia.

Persistent infections of cells *in vitro* with murine leukaemia viruses were reported previously[14–16]. Manaker *et al.*[16] described tumour induction after transplantation of the Moloney virus-carrier cells into the isologous mouse strain. Since they used an established mouse spleen cell line to obtain the virus-carrier culture and since normal mouse cells carried in culture for long periods are known to undergo neoplastic change, they could not conclude that the virus was responsible for the induction of the neoplastic state. In contrast, data presented in this report support the idea that Friend virus can directly induce a neoplastic transformation of primary mouse embryo cells *in vitro*. Properties of the transformed cell line thus obtained have been stable for 1·5 years.

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EFFECT OF DEOXYGUANOSINE ON SKELETAL DEVELOPMENT IN THE CHICK EMBRYO

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SEVERAL investigators have reported that deoxyguanosine can interfere with growth and development of the chick embryo[1–3]. Deoxyguanosine treatment of 4-day embryos resulted in rather severe stunting of growth, abnormalities of the eyes and facial skeleton, severe oedema, inhibition of feather development, and still other miscellaneous defects[1]. Under *in vitro* conditions, deoxyguanosine inhibited growth and development of the 11–13 somite chick embryo[2]. We have previously reported that as little as 25 μg deoxyguanosine is capable of interacting with actinomycin *D* in such a way as to increase the number of embryos which respond to the teratogenic action of the antibiotic[3]. Our continued investigation of the interaction in teratogenesis of the two compounds has of necessity included a detailed investigation of the effects of deoxyguanosine on skeletal development, the results of which are presented here. Apart from their potential significance for the elucidation of the interaction between deoxyguanosine and actinomycin *D*, the observations reported are of interest in that they illustrate that a measure of spurious regional specificity can be superimposed on the rather general inhibitory action of deoxyguanosine. Deoxyguanosine treatment of the chick embryo results in the death of large numbers of cells in a variety of tissues, including the brain and nerve cord, the scleratome and derma-myotome, the mesenchyme of the limb buds, the maxillary processes and visceral arches, the optic cup and head mesenchyme. However, readily predictable patterns of response can be elicited under appropriate conditions of timing and dosage. In one case, the induction of rumplessness, the response can be equated with the presence or absence of induced cell death in a spatially restricted region. The other response patterns elicited appear to be related to subsequent intra-organ developmental events, as yet undefined, which are precipitated by the widespread cell destruction.

In the experiments carried out, varying amounts of deoxyguanosine were injected into the yolk of fertile White Leghorn eggs which had been previously incubated for periods ranging from 0 to 120 h. Aqueous solutions were used, and the volume of fluid injected was never greater than 0·2 ml. After injection, eggs were re-incubated under standard conditions of temperature and humidity. Experiments were terminated at various stages, depending on their purpose. Dead embryos were detected by candling on the 6th, 9th and 13th days of incubation, unless experiments were terminated prior to these stages. Embryos dying before the 6th day were discarded; all embryos which survived beyond the 6th day were examined. In experiments which were terminated at the end of the 17th day of incubation, alizarin preparations of the surviving embryos were made in order to facilitate the detection of skeletal anomalies. As much as 50 μg of deoxyguanosine was found not to be teratogenic when injected into fertile eggs either prior to incubation or at 24 h. Data obtained from experiments in which embryos 48 h old or more were treated and allowed to develop until the 17th day have been summarized in Table 1.

48-h embryos. Injection of 50 μg of deoxyguanosine into the yolk sacs of 48-h embryos resulted in the death of approximately 50 per cent of the embryos before the 17th day of incubation. Most of the deaths occurred after the 6th day, and virtually every one of the dead embryos showed some axial defect. The most common defect involved a constriction of the trunk in the thoracic region with an accompanying malposition of the hind limbs.

Among the survivors of the 17th day of incubation, 30·7 per cent showed at least one defect, and most embryos were multiply affected. The most seriously defective embryos (3·8 per cent) were classified as phocomelic dwarfs. These embryos had extremely reduced limbs, facial coloboma and cleft palate, a shortening of either or both the upper beak or mandible, a shortening of the neck and trunk, and generalized oedema. Their tails

appeared to be normal, however. The remainder of the abnormal embryos were generally in the same size range as were control embryos and the treated embryos which appeared to be unaffected by deoxyguanosine treatment. Among these embryos defects involving the axial skeleton were approximately twice as prevalent as were facial defects and limb anomalies.

Axial defects observed among embryos treated at 48 h included a shortening of the neck and a shortening and constriction in the thoracic region. Examination of alizarin preparations of these abnormal embryos showed a reduction in the size but not in the number of cervical and/or thoracic vertebrae. The reduced vertebrae were compressed anteroposteriorly and partially fused. As might have been anticipated, rib anomalies were also seen in embryos showing abnormal thoracic vertebrae. Both types of axial defects were observed in the same embryo approximately as frequently as they were found separately.

Facial defects were found in 15·3 per cent of the embryos which survived the 17th day of incubation. These include facial coloboma, cleft palate and a reduction in either the upper or the lower beak. After treatment at 48 h, short upper beak was found in approximately three times as many embryos as was short lower beak. With rare exception, an embryo showing one of the facial defects also showed the other two.

In addition to the phocomelic embryos, some reduction in length of the wings and/or legs was seen in another 9·2 per cent of the survivors of the 17th day. Most of these micromelic embryos were affected bilaterally, but in a few cases only the limbs on the right side were affected.

Injections of 25 μg of deoxyguanosine were considerably less toxic to the 48-h-old chick embryo, and were also less teratogenic. The frequency of abnormal embryos was approximately one-half that observed after treatment with 50 μg; however, the incidence of axial defects was reduced by approximately two-thirds, and no phocomelic dwarfs were produced.

72-h embryos. Injections of 75–100 μg of deoxyguanosine approximated the LD_{50} dosage for embryos of 72-h incubation age. Again, most of the embryonic deaths occurred after the 6th day and abnormalities of the axial skeleton were found among the dead embryos. These included constrictions in the thoracic region, and also rumplessness. About 50 per cent of the embryos which survived the 17th day of incubation were abnormal, most being multiply affected. Phocomelic dwarfs similar to these encountered after treatment with 50 μg at 48 h accounted for approximately 5 per cent of the embryos. The remaining embryos showed defects of all the main skeletal regions.

Axial defects observed among survivors of the 17th day included short neck, reduced thorax and rumplessness. Short neck and rumplessness were each found in about 20 per cent of the embryos, and were frequently observed in the same embryo. Reduced thorax was found in less than 4 per cent of the embryos. As indicated in Table 1, facial defects were found in about 40 per cent of the embryos and micromelia in about 22 per cent. In some of the micromelic embryos, either only the limbs of the right side were affected, or the limbs of the right side were more drastically reduced than were those of the left side. A small number of embryos with foot defects were also observed.

In the group which had received only 50 μg of deoxyguanosine, 64·6 per cent of the embryos survived the 17th day and 17·7 per cent of these were abnormal. No phocomelic dwarfs were observed, and rumplessness was the only axial defect seen. The frequencies of axial, limb and foot defects were approximately one-half those found in the groups which had received 75–100 μg, whereas the frequency of facial defects was reduced by approximately three-fourths.

96-h embryos. Embryos of 96-h incubation age tolerated dosages of 100–200 μg of deoxyguanosine fairly well. Even in the 200 μg group, approximately 90 per cent of the embryos survived the 17th day of incubation, and only 3 per cent were phocomelic dwarfs. The frequency of abnormal embryos rose with increasing dosage from 22·4 per cent to 65·7 per cent and limb defects constituted the major class of abnormality. Axial defects, including rumplessness and short neck only, were obtained after treatment with 150–200 μg. Significant frequencies of facial defects were also found in these two experimental groups. A high incidence of foot defects was encountered for the first time. These included clubbing, microdactyly, ectrodactyly and syndactyly. In a number of embryos the feet were affected asymmetrically, but there did not seem to be any difference in susceptibility of right and left feet.

120-h embryos. Of 65 embryos of 120-h incubation age which had received 200 μg of deoxyguanosine, 60 survived the 17th day of incubation. Among these survivors, 63·3 per cent showed foot defects similar to those observed after treatment at 96 h, and 1·6 per cent had short mandibles. No other abnormalities were observed.

Axial Skeleton

When the particular axial defects produced were considered in relation to the time of treatment of the embryo, characteristic patterns of response were indicated (Table 2). Thoracic defects appeared to be characteristic of deoxyguanosine interference with axial development in 48-h embryos. Alternatively, rumplessness was a characteristic response of axial development when 72-h embryos were treated. On the other hand, cervical defects were fairly readily produced by deoxyguanosine treatment of either 48- or 72-h embryos.

An explanation for the pattern of response induced in axial tissues was sought in histological investigations of deoxyguanosine-treated embryos. Fertile eggs of 48- and 72-h incubation age were injected with 50 and 100 μg of deoxyguanosine, respectively, and re-incubated for periods of 1–7 days. Histological examination of embryos in both experimental groups which were killed 1 day after treatment showed clusters of degenerate cells in a variety of embryonic regions. Restricting attention

Table 1. Effects of Deoxyguanosine on Skeletal Development in the Chick Embryo
(Incubation Age and Dosage varied for White Leghorn Fowl)

Incubation age (h)	48		72			96			120
Dosage (μg)	25	50	50	75	100	100	150	200	200
Fertile eggs	198	255	175	176	149	6[illegible]	121	109	65
Embryonic deaths									
0–6 days	7	45	15	21	15	2	6	5	5
7–17 days	16	78	47	64	59	1	5	6	0
Survivors of 17 days	175	130	113	91	75	58	110	98	60
Abnormal embryos (%)	17·1	30·7	17·7	57·1	49·3	22·4	47·3	65·7	63·3
Axial defects	8·6	30·7	14·1	27·4	42·6	0·0	2·2	9·1	0·0
Facial defects	7·4	15·3	7·9	38·4	41·3	3·4	16·3	35·7	1·6
Phocomelia	0·0	3·8	0·0	4·4	5·3	0·0	0·9	3·1	0·0
Micromelia	2·2	9·2	11·5	21·9	22·7	12·1	35·4	48·9	0·0
Foot defects	1·1	2·3	2·0	4·4	5·3	22·4	37·3	50·0	63·3

to axial structures, degenerate cells were abundant in the brain and nerve cord, and among the sclerotomal and derma-myotomal cells. They were not seen in the notochord, nor in the undifferentiated nodal mass and the unsegmented somite mesoderm. Examination of subsequent stages of development indicated that the axial defects were related to the destruction of cells in the nerve cord and in the scleratome. A description of the aberrant morphogenesis will not be presented here, but it is pertinent to note that in the defective regions the nerve cord was unable to elongate properly. For example, in the abnormally developing tail buds the nerve cord ended abruptly some distance anterior to the terminus of the notochord which was displaced dorsally until it virtually contacted the dorsal surface. Abnormal tail buds ceased to elongate and were afterwards resorbed, presumably due to the absence of a normal pattern of dorsal axial tissues[4]. In the thoracic and cervical regions, the failure of the nerve cord to increase in length was so marked in some embryos that the notochord which had increased in length so much more rapidly was folded back on itself. Presumably these embryos represented extreme cases and would have been among the early dead. It should also be noted that in all affected axial regions the pre-cartilaginous mesenchymal condensations were considerably reduced in size and cartilage formation was delayed by several days.

These investigations suggested a rather simple explanation for the failure of deoxyguanosine to induce rumplessness in 48-h embryos. No cell deaths were produced in the tail buds of 48-h embryos since the laying down of the nerve cord and the differentiation of the sclerotome and derma-myotome had not proceeded as far caudally as the tail bud by that stage. On the other hand, degenerate cells were present in the tail buds of embryos treated at 72 h, because by that stage the cells of the axial tissue had differentiated sufficiently to be sensitive to deoxyguanosine treatment. The explanation for the absence of thoracic defects in embryos treated at 72 h was not so obvious, however. Degenerate cells were found in the nerve cord and somite derivatives in embryos treated at 72 h. Quite possibly quantitative differences could have accounted for the different response of the thoracic regions of 48- and 72-h embryos, but cell counts were not attempted.

Facial Skeleton

An alternation of response was also suggested by the distribution of anomalies within the facial defect group. Short upper beak was a more frequent consequence of treatment of 48-h embryos than was short lower beak (10·5 and 2·9 per cent, respectively, after 50 μg; 5·0 and 0·0 per cent, respectively, after 25 μg). On the other hand, short lower beak was encountered more frequently than short upper beak after treatment of 72- or 96-h embryos (for example, 32·8 and 3·3 per cent, respectively, after 75 μg at 72 h; 19·2 and 1·1 per cent, respectively, after 150 μg at 96 h). As in the case of the differential response of the thoracic region, the explanation for this situation was not immediately apparent. Numerous degenerate cells were found in both the maxillary processes and the mandibular and other visceral arches after treatment at either the 48- or the 72-h stage. Again the need for quantitative studies was suggested, particularly since cell death has been implicated as a factor in normal development of the lower beak[5].

Limbs

According to the data presented in Table 1, the incidence of micromelia was approximately proportional to dosage at all stages from 48 to 96 h. Embryos were classified as micromelic whenever either the wings or the legs were shortened, however, and in fact it was only for the leg defect that this statement was true. The wings were shortened in the same percentage of embryos as were the legs after treatment at 48 h, but fewer embryos had shortened wings after treatment at 72 or 96 h. The susceptibility of the leg buds, on the other hand, appeared to remain fairly constant between 48 and 96 h, and even until 120 h, since the micromelic embryos were quantitatively replaced by embryos with foot defects after treatment at the latter state.

Although the susceptibility of the leg bud remained constant from 48 to 120 h, the response elicited by deoxyguanosine did show significant variation depending on the stage of the embryo at the time of treatment. The pattern observed was consistent with a distal shift with age of the region of maximal susceptibility to deoxyguanosine, except for some complication after treatment at 72 h. At all stages from 48 to 96 h, a number of treated embryos were found in which combinations of two or more long bones were reduced in length. These were most frequent after treatment with the higher dosages at each stage, and included the phocomelic embryos already described. In addition, embryos were found in which only one of the long bones of the leg was appreciably shortened. Thus, after treatment of 48-h embryos with 25–50 μg of deoxyguanosine the tibiotarsus alone was reduced in all sixteen micromelic survivors of the 17th day of incubation. Examination of alizarin preparations showed that the fibula was approximately normal size, although sometimes bent, in these embryos. After treatment of 96-h embryos, on the other hand, the tarsometatarsus bone alone was reduced in 92 out of 94 micromelic embryos. After treatment of 72-h embryos with 50 μg of deoxyguanosine in twelve out of thirteen micromelic embryos only two long bone rudiments were present, one of which was the tarsometatarsus. The fibula was missing, and either the femur was also missing, or the femoral and tibiotarsal rudiments had been so reduced that the two had undergone tandem fusion at some time during their development. In the groups of micromelic embryos produced by treatment with 75–100 μg of deoxyguanosine at 72 h, 26 of 37 embryos also had only two long bone rudiments present in at least one of their legs. Of the remaining eleven embryos, the tibiotarsus was reduced in four, the tarsometatarsus in one, and 6 were multiply affected. An apparently similar situation was observed in the reduced wings of some embryos; for example, the most proximal long bone appeared to be missing. In the wing, two adjacent bony rudiments of unequal size were found proximally. These could have resulted from a situation in which the femoral, ulnar and radial rudiments were all reduced, followed by tandem fusion of the femoral and ulnar rudiments. A possibly comparable situation has been described by others[6], but has been explained as being due to the absence of the humerus and marked reduction of the radius. This

Table 2 Distribution of Axial Defects in Deoxyguanosine-treated Chick Embryos (Incubation Age and Dosage varied for White Leghorn Fowl)

Incubation age (h)	48		72			96		
Dosage (μg)	25	50	50	75	100	100	150	200
Survivors of 17 days	175	130	113	91	75	58	110	98
Axial defects (%)	8 6	30 7	14·1	27·4	42·6	0 0	2 2	9 1
Short neck	6 8	26 8	0 0	22 2	20·4	0 0	2 2	5 1
Thoracic defect	6 8	24 8	0 0	0 0	3 8	0 0	1 1	3 0
Rumplessness	1 1	0 0	14 1	17 6	28 1	0 0	1 1	2 0

appears less likely as an explanation for the present results, but further investigations are needed.

In view of the availability of 'fate maps' for the various components of the developing limbs[7], investigations were undertaken in order to determine if distinctively located populations of deoxyguanosine-sensitive cells could be detected. Fertile eggs which had been incubated 48, 72, 96 or 120 h were injected with 50, 50, 100 and 200 μg of deoxyguanosine, respectively, and embryos were collected at various times after treatment. Live embryos were then vitally stained with Nile blue sulphate according to the procedure of Saunders *et al.*[8], and the location of degenerate cells in the leg buds noted. With only rare exceptions, embryos killed 24 h after treatment showed a fairly even distribution of degenerate cells throughout their leg bud mesenchyme. The response pattern then did not appear to be related to the spatial distribution of sensitive cells. Presumably it was determined by subsequent internal rearrangements possibly directed by factors such as the competitive interactions among the long bone rudiments which have been reported in recent years[9].

The investigations described here confirm and extend the earlier observations of Karnofsky and Lacon[1] regarding the teratogenic capability of deoxyguanosine. Failure to record such a capability in our previous investigation[3] can presumably be traced to a combination of factors such as duration of experiments, small dosage of deoxyguanosine and sampling error. Previous experiments were terminated at the end of 8 days, a time at which it is difficult to detect either the relatively slight effect of 25 μg of deoxyguanosine on thoracic development or even the eventually more marked shortening of the neck. The reasons why deoxyguanosine is cell toxic and consequently teratogenic are not known. That embryos can be protected against the effects of deoxyguanosine by supplementary treatment with cytidine has been reported by several investigators[2,10]. It has been suggested that deoxyguanosine inhibits the enzyme deoxycytidylate deaminase[2], and thereby interferes with DNA synthesis, but direct evidence for this inhibition is lacking. Deoxycytidylate deaminase has been shown to be inhibited in an *in vitro* test system by deoxyguanylic acid, however, and it is possible that deoxyguanosine becomes teratogenic only after phosphorylation *in vivo*[2]. We have found that the latter is not so effective a teratogen in our *in ovo* test system as is deoxyguanosine, but the possibility that permeability to the phosphorylated compound is reduced has yet to be examined.

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PSILOCIN: EFFECTS ON BEHAVIOUR AND BRAIN SEROTONIN IN MICE

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PSILOCYBIN and psilocin have recently been included on the list of psycho-active agents. Psilocin, or dephosphorylated psilocybin, has been structurally identified as 4-hydroxy-*N* : *N*-dimethyltryptamine[1]. Clinical reports have indicated that the hallucinogenic effects of psilocin in man are quite similar to those of LSD-25, but of shorter duration[2], and that its distribution in the brain and other organs is more uniform[3]. Other findings have indicated that psilocin is an analogue of 5-hydroxytryptamine and possesses certain serotonin and anti-serotonin properties[4].

Although a number of investigations have reported behavioural differences in laboratory animals to experimental serotonin alteration[5,6], and others have indicated brain amine variations following administration of psychotomimetic compounds[7], few multidisciplinary investigations have attempted to relate changes in brain serotonin and behaviour following psychotropic drug treatment in a single experimental design.

The present experiments were conducted to determine specific directional behavioural changes in mice to psilocin administration, to correlate these changes to possible directional variations in total-brain serotonin, and to separate the effects of psilocin on serotonin-levels from possible non-pharmacological effects.

Groups of 20 adult female mice from the highly inbred *C57BL/10J* strain were used as subjects. All experimental mice were maintained under rigid laboratory conditions with free access to Purina mouse chow and water until 90 days of age. At the beginning of experiments, subjects were matched for age and weight and then randomly assigned to experimental groups. Groups designated '*A*' and '*B*' received 10 mg/kg psilocin or an equal volume of placebo, respectively. Psilocin was dissolved in dilute hydrochloric acid and administered orally by means of a beaded-tip syringe. On each of five successive test days, groups received psilocin or placebo 1 h before testing. To evaluate possible carry-over effects as well as changes in performance following psilocin withdrawal, both groups received oral placebo during five additional days of testing. Naive groups were used in each behavioural test.

Psilocin-induced changes in behaviour were evaluated in the following tests: (1) shock-elicited escape-avoidance conditioning; (2) open field exploration; (3) locomotor activity wheel running. Details concerning apparatus and procedure have previously been presented[8].

Serotonin content (5-hydroxytryptamine, 5-HT) in individual whole brains was determined by the spectrofluorometric technique of Bogdanski *et al.*[9]. To ensure that serotonin measurement would not be confounded with systematic differences in daily runs, 5-HT-levels were evaluated from randomized treatment group samples on an individual day. Concurrent daily standards were run, and all scores corrected for daily percentage recovery. Recovery ranged from 87 to 93 per cent. Psilocin was run under identical spectrofluorometric conditions but it could not be detected.

Serotonin content in individual whole brains was determined for six groups of mice. Groups 1 and 6 were identically matched non-handled controls; these mice were obtained directly from laboratory stock and immediately killed. Brains from Group 1 subjects were analysed concurrently with those of the treatment groups, while Group 6 was added at the completion of the analysis to

further verify serotonin-levels in non-handled mice. Group 2 received 50 mg/kg psilocin. Group 3 received an equal volume of HCl vehicle. Group 4, designated sham placebo, received experimental manipulation alone without oral administration of either drug or vehicle. Group 5 received 5 mg/kg reserpine. With the exception of non-handled mice, all treatment subjects were returned to individual cages for 1 h before being killed. A total of sixty whole brain serotonin determinations were made.

Analyses of variance were used to detect differences in behaviour, while an unweighted means analysis of variance was used to assess the serotonin data in which there were slightly unequal sub-class numbers[10]. In addition, an extension of the Newman–Keuls' multiple range test was used to perform all intergroup serotonin comparisons (carried out in pairs)[11]. Unless otherwise noted, degrees of freedom for F-ratios were 1/190.

Table 1. SUMMARY OF MEAN BEHAVIOURAL PERFORMANCE FOR GROUP *A* (10 MG/KG PSILOCIN) AND GROUP *B* (PLACEBO)*

	Open field exploration				Avoidance conditioning			
	Latency (sec)		Squares traversed		Latency (sec)		Avoidance number	
Group	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Day 1	3·06	2 44	46 0	52·1	4·88	4·55	0·7	0·6
2	2·42	2·25	46·7	63·6	3 70	3 37	5·7	6·3
3	2·83	2·34	47·4	61·7	3·11	2·60	9·2	11·3
4	2·62	2·26	38 9	59·2	2·53	2·27	12·9	13·7
5	2 39	2·21	59·3	79·5	2·22	1·90	14·7	15·3
Mean	2·66	2·30	47·7	63·2	3·29	2·94	8·6	9·4
Day 6	2·88	2·37	70·5	64·8	2·12	2·21	15·3	14·5
7	2·71	2·05	76 6	66 4	2·10	2·06	15·7	15·3
8	2·94	2 32	66 9	66·2	2·11	1·91	15·7	15·3
9	2·81	2·14	72·3	61·3	1·94	2 02	16·6	16·8
10	2·10	2·14	69 9	64·2	2·22	2·15	15·3	15·3
Mean	2·69	2 20	71·2	64·6	2 10	2·07	15·7	15·8

* Both groups received oral placebo during days 6–10.

Table 1 summarizes the mean performance scores for open field exploration and avoidance conditioning for psilocin and placebo groups. During the first 5 days, psilocin-treated mice explored less in an open field than placebo mice ($P < 0{\cdot}0001$; $F = 17{\cdot}4$). On days 6–10, during which all mice received oral placebo, the exploration mean of Group *A* was higher than Group *B*. This reversal suggested that cessation of psilocin facilitated exploration performance. However, the statistical analysis did not reject the null hypothesis that this shift was a chance event ($P \simeq 0{\cdot}08$; $F = 3{\cdot}15$). Psilocin mice exhibited longer latencies to move from the middle square during the first week ($P < 0{\cdot}025$; $F = 5{\cdot}83$), as well as during the second week ($P < 0{\cdot}005$; $F = 9{\cdot}01$). Thus, the effect of psilocin on open field latencies persisted beyond drug withdrawal.

Both groups learned to avoid shock to an asymptotic level by 5 days of testing. However, mice which received psilocin traversed the runway more slowly ($P < 0{\cdot}001$; $F = 12{\cdot}6$). This 0·35 sec mean decrement was relatively constant during the 5 days of psilocin administration. During days 6–10, Group *A* performance did not differ from that of Group *B* ($P > 0{\cdot}5$; $F = 0{\cdot}3$). To distinguish whether the increased latency of psilocin-treated mice during the first week reflected a learning deficit or decreased locomotor ability, the mean number of avoidances per session were analysed for both groups. During the first 5-day period, Group *A* averaged 8·64 avoidances and Group *B* 9·44 avoidances. The difference between these averages was not significant ($P > 0{\cdot}25$; $F = 1{\cdot}17$). During the final 5-day period, the averages for Groups *A* and *B* were 15·7 and 15·8 avoidances, respectively. Whereas the analyses indicate that psilocin resulted in a decreased ability to traverse the runway, they did not indicate a differential effect on avoidance acquisition.

To evaluate the effect of psilocin and reserpine on spontaneous activity, four groups of mice were pretrained in activity wheels until a stable performance of 4,000 rev per 2 h period was attained. On each of five successive test days, treatment groups received oral administration of 50 mg/kg psilocin, 10 mg/kg psilocin, 5 mg/kg reserpine, or placebo 1 h before testing. Fig. 1 illustrates the group mean locomotor performance during five test days.

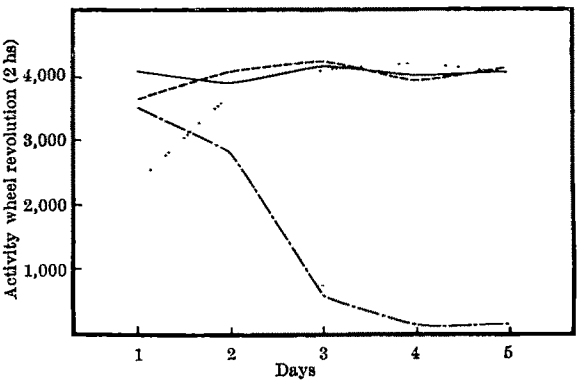


Fig. 1 Daily performance averages of spontaneous locomotor activity for four groups of mice administered psilocin, reserpine, or placebo. ———, placebo; – – –, psilocin, 10 mg/kg; ·····, psilocin, 50 mg/kg; – – –·–, reserpine, 5 mg/kg

Data analyses by simple-effects analyses of variance and multiple range tests indicated that the day 1 locomotor performance of both psilocin groups was significantly lower than placebo scores ($P < 0{\cdot}0001$; $F = 35{\cdot}3$, d.f. = 2/285). By the second day, groups which received psilocin regained asymptotic levels and did not differ from the control group ($0{\cdot}25 < P < 0{\cdot}5$; $F = 1{\cdot}02$, d.f. = 2/285). By contrast, the reserpine-treated group did not differ from controls on the first day of testing, but differed from the control group as well as psilocin-treated mice, beginning on the second day ($P < 0{\cdot}001$; $F = 11{\cdot}8$, d.f. = 3/380). These data indicate that psilocin administration produced decrements in locomotor performance on the initial treatment day, that the degree of decrement was a function of the dosage, and that psilocin had no effect on wheel running from the second day onward.

The results of the sixty serotonin determinations are summarized in Table 2. An unweighted means analysis of variance demonstrated highly significant differences in whole brain serotonin levels among the six groups ($P < 0{\cdot}0001$; $F = 46{\cdot}2$, d.f. = 5/54). Application of the Kramer–Newman–Keuls' multiple range test demonstrated that the mean serotonin-levels fell into three non-overlapping major groupings ($P < 0{\cdot}01$). Groups 4, 3, and 2 did not differ among themselves, but differed from all remaining groups. Both groups of non-handled controls did not differ between themselves, but differed from all other groups. In addition, the group which received reserpine differed from all other groups. The mean serotonin-level of the psilocin group did not differ from either the HCl or sham-placebo groups. By contrast, the serotonin content in both groups of non-handled controls was 11 per cent lower than sham-placebo, HCl, and psilocin scores. The analyses suggest that experimental handling alone may have been responsible for the significant elevation of 5-HT for Groups 4, 3, and 2, since one treatment condition common to these groups was experimental manipulation. The highly significant depletion of serotonin in Group 5 which received reserpine is consistent with many previous findings[12].

Some recent evidence has suggested that central nervous system amines are responsive not only to pharmacological alteration, but also to environmental stressors requiring extensive adaptation. Whereas Barchas and Freedman[13] reported an average 13 per cent increase in whole brain serotonin in rats exposed to prolonged forced activity or a 15 min cold swim, the present findings demonstrate that

Table 2. MEAN WHOLE BRAIN SEROTONIN-LEVELS (μG 5-HT/G WET TISSUE)

Treatment	Sham placebo	HCl vehicle	Psilocin (50 mg/kg)	Non-handled controls		Reserpine (5 mg/kg)
Group number	4	3	2	1	6	5
No. of subjects	10	10	9	11	11	9
5-Hydroxytryptamine	1·27	1 21	1·21	1·12	1·05	0·90

Group means underscored by a common line do not differ statistically among themselves, but differ significantly from remaining groups ($P < 0{\cdot}01$). Average standard error for treatment means equals 0 022 μg 5-HT/g wet tissue.

a comparable elevation in brain serotonin may result from relatively mild handling stress in mice. Since these results indicate that total brain serotonin content may be confounded with levels of adaptation or arousal, future pharmacological investigations which utilize neurohumour levels as dependent variables should incorporate adequate control procedures to separate possible treatment differences from placebo effects.

The behavioural findings observed in this investigation indicate that psilocin produced consistent decrements in open field exploration parameters and avoidance conditioning running, but did not appreciably influence avoidance acquisition. The data suggest that open field exploration was enhanced following psilocin withdrawal, and that latency decrements persisted. In addition, psilocin treatment was associated with a dose-dependent decrement in spontaneous locomotor activity on the initial day of treatment, with rapid tolerance development by day 2.

The comparison of the biochemical and behavioural findings is noteworthy in several respects. For example, the significant decrease in day 1 activity wheel performance of both psilocin-treated groups was not associated with a corresponding variation in endogenous whole brain serotonin. Thus, psilocin significantly altered behavioural performance without altering serotonin-levels. Conversely, the locomotor performance of mice administered reserpine did not differ from placebo mice on the first day of testing, although brain serotonin-levels were depleted 29 per cent. Thus, a dramatic change in brain serotonin-level was not associated with a concurrent alteration in behaviour. These results indicate a lack of correlated response between brain serotonin content and certain behavioural criteria.

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EVOKED POTENTIALS CORRELATED WITH A VISUAL ANOMALY

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An incoherent succession of randomly patterned optical images ('dynamic visual noise') offers a useful stimulus to probe the visual system and reveal any bias present in perceptual mechanisms[1–3]. The present investigation concerns the physiological correlates of a curious visual anomaly discovered some years ago when using a stimulus of this kind[4]. If each frame of such a visual noise sequence is followed by a short bright flash of light in the same eye, illuminating the same area of the visual field, then with a certain critical time interval between frame and flash, and at frame repetition rates of the order of 5–20 per sec, the random 'Brownian movement' normally perceived in the visual noise field is disrupted. In its place (or in addition), the screen appears to be filled with elongated objects resembling maggots, which seem to wriggle to and fro at random and link up to form long chains or chain-mesh patterns. The critical lag between the onset of each noise frame and the blank flash varies with frame duration, repetition frequency and intensity[5], but is typically of the order of 18–25 msec.

This is not the only visual anomaly that may be observed with such interlaced sequences of stimuli. With a somewhat longer time interval between frame and flash, Dr. J. P. Wilson[6] has reported that spurious impressions of contour motion are produced with stationary stimulus figures. A stationary black disk on a stroboscopically-illuminated white ground, for example, may appear to be continually expanding or contracting according to the time interval between the illuminating flashes and the blank flashes[6]. (Explanations in terms of eye movements are ruled out, for with a more complex field such as a black annulus one may see simultaneously a contraction of the inner edge and expansion of the outer.)

On both scores it seemed worth while to ask whether the occipital evoked potentials, as recorded from scalp electrodes, showed any corresponding anomalies at the inter-stimulus intervals giving rise to these phenomena. The preliminary investigation to be reported here indicates that they do, and appears to give evidence of a specific sensitivity, at the critical phase, to the presence of spatial patterning in the visual stimulus.

In place of the cine projector used in the earlier investigations[4,5] a high-powered electronic stroboscope was used to illuminate an area of 'static visual noise'—an enlarged photograph of sand paper, mounted on a turntable, and viewed through a circular (non-concentric) aperture. The turntable could be rotated asynchronously, so as to present a substantially unrelated sequence of visual noise samples when required[2]. A second stroboscope illuminated a plain white disk of the same size as the exposed area of visual noise, the two being optically superimposed by means of a half-silvered mirror. These fields subtended an angle of about 10° at the observer's eye. The repetition period and relative timing of the two stroboscopes were adjustable in steps of one msec.

Scalp electrodes were positioned as shown in Fig. 1, with the reference electrode 2 cm above the inion and inter-electrode distances of 5 cm, so that longitudinal (*A*) and transverse (*B*) components of the electric field could be simultaneously recorded and compared. Conventional electronic computing equipment (a Mnemotron '*CAT*'), triggered synchronously with the visual noise exposure, enabled the two evoked components *A* and *B* to be averaged on separate channels; a typical run lasted for 1 min and represented the sum of 500–1,000 responses.

To summarize the results (to be presented more fully elsewhere), the critical phase of our double-flash stimulation was indeed found to be accompanied by anomalies in the evoked potential. A sample run with a repetition period of 63 msec is shown in Fig. 1, where the number on the left of each pair of tracings represents the lag in msec between noise and blank flashes, and that on the right shows the order in which runs were made (subject A. F.). Since the perceptual anomalies in question are visible only at repetition rates above 5 or 6 per sec, their corresponding evoked potentials have a large continuous and oscillatory component, and the waveforms shown represent one complete cycle of a repetitive train. Slow changes in

form occur even under steady conditions, but in view of the order in which runs were made the general trend emerges clearly in Fig. 1. With a cycle period of 63 msec, the most striking change may be seen to occur in the transverse (*B*) component at values of lag between 13 and 23 msec, where the amplitude grows markedly and the phase also changes more rapidly than elsewhere. Striking also is the change in relationship between the longitudinal and transverse components, reflecting as it does a shift from virtual synchrony into near antiphase, and the appearance of a marked third harmonic in the longitudinal component. The psychophysically observed delay range for the 'maggot' effect in this case was 15–24 msec, with an optimum at 18 m.sec.

In order to disentangle the factors that might be responsible for the electrophysiological anomaly, a series of further experiments were performed. First, the rotating noise field was brought to rest, so that successive noise frames were identical. Subjectively, the effect at the critical phase was to replace the 'maggots' by a (much fainter) impression of a pulsating lace-work structure in the static noise pattern. The corresponding evoked potentials, however, showed remarkably little difference from those of Fig. 1, apart from a suggestion of an increased lag in the longitudinal component at the critical interval of 18 m.sec.

This raised the question whether the anomalies in the evoked potentials were related at all to spatial patterning in the stimulus, or whether only the temporal rhythm of the light entering the eye was responsible. Accordingly, the responses with the visual 'noise' pattern were compared with those obtained with a uniform grey surface of comparable overall reflectance, under two conditions: (1) blank flash following each frame at the critical interval for the 'maggot' effect; (2) blank flash preceding each frame by the same interval.

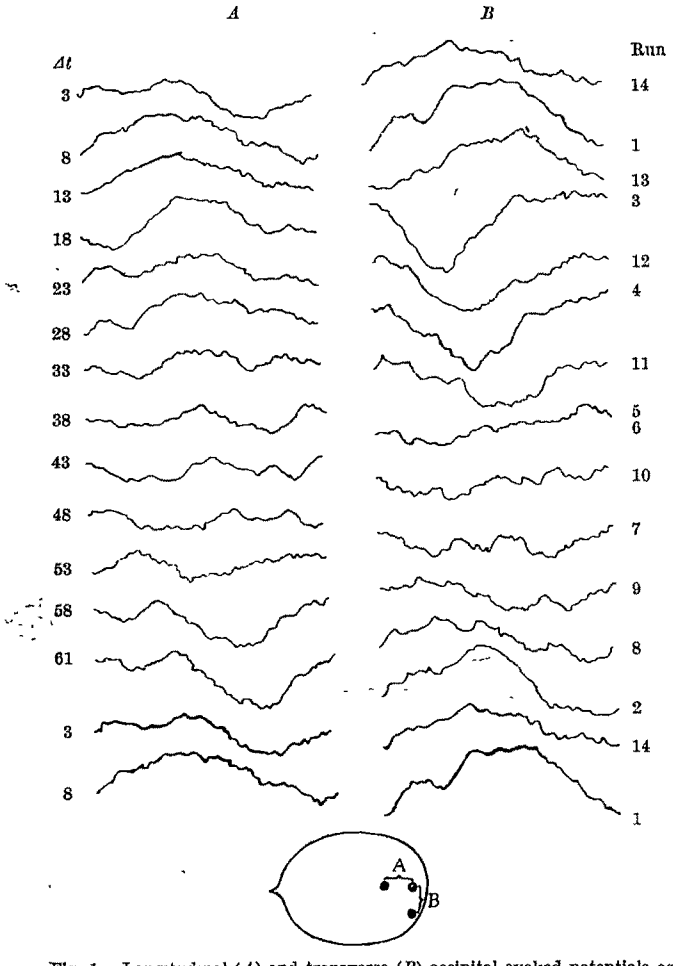


Fig. 1. Longitudinal (*A*) and transverse (*B*) occipital evoked potentials as a function of interval Δt between noise frame and blank flash. Intervals are shown on left; run numbers on right. Electrode placements were as indicated, with bipolar connexions. Period of stimulus cycle 63 msec

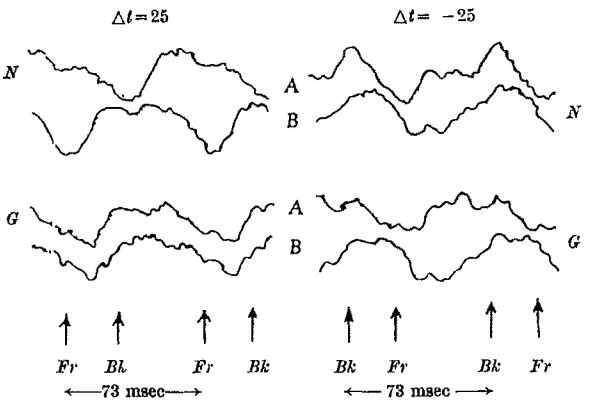


Fig 2. Effects of stimulus patterning on evoked potentials for $\Delta t = +25$ ('critical interval') and -25 msec. *N*, With noise field; *G*, with uniform grey field. Note that almost two complete cycles are shown, with a cycle period of 73 msec

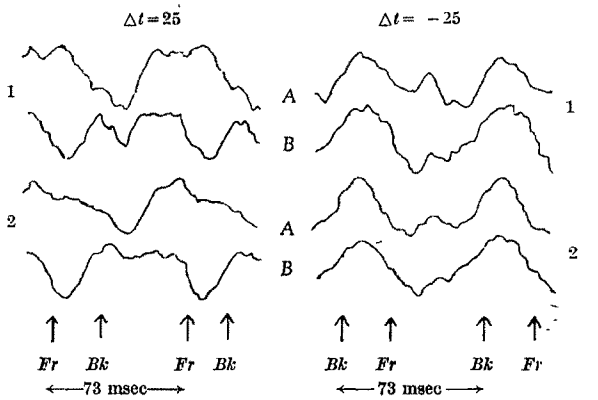


Fig 3. Control runs showing effects of intensity change on evoked potentials. Intensity for run (1) was greater by 0·5 log unit than for run (2)

The results with a repetition period of 73 msec, for which the critical interval (Δt) was 25 msec, are shown in Fig. 2, where the longitudinal (*A*) and transverse (*B*) response components in each case are presented one above the other. It will be seen that under the critical condition for the 'maggot' effect ($\Delta t = 25$) the change from noise (*N*) to uniform grey (*G*) made a marked difference to the phase relationship between the longitudinal and transverse components; but that when the blank flash (*Bk*) preceded the frame (*Fr*) by the same time interval ($\Delta t = -25$), the same change from noise to grey had a much smaller effect.

Though care had been taken to match the overall subjective brightnesses of noise and grey fields, the possibility that a brightness difference was responsible for the contrast in evoked potentials was checked by performing two runs with the same noise frame, but at frame brightness levels differing by approximately half a log unit. As will be seen from Fig. 3, the effects of this (subjectively large) change in brightness were both smaller than, and quite different from, those produced by the change from noise to patternless stimulation at subjectively constant brightness. It seems fair, therefore, to conclude that at the critical phase the evoked potentials show an enhanced response to the introduction of patterning as such into the visual field, and that this response takes the form of a change in the relative phase of longitudinal and transverse evoked potentials. Further work is needed, however, with more subjects, to test the generality of this conclusion.

It seems clear from these observations that stimulation of the visual system in specific uneven temporal rhythms calls out anomalous neural responses which are reflected

physiologically in a change of spatial and temporal activity patterns at the cortical level, and perceptually in the imposition of spurious structure on an otherwise incoherent visual field. Unlike the well-known 'stroboscopic patterns'[7] evoked by regularly repetitive flashes, which are most noticeable against a blank background, these perceptual effects are seen most clearly when optical contours are present in at least the first of each pair of stimuli. In this respect they resemble the familiar after-effect of visual image motion, which may be interpreted as due to the activity of a sub-system responsible for signalling motion-as-such, the output of which is perceptually 'attributed' to whatever structure is present in the visual field. In the absence of a structured stimulus the effect has only the residual entoptic irregularities on which to manifest itself.

The suggestion here would be that the spurious contours imposed on incoherent stimuli at the critical phase may result from the excitation of a sub-system the elements of which are normally set to detect local contours in the retinal image[8], but at the critical phase (as the result of a kind of resonant response to the rhythm of the stimulus) become abnormally excitable. A similar temporal rhythm-sensitivity in the motion-detecting sub-system could account for Wilson's effect[6].

The results shown in Figs. 2 and 3, though far from conclusive, seem to be in line with this suggestion, since they indicate that a major change in the sensitivity of cortical activity to temporal rhythm in the stimulus occurs when a spatially patterned stimulus is substituted for a patternless one of similar overall luminance. The fact that no similar effects are observed when the frame and flash are presented to different eyes would suggest that the mechanisms involved are fairly near the periphery of the visual system.

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ACTIVE TRANSPORT OF GLUCOSE BY SUSPENSIONS OF ISOLATED RAT INTESTINAL EPITHELIAL CELLS

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THIS article presents data on the active transport of glucose into the isolated, intact, functioning rat intestinal epithelial cell. Much of our knowledge of the mechanism of amino-acid and sugar transport in the intestine has been derived from observations with various types of *in vivo* and *in vitro* intestinal preparations[1,2]. The use of suspensions of isolated intestinal epithelial cells prepared by the method developed by Harrer, Stern and Reilly[3] affords an advantage over other preparations in that they are uncontaminated by mesenchymal elements and are free of the normal homoeostatic mechanisms that operate when the cells are organized into a tissue. Huang[4] recently reported on the energy-dependent uptake of L-tyrosine and 3-*O*-methyl glucose by intestinal epithelial cell suspensions from the rabbit. Our method appeared to produce much purer cell preparations than did that used by Huang, as his cells were obtained after chopping up the entire intestine, incubating with lysozyme, filtering through gauze and washing. It is likely that his cell preparations were contaminated with other types of cells and debris. With the method we used, only cells from the lumen of the intestine were obtained, and careful microscopic examination revealed that the basement membrane of the intestine was intact.

Suspensions of rat intestinal epithelial cells were prepared as described by Harrer, Stern and Reilly[3] and Stern and Reilly[5] with the following modifications: citrate was used to dissociate the epithelial cells from the mucosa. The composition of the medium was: 0·096 M sodium chloride, 0·008 M potassium phosphate, 0·027 M sodium citrate, 0·0056 M dibasic sodium phosphate, 0·0015 M potassium chloride—final *p*H 7·2. 15,000 U. of penicillin and streptomycin were added per 100 ml. of all solutions used. The lumen of the small intestine was filled with this solution and incubated for 11 min at 33° C. All subsequent operations were carried out at 0°–4° C unless otherwise indicated. This solution was removed following the incubation and either a solution composed of 0·137 M sodium chloride, 0·0115 M potassium phosphate, 0·008 M dibasic sodium phosphate, 0·0022 M potassium chloride—final *p*H 7·2, or 0·2 M sucrose, 0·05 M sodium potassium phosphate, *p*H 7·4 (sodium ion = 0·076 M potassium ion = 0·019 M), was washed through the lumen of the intestine. The cells were released from the mucosa by gently pressing the filled intestine with one's fingers. The cells were suspended in 40 ml. of either the inorganic salt solution or the sucrose phosphate solution and centrifuged at 500*g* for 10 min. 0·4–0·8 ml. of packed cells were suspended in 7·7 ml. of one or other of the foregoing media. To this suspension was added 30 μg of crystalline DNase (Nutritional Biochemical Corp.), 0·07 ml. of 0·25 M D-mannitol-1-^{14}C, 0·02 ml. of 0·4 M $MgSO_4$ to a final volume of 7·82 ml. D-Mannitol-1-^{14}C was used to measure extracellular space since it is not absorbed by the intestine[6]. DNase was added to reduce the viscosity of the reaction mixture. Bare nuclei, and some lysed cells and mucus contributed to the relatively high viscosity. The initial glucose content of the cells, as determined on a portion of the reaction mixture, was negligible. The reaction mixture was transferred to a Warburg flask for the incubation. When glucose uptake was measured in the balanced salt solution, 0·11 ml. of 0·0762 M glucose was added from the side arm. When 4,6-dinitro-*o*-cresol was present, the reaction mixture was pre-incubated for 5 min under argon before the glucose was introduced. The cell suspension was then incubated for 15 min with continuous gassing with oxygen or argon and while shaking slowly. When sucrose phosphate was used as the suspending medium, no glucose was added. After incubation, the suspension was transferred to a McNaught tube (A. S. Aloe Co., St. Louis, Missouri), dispersed by forcing through a 14 gauge needle, centrifuged at 270*g* for 20 min, re-dispersed and centrifuged at 1,100*g* for 15 min. The supernatant was removed and the volume of the cell pellet read with an accuracy

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of ± 5 μl. The inside of the tube above the pellet was wiped many times alternately with damp and dry swabs. The supernatant and cell pellet were diluted with water and the glucose and mannitol extracted by heating in a boiling-water bath for 5 min and the solids removed by centrifugation.

Glucose was determined by the glucose oxidase method ('Glucostat Reagent' from Worthington Biochemicals). When 4,6-dinitro-*o*-cresol was present, appropriate corrections were made for the absorbancy produced by this compound at 420 mμ, the wave-length at which the colour produced in the glucose oxidase reaction is read. The extracellular volume of the packed cell pellet was determined by counting aliquots of the supernatant and cell pellet dissolved in a liquid scintillation mixture in a Nuclear Chicago liquid scintillation counter. The extracellular volume ranged from 83 per cent to 95 per cent. Crane and Mandelstam[2] have reported the extracellular volume of packed villi from hamster intestine to be about 80 per cent.

The millimolar concentration of glucose in the cells was calculated on the basis of the following equation as used by Crane[2]:

$$\text{mM glucose cells} = \frac{\text{Total glucose content of pellet } (\mu\text{moles}) - \left(\text{extracellular space} \times \text{pellet volume} \times \text{mM medium glucose}\right)}{(1 - \text{extracellular space})\,(\text{pellet volume})}$$

The data in Table 1 show that the cells accumulate glucose against a concentration gradient by a process which is energy dependent. The accumulation of glucose varied with different cell preparations which is as expected, and the rate of glucose transport varies along the length of the intestine. Cells were removed from the entire small intestine and in separate experiments various proportions of cells from different regions of the intestine were obtained. Glucose utilization by the cells is much slower than glucose transport. 4,6-Dinitro-*o*-cresol is known to inhibit aerobic phosphorylation[7]. When available cell energy supplies were reduced by anaerobiosis and the presence of 4,6-dinitro-*o*-cresol, transport was inhibited.

We have previously reported on some characteristics of the respiratory metabolism of intestinal epithelial cells suspended in a sucrose–phosphate medium[5]. The sucrose

Table 1. ACTIVE TRANSPORT OF GLUCOSE BY INTESTINAL EPITHELIAL CELLS

Exp. No.	Experimental conditions	Glucose concentration medium (mM)	Glucose concentration cells (mM)	Glucose concentration cells / Glucose concentration medium
(1)	Oxygen	0·8	2 0	2·5
(2)	Oxygen	0·7	5 4	7·7
(3)	Oxygen	0·8	5·0	7·4
(4)	Oxygen	0·8	20 2	25·4
(5)	Argon + 10^{-3} M dinitro-*o*-cresol	0·8	0·5	0·6
(6)	Argon + 10^{-3} M dinitro-*o*-cresol	0·9	1 0	1·1
(7)	Argon + 10^{-3} M dinitro-*o*-cresol	0·9	0 9	1·0

Experiment details are given in the text. The initial glucose concentration of the medium was 1 mM. The cell suspension was incubated at 36° C.

Table 2 GLUCOSE UPTAKE BY INTESTINAL EPITHELIAL CELLS SUSPENDED IN SUCROSE-PHOSPHATE MEDIUM

Exp No.	Cell glucose (mM)	Medium glucose (mM)	Cell glucose / Medium glucose
(1)	79·8	11·5	6 9
(2)	73·0	11·3	6·5
(3)	217·0	4·4	49·3
(4)	42·0	0·7	60·0

Experiments (1) and (2) were carried out at 30° C. Experiment (3) was carried out at 36°. In experiment (4), 0 0058 M sucrose was added to the medium used for cell dissociation and the incubation period in the Warburg was omitted. Other experimental details are given in the text.

was rapidly hydrolysed by invertase present in the brush borders of the cells. It was of interest to see whether the cells would accumulate the glucose formed against a concentration gradient. The results given in Table 2 show that this is indeed the case, though some of the glucose leaked out of the cells or was released from cells that lysed.

Experiments (1) and (2) were carried out at 30° C, while experiment (3) was carried out at 36°. There was a much greater accumulation of glucose at 36° than 30°. In experiment (4) the accumulation of glucose by the cells during cell dissociation was measured. The sodium citrate salt solution contained sucrose. For experiment (4) the cell suspension was centrifuged without any incubation period. The results show that the cells do accumulate glucose against a concentration gradient during cell dissociation and they retain this glucose during centrifugation.

In other experiments not shown, sodium was omitted from the sucrose phosphate medium and glucose uptake measured after 15 min incubation. The cells still accumulated glucose against a concentration gradient. It is not known whether all the sodium ions were washed out as the cells were removed in a sodium-containing medium. Further experiments will be carried out to determine whether the uptake of glucose in the sucrose–phosphate medium is an energy-requiring process.

The results presented in this article indicate that the intact, isolated, intestinal epithelial cells can accumulate glucose by a process that depends on a normally functioning aerobic metabolism. The information obtained here and that which will result from further experimentation will shed much light on the mechanism of cellular transport.

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BIOPHYSICAL STUDIES OF A RHINOVIRUS

Ultracentrifugation and Electron Microscopy

THE picornavirus group includes viruses which are very small, ether resistant and contain ribonucleic acid[1]. The rhinoviruses form a sub-group of the picornaviruses[1]. The properties that characterize rhinoviruses are acid lability and optimal growth at 33° C in rolled cultures containing medium with almost neutral *p*H rather than stationary cultures with medium of slightly alkaline *p*H. Some physical properties of rhinoviruses were described by Dimmock and Tyrrell[2,3] and Ketler *et al.*[4]. Using indirect methods, they showed that the size, density and type of nucleic acid were the same as the other picornaviruses. In this and the following article we report further investigations of these biophysical properties by more direct methods. In addition, methods are described for the production of high titre rhinovirus and its partial purification.

The strain of rhinovirus *HGP*[5] used was grown from a terminally diluted preparation. Diploid human embryo lung fibroblast cell strains were used for production of some virus pools and for all assays of infectivity. The cell

strains used were established in this laboratory by Mrs. P. K. Brown. In addition, a line of *KB* cells was obtained from Dr. M. A. Mufson (National Institutes of Health, Bethesda, Maryland) and used for the production of high-titre virus.

Both types of cell were grown in 90 per cent Eagle's minimal essential medium containing 1·1 g/l. of sodium bicarbonate, 100 U./ml. of penicillin *G*, 100 μg/ml. of streptomycin and 100 U./ml. of neomycin and 10 per cent inactivated calf serum. The maintenance medium was identical to the growth medium except that 0·5–2 per cent inactivated calf serum was used.

Production of High Titre Virus

For the success of this work it was necessary to increase the yield of virus per culture. In the first experiment the material collected from one set of cultures was passed serially, without dilution, into further cultures. In addition, we reduced the volumes of medium in the culture vessels (namely, 6 oz. medical flat or 8-oz. baby's feeding bottles, the latter with cells on all sides). Culture fluids and cells were gathered at the time of maximum cytopathic effect, namely 2–3 days after inoculation. The material collected was subjected to cycles of freezing and thawing and sonication to release virus from the cells. Freezing and thawing and sonication resulted in an increase in titre of about $10^{0.5}$ TCD_{50}/ml. only when the cells had not been completely disrupted by the virus. Virus titred to about 10^7 TCD_{50}/ml. in contrast to the titres of about 10^5 TCD_{50}/ml. obtained previously. About this time the virus was adapted to grow in *KB* cells. With a multiplicity of infection of about two infectious units per cell stationary *KB* bottle cultures consistently produced pools with titres of 10^6–10^7 TCD_{50}/ml. Titres were increased by inoculating virus at a multiplicity of about five. By collecting after 1–2 days' incubation and with repeated passages at a high multiplicity, titres of 10^9 TCD_{50}/ml. were achieved. Neutralization tests showed that the virus propagated was in fact *HGP*.

Use of Aluminium Phosphate for Concentration and Purification

Aluminium phosphate was prepared and supplied by Dr. Karl Fantes (Glaxo Laboratories, Ltd.). Fantes[6] has described the use of aluminium phosphate for the concentration and purification of poliovirus. Fantes and Tyrrell (unpublished) found that it was possible to adsorb rhinoviruses to aluminium phosphate. The conditions for the absorption to and elution from aluminium phosphate of *HGP* have now been investigated. The following have been found to give the best results:

(*a*) A final concentration of 0·9–1·0 mg/ml. of aluminium phosphate adsorbs about 95 per cent of the virus from tissue-culture fluids pretreated with fluorocarbon or grown with low concentrations (0·5 per cent) of serum. In the presence of greater amounts of cellular protein or serum more aluminium phosphate is required. Adsorption is performed at a *p*H of 6·8–7·0 at the temperature of melting ice for about 2 h; the mixture is stirred magnetically. The *p*H is adjusted with either 0·1 M sodium phosphate buffer or N/10 hydrochloric acid. In the case of the latter, care must be exercised to add the acid slowly in order to avoid producing a local high concentration of acid which would inactivate the virus.

(*b*) After adsorption the complex of virus and aluminium phosphate is pelleted by centrifugation at 600*g* for 15 min and resuspended in not less than 1/20th of the original volume of 0·5 M sodium phosphate buffer of *p*H 7·9–8·0. Under these conditions and with intermittent shaking, almost all the adsorbed virus elutes at 4° C in 15–30 min. Aluminium phosphate is removed by centrifugation.

Purification and Concentration of a Rhinovirus

About 300 ml. of virus suspension derived from human embryo lung diploid fibroblast cell cultures was filtered through a sintered glass filter to remove cellular debris and ensure sterility. The filtrate was adsorbed with 30 ml. of a suspension of aluminium phosphate gel (containing 9·0 mg/ml. aluminium phosphate) as already described and eluted into 150 ml. of sodium phosphate buffer. The eluted virus was deposited by centrifugation at about 78,000*g* for 3 h at 4° C. The deposit was resuspended by rubbing with the tip of a sterile glass rod and sonication. The virus was finally resuspended in Hanks's saline containing 1 mg/ml. gelatine as a stabilizing agent. The virus suspension was blended with an equal volume of fluorocarbon ('Arcton 113'—Imperial Chemical Industries, Ltd.) for 2–4 min at ambient temperature and the aqueous and fluorocarbon layers were separated by centrifugation at 600*g* for 15 min. Caesium chloride was added to the aqueous layer. The solution was then centrifuged in an M.S.E. 'Superspeed 40' preparative ultracentrifuge in the 3 × 5 ml. swing-out rotor for 38 h at 4° C at about 111,000*g*. No brake was used. By this means an equilibrium density gradient was established. Fractions of 0·2 ml. were removed from the meniscus, from each centrifuge tube. One tube was used for infectivity measurements, one for density measurements and the third for electron microscopy. All these manipulations were performed without delay.

Densities of the caesium chloride gradient fractions were calculated from their refractive indices measured in an Abbé refractometer at room temperature, using the formula: $\rho 25 = 10{\cdot}8601.\ \eta_D^{25} - 13{\cdot}4974$ where $\rho 25$ = density at 25° C, η_D^{25} = refractive index of the *D* line of sodium at 25° C. The reading of refractive index was corrected to 25° C from room temperature using the correction of 0·0001 refractive index units for every degree centigrade difference in temperature.

Titrations of infectivity at the various stages of purification and of all the fractions from the density gradient were carried out by the microplaque technique[7].

Fractions from the gradient were examined in a Siemens 'Elmiskop I' electron microscope at an accelerating voltage of 80 kV with 200μ condenser and 50μ objective apertures. Fractions for electron microscopy were prepared using Harris's vibrating reed technique[8]. After the samples had dried on the carbon-coated 'Formvar' grids they were washed in distilled water to remove caesium chloride and then stained with 0·5 per cent phosphotungstic acid at *p*H 6·8 (ref. 9). Survey examinations were carried out at magnifications of 20,000 and micrographs taken at 80,000. The results obtained are shown in Figs. 1 and 2.

A gradient was produced which had a range of density of 1·264–1·459. Virus-like particles were seen in fractions with a density of 1·307. They were associated with no infectivity. A large number of virus-like particles were seen in the fraction with a density of 1·404. Most of the infectivity was detected at this point.

Similar results were obtained in a second experiment which differed from the first experiment in the following particulars. The virus suspension was grown in *KB* cells and cellular debris was removed by centrifugation at 600*g* for 15 min. Before adsorption to aluminium phosphate the tissue-culture fluid was blended with an equal volume of fluorocarbon. Aluminium phosphate was added to the aqueous layer to a final concentration of 2·0 mg/ml. and the mixture stirred for 2 h. The aluminium phosphate and adsorbed virus were deposited by centrifugation and eluted for 2 h at 4° C. An equal volume of Hanks's saline containing gelatine was added to the eluate, which was clarified by centrifugation at 7,000*g* for 15 min. After deposition and resuspension of the virus, and treating it with fluorocarbon, 6·3 g caesium chloride was added to 15 ml. of virus suspension. The mixture was centrifuged to equilibrium at 111,000*g* for 24 h. Fractions were removed as before, but instead of keeping three sets of fractions (one from each centrifugation tube), equivalent fractions were pooled and 0·2 ml. of each pool was put into 1·8 ml. of Eagle's minimal essential medium con-

taining 2 per cent heat-inactivated calf serum and kept for infectivity measurements. A gradient of 1·314–1·490 was achieved. Once again two groups of particles were seen in the electron microscope and these occurred at about the same densities as in the previous experiment. A single peak of infectivity was found in the fraction with a density of 1·401.

In both experiments infectivity was lost during the density gradient run. It has been found in other experiments that *HGP* is unstable in caesium chloride solutions. Measurements of the diameter of virus particles (Table 1) confirm the value previously found by ultrafiltration[3].

Poliovirus has a buoyant density of 1·32 (ref. 10). Because of the similarity in size and the fact that the sedimentation rates are identical[3], it has been assumed that *HGP* must have a density of the same order as poliovirus. However, direct measurement of the buoyant density in the present work seems to indicate that *HGP* is more dense than poliovirus. Indirect evidence indicates that capsomeres of *HGP* are not held together so tightly as those of poliovirus. For example, the acid lability[3] and the sensitivity of *HGP* to urea[11] contrast with the relative stability of poliovirus under these conditions[3,12]. Thus it is possible that caesium chloride can penetrate *HGP* more readily than poliovirus and by this means increase its buoyant density. The density of the non-infectious *HGP* was much nearer that observed for poliovirus[10]. The differences in density observed between infectious and non-infectious *HGP* are much greater than observed for other picornaviruses, for example, echovirus 11 (ref. 11) and echovirus 12 (ref. 13). It is interesting to note that

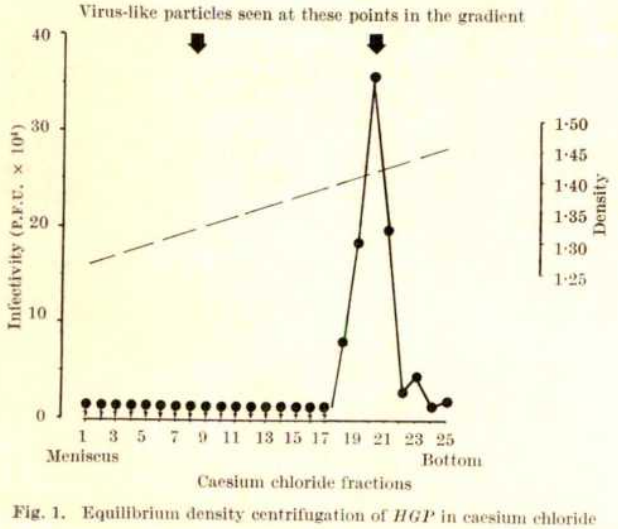


Fig. 1. Equilibrium density centrifugation of *HGP* in caesium chloride

Table 1. Measurements of the Diameter of *HGP* Virus Particles from Electron Micrographs

Experiment	'Full' No. counted	'Full' Mean ± 3 *S.D.*	'Empty' No. counted	'Empty' Mean ± 3 *S.D.*
1	9	315 ± 16 Å	6	302 ± 27 Å
2	40	295 ± 10 Å	35	304 ± 14 Å

S.D. = standard deviation of the distribution of particle diameters.

after staining with phosphotungstate, samples of the high- and low-density fractions both contained approximately equal numbers of apparently 'full' and 'empty' particles. This would suggest that some particles appear 'empty' because they are permeable to phosphotungstic acid and not because they lack ribonucleic acid.

The micrographs obtained so far reveal little of the substructure of the virus, although it has been reported that another rhinovirus probably has icosahedral symmetry[14].

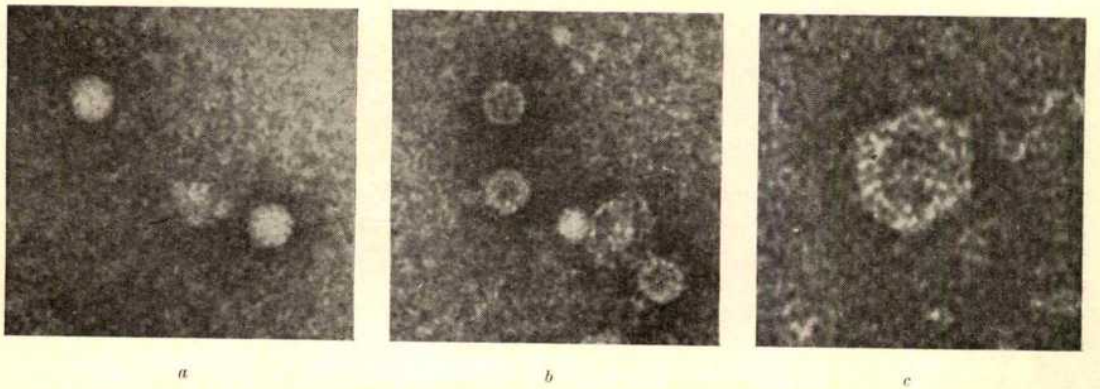
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Extraction and Assay of Infectious Ribonucleic Acid

It is believed that all picornaviruses contain infectious ribonucleic acid (RNA), which has indeed been extracted from many enteroviruses. Rhinoviruses are also classified within the picornavirus group and it has been shown that halogen-substituted pyrimidine analogues failed to inhibit their replication. It is therefore assumed that they also contain RNA. Detailed accounts of the extraction and assay of infectious RNA from representatives of the polioviruses, coxsackieviruses and echoviruses have been given[1,2], but there is only a brief report[3] on the recovery of infectious RNA from one of the rhinoviruses (*C.V.*11), which grows only in human cells (an *H* strain). This article presents direct evidence that the rhinovirus *HGP*,



a *b* *c*

Fig. 2. Phosphotungstate preparation of rhinovirus particles. (*a*) Typical 'full' particles (× 200,000); (*b*) typical 'empty' particles, one of which is apparently unfolded (× 200,000); (*c*) a higher magnification of an 'empty' particle showing a hexagonal outline and a roughly circular inner cavity

which grows in both human and monkey cells and is therefore an *M* strain, possesses infectious RNA. Its extraction from infectious tissue-culture fluid and its assay are described and it is demonstrated that its infectivity has properties that are quite different from those of the original virus.

HGP was grown in monolayers of *KB* cells held stationary at 33° C (ref. 4). The majority of cells showed cytopathic effect within 48 h. Culture fluids were collected, subjected to ultrasonics and clarified by centrifugation at 500*g*. The virus titred about $10^{6.7}$ $T.C.D._{50}$/ml. in a sensitive human embryo lung (*HEL*) cell strain.

Before extraction the virus was mixed with washed bentonite (Hopkin and Williams, Ltd., Chadwell Heath), which has been shown to inhibit the action of contaminating ribonuclease[5]. The quantity of bentonite used (about 8 mg/ml.) was sufficient to preserve poliovirus RNA infectivity in the presence of non-infectious culture fluid prepared in parallel with virus. The extraction process was a modification of the cold phenol method of Gierer and Schramm[6] and was carried out so far as possible at 4° C.

Ethylenediamine tetraacetic acid ('AnalaR', B.D.H., Ltd., Poole) was added to the virus to a final concentration of 0·001 M before extracting with phenol. Bentonite was present at all times. Dissolved phenol ('AnalaR') was removed with freshly distilled ether ('AnalaR') and the ether blown off with nitrogen. The extract was stored with bentonite at −70° C.

The choice of cells for an infectivity assay was governed by their availability. The 'Bristol' HeLa line (obtained from Dr. S. K. R. Clarke), in which the assay of the infectious RNA of poliovirus had been studied in some detail, was chosen. The cells were grown in Hanks's balanced salt solution containing 10 per cent heat-inactivated rabbit serum, 0·1 per cent lactalbumin hydrolysate, 0·1 per cent sodium bicarbonate and antibiotics. As an assay for infectious RNA is highly specific for a particular cell system[2], the poliovirus RNA-'Bristol' HeLa system was used without alteration for the assay of *HGP* phenol extract. However, *HGP* virus grew poorly in monolayers of these cells held stationary at 33° C and did not produce a visible cytopathic effect. The presence of virus was detected by inoculating HeLa culture fluid into *HEL* monolayers which were rolled at 33° C.

The infection of cells was based on the method evolved by Alexander[1] where the phenol extract was suspended in hypertonic saline. HeLa monolayers in 50-mm plastic Petri dishes (Grade *A*, Esco (Rubber), Ltd., London) were washed twice in Hanks's balanced salt solution containing 0·06 per cent sodium bicarbonate and no calcium and magnesium ions, and a third time in the same medium containing 0·5 M sodium chloride. Bentonite was removed from the *HGP* phenol extract by centrifuging at about 500*g* and the extract was suspended in M sodium chloride in 0·01 M *tris* (hydroxy-methyl) amino methane (*p*H 7·1). Volumes of 0·2 ml. of serial two-fold dilutions were inoculated on to the monolayers for 10 min at room temperature. 5 ml. of 2 per cent heat-inactivated calf serum in Eagle's minimum essential medium with antibiotics was added at the end of this time and the dishes were incubated at 33° C for 4 days in a humidified atmosphere of 5 per cent carbon dioxide in air. The fluids of each pair of monolayers were then pooled and 0·5 ml. was inoculated into each of three *HEL* monolayer tubes. A cytopathic effect in one or more monolayers was taken to indicate that the original extract was infectious. The cytopathic effect resembled that of *HGP*. Infectivity of the phenol extract was abolished by incubation for 15 min at room temperature with 10 μg/ml. ribonuclease (L. Light and Co., Ltd., Colnbrooke). The virus which grew up in *HEL* monolayers was neutralized by antiserum to *HGP*.

The titre of the extract was usually about 4 $\log_{10}$ less than that of the original virus. Other phenol extracts of picornaviruses which have been titrated in a similar manner by other workers showed a comparable loss of infectivity[1,2]. The infectivity of *HGP* phenol extracts persisted without loss for more than 2 months at −70° C.

An attempt was made to neutralize infectivity of the phenol extract to determine whether or not it was associated with active virus antigen. Phenol extract or virus of the same titre was mixed with *HGP* monkey antiserum (*B.W.*195) or phosphate-buffered saline and was incubated at room temperature for 30 min. An equal volume of phosphate-buffered saline or 2 M sodium chloride in 0·01 M *tris* was added to the virus mixtures which were assayed respectively in *HEL* monolayer tubes or exactly as phenol extract. Virus infectivity, assayed by either technique, was completely neutralized and the infectivity of the extract was not affected (Table 1).

The failure to neutralize the infectivity of the extract could have been caused either by damaged antigen, possibly denatured by phenol, or by absence of the antigen. If the latter were correct the infectivity of the extract would sediment at a slower rate than *HGP*. The rate of sedimentation of the extract suspended in phosphate-buffered saline was determined by centrifuging at 75,000*g* for 30 min in the swing-out head of the M.S.E. 'Superspeed 40' preparative ultracentrifuge. (The suspension contained bentonite which would be pelleted within the first minute of centrifugation.) At the end of the experiment, consecutive 0·5 ml. fractions were taken from the top of the tube and immediately placed in bentonite. Table 2 shows that the titre of all fractions was 0·3 $\log_{10}$ less than that of the uncentrifuged control. This may have been caused by aggregations, which would sediment more rapidly, or by infectivity being carried down with the bentonite. Otherwise, the infectivity of the extract was not sedimented appreciably. In comparison, after centrifugation by the same method in a concentration gradient of 0–9 per cent sucrose, all virus infectivity was found in the lower half of the tube. It is concluded that the infectivity of the phenol extract is associated with little virus antigen.

In summary, the infectivity remaining after extraction of *HGP* with cold phenol was about 4 $\log_{10}$ less than the original virus. It was inactivated by ribonuclease, was unaffected by antiserum which neutralized the virus and was not sedimented under conditions which sedimented the virus. Culture fluids from HeLa cells inoculated with the extract produced a cytopathic effect in *HEL* cells resembling that of *HGP*. The resulting infectivity was neutralized by antiserum to *HGP*. It is concluded that the infectious agent was *HGP* ribonucleic acid.

Table 1. Effect of *HGP* Antiserum on the Infectivity of an RNase-sensitive Phenol Extract of *HGP*

	Virus				Phenol extract	
	Titrated as virus†		Titrated as phenol extract‡			
	+anti-serum*	+PBS	+anti-serum	+PBS	+anti-serum	+PBS
Titre ($\log_{10}$ $T.C.D._{50}$/0·2 ml.)	<0·3	1·4	<0·3	1·5	2·1	2·1

*Antiserum diluted 1/5 in phosphate-buffered saline (PBS) and mixed with virus or phenol extract immediately. Controls mixed with PBS only. Incubated at room temperature for 30 min. An equal volume of PBS was added to the virus mixtures (†) which were titrated conventionally in *HEL* monolayers. An equal volume of 2 M sodium chloride in 0·01 M *tris* buffer *p*H 7·1 was added to the other virus mixtures (‡) and to the phenol extract mixtures. Both were titrated as phenol extract in HeLa monolayers as described in the text.

Table 2. Centrifugation of an Infectious, RNase-sensitive Phenol Extract of *HGP* at 75,000*g* for 30 min

Fraction (taken from the top)	Centrifugation of phenol extract*		Centrifugation of virus†	
	Titre ($T.C.D._{50}$/0·2 ml.)	Reduction of titre	Reduction of titre	
1	1·4	0·3	>1·8	
2	1·4	0·3	N.D.	
3	1·1	0·6	>1·8	
4	1·1	0·6	0·9	
5 + 6	1·4	0·3	0·1	
7	N.D.		−0·1	
Uncentrifuged	1·7	—	—	

* Suspended in 3·5 ml. phosphate-buffered saline containing bentonite.
† Suspended in 3·5 ml. of sucrose forming a concentration gradient from 0–9 per cent.
All measurements of titre are expressed as $\log_{10}$.
N.D. = not done.

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EXPERIMENTAL TRANSMISSION OF A KURU-LIKE SYNDROME TO CHIMPANZEES

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A CLINICAL syndrome astonishingly akin to kuru in man has developed in three chimpanzees from 18 to 21 months after intracerebral inoculation with brain suspension from different kuru patients. This fatal syndrome with progressive cerebellar ataxia and incoordination has not been seen as a spontaneous disease of apes, and is the first convincing indication of the transmissibility of one of the sub-acute or chronic human central nervous system diseases under investigation in our programme.

In an earlier communication[1] we described our programme of work on the possible infectious aetiology of sub-acute and chronic human central nervous system diseases and presented negative results on isolation and experimental transmission following 19 months of observation. The purpose of this article is: (*a*) to present details of the apparent transmission of kuru to chimpanzees and of our present efforts at virus isolation from tissues from these animals and from kuru patients (Table 1); (*b*) to provide further information and generally negative results on our expanded experimental studies after an additional 17 months of observation, that is, at 3 years after initiation of primate inoculations (Table 2). Because of continuing reports from other laboratories[2] of transmission of chronic progressive nervous system disease to animals from certain of the neurological diseases under investigation, this second progress report of our negative results, with the notable exception of kuru, is in order.

A year ago at a 'workshop-symposium' on "Slow, Latent and Temperate Virus Infections", held at this laboratory[3], details of the inoculation of laboratory animals, particularly primates, and tissue cultures with tissue from patients suffering from a number of chronic neurological diseases were presented. The diseases under study included kuru, amyotrophic lateral sclerosis, parkinsonism, parkinsonism-dementia, sub-acute inclusion encephalitis (Dawson's), myasthenia gravis, multiple sclerosis, Schilder's disease, progressive multifocal leucoencephalopathy, and necrotizing encephalitis. However, at that time there was still no evidence of a progressive degenerative nervous system syndrome developing in any of the primates under observation[4,5].

Our techniques have emphasized the collection and optimal preservation of blood, brain and other tissues at early autopsy or by surgical biopsy procedures for animal and tissue culture inoculation, for cultivation of live cells from explants, or for use as sources of antigens. Suspensions of these tissues are then inoculated into a variety of primates and other experimental animals, as well as an extensive array of tissue and cell cultures. Animals are held in isolation and observed for periods of more than 5 years. Additional virological techniques, for example, blind passages, the search for sero-conversions by estimation of antibodies to known viruses, and the search for viral antigens or reagents in tissues and tissue cultures by a variety of immunological methods, including fluorescent antibody, are being used in an effort to detect the presence of infectious entities.

The intracerebral inocula used in and the date of inoculation of the three chimpanzees which have developed kuru-like clinical syndromes, and the data on the remaining five animals still under observation without illness are summarized in Table 1. It is noteworthy that the incubation period in the three affected animals has ranged only between 18 and 21 months and that the course to killing in the terminal stages has been 5 and 9 months in the first two animals. Most remarkable, however, is the close similarity in the clinical appearance of the syndrome in the chimpanzee to kuru in human patients. To document and preserve this clinical record extensive use has been made of the 'research cinema film' technique[6], and several research films are in preparation documenting the progress of the disease in chimpanzees[7].

First chimpanzee (Georgette, tattoo No. 4). Female, about 2 years old. Inoculated with Eiro 10 per cent brain suspension, 0·2 ml. intracerebrally, in September 1963. Eiro (Eiru) (male, aged 13) died in September 1962; brain material was taken and frozen within 4·5 h of death, kept in a refrigerator (−10° C) or dry ice until placed at constant −70° C 60 h after death; the pathology of this patient has been reported[8]. In May 1965 the chimpanzee first showed signs of apathy and lassitude, which progressed and within a month was accompanied by a pendulous lower lip, increased sensitivity to cold, and pilo-

Table 1. Summary of Inocula and Results in Primates inoculated intracerebrally with Kuru Human Brain

Patient	Date of inoculation and No. of animals inoculated					Kuru-like syndrome in chimpanzee	
	Species of primates inoculated					Months post-inoculation	
	Chimpanzee	*M. rhesus*	*M. cynomolgus*	*C. aethiops*	*S. sylvania*	Onset	Killed (terminal)
Eiro (Eiru)	Sept 1963 (1)*	Jan. 1963 (1)	Sept. 1963 (5)	Sept. 1963 (4)		20	25
Enage	Aug 1963 (1)	Aug 1963 (5)	Sept. 1963 (4)	Sept 1963 (3)			
Kigea	Aug 1963 (1)	Aug. 1963 (5)	Aug. 1963 (7)	Aug 1963 (3)	Aug. 1963 (2)	21	30
Moba	Sept. 1963 (1)		Sept. 1963 (5)	Sept. 1963 (3)			
Kariwani	Nov. 1963 (2)	Nov. 1963 (6)					
Igierakaba	Feb 1964 (1)	Feb. 1964 (8)					
Kabuinampa	Feb 1964 (1)	Feb 1964 (8)				18	
Uninoculated controls†	(1)	(8)	(14)	(7)			

* In addition to the primary isolation inoculation a further three chimpanzees were inoculated in October 1965 in an attempt at re-isolation and three other chimpanzees were inoculated with brain from the first killed animal

† In addition all primates inoculated with suspension of human brain tissue as summarized in Table 2 serve as controls. All chimpanzees and more than 100 monkeys are housed in the same building. The facilities provide the animals with adequate space for daily exercise outside of their individual cages.

erection. Within two months she demonstrated titubation and trunkal unsteadiness, a wide-based, lurching gait and, later, marked dysmetria of hand movements. Her ataxia became more severe and she suffered many stumbles and falls. Eventually she was unable to sit up without external support and spent her time huddled at the bottom of her cage. She required encouragement to eat and drink, but only terminally was there any difficulty with swallowing. Visual fixation became impaired terminally. Hearing appeared normal. There was no spontaneous nystagmus but it could be elicited by vestibular stimulation. At no time was there any motor paresis, though power was poorly sustained; passive limb tone was normal or decreased. There was no loss of pain sensation; hyperaesthesia to touch occurred over the last 6 weeks of her illness. Detectable reflex changes, changes in the fundi, convulsions, impairment of consciousness or obvious disorientation did not occur. Haematological, biochemical and trace metal determinations on the blood were considered normal. Cerebrospinal fluid collected just before death was clear with a protein level of 237 mg/100 ml. Progressive ataxia involving the trunk and all limbs and leading to increased disability remained the chief feature of the syndrome. She was killed in October 1965, when her terminal debility, dysphagia and respiratory distress caused our concern lest sudden death from secondary infection should intervene.

Second chimpanzee (Daisey, tattoo No. 1). Female, about 2 years old. Inoculated with Kigea 10 per cent brain suspension, 0·2 ml. intracerebrally, in August 1963. Kigea (female, aged 11) died in March 1963; brain material was taken 1 h after death, frozen within 6 h, and kept frozen in a refrigerator (− 10° C) or dry ice until placed at constant −70° C 80 h after death; the pathology of this patient will be reported[9]. In May 1965 the chimpanzee first showed definite signs of withdrawal and apathy having previously been a very friendly, open and affectionate animal. This stage lasted for 3 months without significant change. Then titubation, trunkal instability, ataxia of gait and dysmetria of hand movements began and slowly increased in severity. Lethargy, apathy and poverty of movement persisted; at times, huddled on the shelf of her cage, she maintained slowly evolving postures in an almost catatonic state. Small stimuli, however, would lead to an exaggerated startle reaction. Tendon reflexes were diminished but not absent; passive limb tone was increased. There were no detectable sensory deficits, nor motor pareses. Terminally, an inconstant strabismus appeared; there was no nystagmus. Vestibular function and hearing appeared normal. There were no changes in the fundi, and no convulsions occurred. By late December 1965 she reached the same terminal state as the first chimpanzee, huddled in the bottom of the cage with her arms around her trunk, grossly ataxic on any attempted movement, and showing impairment of visual fixation and increased dysphagia.

Third chimpanzee (Joanne, tattoo No. 9). Female, about 2 years old. Inoculated with Kabuinampa 10 per cent brain suspension, 0·2 ml. intracerebrally, in February 1964. Kabuinampa (female, aged 23) died in December 1963; brain material was taken within 1 h of death and placed in liquid nitrogen; the pathology of this patient will be reported[9]. In August 1965 the chimpanzee first appeared lethargic and apathetic, had a pendulous lower lip, sustained pilo-erection, and frequent shivering which never became paroxysmal. Within a few weeks signs of motor inco-ordination, titubation, trunkal instability, dysmetria and an unusual wide-based stamping gait, in which she never rose much above her haunches, developed. Muscles undergoing sustained contraction showed a marked clonic tremor. Occasional shivering-like tremors were seen. There have been no motor pareses or reflex changes. Hearing, vision and extraocular movements have appeared normal. She has had no convulsions. She has remained lethargic and shows little spontaneous voluntary movement; but she greatly resents handling and examination, to which she responds with wide facial grimacing and a monotonous shrill cry. Her ataxia has progressed steadily and she has followed a course strikingly similar to that of the first chimpanzee.

On the 160th day after the recognized onset of disease in the first chimpanzee (in October 1965) the original 10 per cent brain suspension (stored at −70° C) which had been inoculated into this animal was re-inoculated into three new young chimpanzees (two male, one female). When this first affected chimpanzee was killed on the 163rd day of illness, generous portions of blood, brain and visceral tissue were taken and stored in liquid nitrogen, using separate sterile instruments for each tissue. A freshly prepared 10 per cent suspension of unfrozen brain material, pooled from three brain areas, was inoculated intracerebrally into three young male chimpanzees and a number of individual monkeys of several species. Further chimpanzees will be inoculated with blood and with suspensions of visceral tissue and these tissues and brain will be titrated as the supply of animals permits. Filtrates of brain suspension through filters of various pore size will be inoculated into chimpanzees in an attempt to establish whether the agent involved is a filterable virus, for to date only centrifugally clarified tissue suspensions (2,000 r.p.m./20 min) have been used and thus non-viral micro-organisms remain an aetiological possibility, although conventional bacterial and fungal cultures as well as tissue culture passage of the inocula have proved sterile. A few chimpanzees will be inoculated only subcutaneously, not intracerebrally, with brain suspension.

When the first chimpanzee was killed no gross neuropathological lesion other than a fine needle scar at the site of the inoculation in the frontal lobe was observed. Preliminary histological study by Mrs. Elisabeth Beck (Department of Neuropathology, The Maudsley Hospital,

Table 2. Summary of Primate Inoculations with Human Tissues from Other Sub-acute and Chronic Nervous System Degenerative Diseases*

Disease	Tissue	No. of patients	Date of inoculation and No of animals inoculated‡			
			Species of primates inoculated			
			Chimpanzee	*M. rhesus*	*M. cynomolgus*	*C. aethiops*
Amyotrophic lateral sclerosis (Guam and U.S.A.)	CNS†	6		Sept. 1963 (7)	Sept. 1963 (16)	Sept. 1963 (8)
	Blood	6		Oct. 1964 (7)		
Parkinsonism	CNS	2	May 1965 (1)	May 1965 (4)		
Parkinsonism-dementia (Guam)	CNS	5	Aug 1963 (1)	Aug. 1964 (4)	Aug 1963 (10)	Aug 1963 (5)
Dawson's inclusion encephalitis	CNS	1		Aug. 1964 (2)		
Myasthenia gravis	Thymus	1		Aug. 1964 (2)		
Multifocal leuco-encephalopathy	CNS	1		May 1965 (3)		
Multiple sclerosis	CNS	4	Mar 1965 (2)	Mar. 1965 (13)		
Schilder's disease	CNS	1		May 1965 (3)		
Necrotizing encephalitis§	CNS	1		Apr. 1965 (1)		
Uninoculated controls			(1)	(8)	(4)	(5)

* All animals have received intracerebral inoculation of suspended tissues; many have, in addition, received intravenous inoculations

† CNS, Central nervous system.

‡ Date given is that of the initiation of the inoculation programme with each disease; the number is the total number of animals inoculated up to February 1966.

§ A strain of herpes simplex virus was isolated in tissue culture from the specimen, the single rhesus monkey inoculated intracerebrally has not developed illness.

London) has revealed the diffuse non-specific changes seen in the brains of kuru victims together with the cerebellar system degeneration characteristic of the disease. Detailed neuropathological studies are in progress[10].

We continue to observe the remaining five kuru-inoculated chimpanzees, inoculated 30, 29, 27, 27 and 24 months ago, respectively, and the many smaller monkeys of other species which have been inoculated with kuru material intending to hold these animals for at least 5 years of observation. The three animals which have developed kuru-like syndromes have all been females, as are three of the five kuru-inoculated chimpanzees still well. (*Addendum.* Since this article was written, one male chimpanzee of the two inoculated in November 1963 with brain material from patient Kariwani has developed, in mid-January 1966, the same kind of progressive ataxia described in the three earlier chimpanzees.) Efforts are being concentrated on cultivating an agent in a wide variety of tissue cultures, in chick embryos and many different young laboratory animals, and on identifying a viral antigen in tissues from the affected chimpanzees and from human patients, and in passage animals and cultures by immunological methods.

In view of the close similarity in the clinical picture and the neuropathological findings of kuru in man with those of scrapie in sheep and mice[11,12], the remote possibility of scrapie infection in the affected chimpanzees must be considered, although so far there is no evidence of the scrapie virus producing disease in primates. All primates inoculated with human material are housed in a remote, separate building from that in which scrapie investigations are being carried out. Of nineteen chimpanzees and more than 200 smaller monkeys in these transmission experiments from human tissue no animals have developed a chronic progressive neurological disorder, other than the three kuru-inoculated chimpanzees described here. *Macaca rhesus* monkeys inoculated 18 months ago with scrapie mouse brain suspension have not yet shown disease. Chimpanzees are only now being inoculated with scrapie material.

To anyone who has had the opportunity of observing the unique syndrome of kuru developing and progressing steadily to fatal termination in patients in New Guinea, the similarity of its clinical picture and course to the experimentally induced syndrome in the chimpanzee is dramatically evident. This remarkable clinical correspondence of a disease developing successively in three chimpanzees each inoculated with brain material from a different kuru patient, the onset in each after a very similar long incubation period, the fact that there is no such syndrome of chimpanzees known to occur spontaneously or seen at present in our many control animals, and the remarkable similarity of the neuropathological findings, in the one case examined, to those observed in kuru victims lead us to believe that kuru has been transmitted experimentally to these chimpanzees.

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KINETICS OF TWO-BREAK CHROMOSOME EXCHANGES AND THE SPATIAL ARRANGEMENT OF CHROMOSOME STRANDS IN INTERPHASE NUCLEUS

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ONE of the consistent observations in radiation cytology has been that the yield of two-hit chromosome exchanges, whether detected genetically as translocations or cytologically as dicentrics and rings, increases not as the square of the dose but by about the 1·5th power of the dose[1-3]. It has been known that for aberration production the breaks in two chromosome strands should occur sufficiently close together in space for rejoining, other than restitution, to occur[4]. Wolff[3,5] has recently developed an equation, to formulate the spatial restriction in terms of a limited number of sites in which the chromosomes come within the rejoining distance, to describe the theoretically expected kinetics for the two-hit chromosome exchanges. Earlier explanations for anomalous kinetics of two-break aberrations considered the occurrence of a large amount of inviability appearing at high doses or a large linear component[6] to be the reasons for falling off of the dose response curves from the dose square approximation at high doses. In the experiments reported here, the dose-response has been examined separately for dicentrics and centric rings. The results have suggested an interpretation of over-all response and permitted us to put forth a model concerning the spatial organization of chromosomal strands in the interphase nucleus.

Dry barley seeds (*Hordeum vulgare cv CN* 292, $2n = 14$; hull-less; moisture content, 10–12 per cent) were used in this investigation. With dry seeds, the post-irradiation environment during germination can be effectively controlled. The seeds were given doses of γ-rays at 5·1 kr./min in cobalt-60 'Gamma Cell 200' (Atomic Energy of Canada, Ltd.) at our laboratory and of fast neutrons (mean energy ≃ 1 MeV, γ contamination negligible) at reactor R_1 of the Swedish Department of Atomic Energy, Stockholm. The irradiated seeds were hydrated in the presence or absence of oxygen at 0°, 20° and 30° C for 24 h and then germinated on moist filter paper at 25° ± 2° C. The yield of dicentrics and rings was evaluated at colchicine-blocked first mitosis, from Feulgen-stained squash preparations. The data from five replications from each post-irradiation treatment were pooled together. For the doses investigated, the distributions of two-break aberrations have been observed to be Poisson at low doses and non-Poisson—with tendency towards normality—at high doses, and this indicates the absence of limitation

Table 1. Effect of Doses of γ-Rays and Fast Neutrons on Yields of Dicentrics/Cell, Rings/Cell and Dicentrics+Rings/Cell

Radiation/dose	Dicentrics/cell	Rings/cell	Dicentrics+rings/cell	Dicentrics/Rings
Fast neutrons				
200	0·221	0 023	0 244	9·6
400	0 361	0 044	0 405	8·2
600	0 433	0 069	0·502	6·3
800	0·589	0·092	0·681	6·4
Kinetics	$D^{0\cdot89}$	$D^{0\cdot95}$	$D^{0\cdot83}$	
	$D^{0\cdot82}$	$D^{1\cdot05}$	$D^{0\cdot84}$	
γ-rays/(kr.)				
25	0·229	0·050	0·279	4·6
37·5	0 340	0 106	0·446	3·2
50	0 610	0·221	0·831	2·8
Kinetics	$D^{1\cdot33}$	$D^{2\cdot20}$	$D^{1\cdot49}$	

on the number of sites for aberration formation in interphase meristematic cells of dry barley seed[7].

The effect of dose on yields of dicentrics/cell, rings/cell and dicentrics + rings/cell is given in Table 1. The yield of rings/cell is proportional to the dose with fast neutrons and to the square of the dose with γ-rays. These data show that in a site forming a ring, where two arms of a chromosome come within the rejoining distance, the two strands are broken by the same ionizing particle to give one-hit kinetics with fast neutrons and by two different ionizations to give two-hit kinetics with γ-rays. However, the kinetics for dicentrics/cell are such that the yield increases by less than a factor of 2 with fast neutrons and by less than a factor of 4 with γ-rays. Since kinetics in terms of initial ionization (or hits) by sparsely—or densely—ionizing particles must be the same irrespective of whether the site forms a ring or a dicentric, the reasons for wide differences in kinetics for rings and dicentrics have to be found in the distribution and structure of nuclear sites for rings and dicentrics.

If the arrangement of chromosomes in the interphase nucleus is random and sites for dicentrics and rings are formed randomly, then in barley, which has a symmetrical karyotype containing 28 chromosome arms, the expected ratio of dicentric-forming sites to ring-forming sites will be 26 : 1. The experimentally observed ratio varies between 9·6 : 1 and 2·8 : 1 (Table 1). This indicates that the arrangement of the two arms of a chromosome may be such that they come near each other more frequently than would be expected on the basis of random arrangement.

The examination of the characteristics of the radiation-induced aberrations in barley revealed that (1) most dicentrics carried a small intercalary segment and were accompanied by a big acentric fragment; (2) dicentric rings carried a very small intercalary segment. The aberration configurations with small intercalary segments can arise only if the centromeres of the chromosome strands involved in an aberration are situated close to one another. These results indicate that the centromeres in the interphase nucleus are strongly polarized. A similar conclusion was arrived at by Sax[8], from observations in *Tradescantia* where unequal interchanges induced by radiation were found to be rare, and more than 80 per cent of the interchanges were found to involve exchanges between the chromosomes or chromatids at corresponding loci in respect of the distance from the centromere. Evans's data on interchanges in *Vicia faba* indicate that 80–90 per cent of the interphase chromosomes are polarized[9]. The model of interphase nucleus under consideration, then, has the following features: (1) the chromosome strands are strongly polarized; (2) the chromosome arms radiate from the centromeric pole at a small angle; (3) the chromosome strands are located in definite and recurrent sites.

This model gains support from the observation that relatively little displacement occurs following uncoiling and elongation of chromosomes and during the succeeding interphase so that chromosome strands reappear in the prophase in the same place as they were at the preceding telophase. If the three characteristics listed here apply to telophase chromosomes, then they must also apply to chromosomes in the static interphase as well.

It follows from the properties of the interphase nucleus that the average strand to strand distance would be least at the polar end and maximum at the free end. The chances, therefore, of chromosome strands coming within the rejoining distance and forming a site are higher near the pole than away from it. We would expect this feature to have an effect both on the number of sites and number of strands forming a site. Thus (1) near the pole end, a far greater number of sites is formed than away from it; (2) a large majority of sites formed near the pole involve more than two strands. Since most of the dicentrics carry small intercalary segment and accompany large acentrics and most rings accompany small acentrics, it can be predicted that a greater proportion of the dicentric-forming sites are formed near the pole and a majority of ring-forming sites occur away from the pole towards the free ends of chromosome strands. It would appear from postulate (2) that a site has to be imagined as a spherical volume of diameter of rejoining distance. Within this sphere two or more than two strands can be included.

Only two chromosomes are required to be broken for an exchange to be produced. The chance of getting two independently broken chromosomes with sparsely ionizing radiation in a bi-stranded site will be different from that in a tri- or a multi-stranded site. The chance will be the product of the probabilities of breaking individual chromosomes multiplied by one less than the number of strands included in the site, that is $(s-1)p^2$, where s is the number of strands included in a site and p is the probability of breaking a chromosome in a site. The yield for two-hit exchanges within site will vary with s, it will be proportional to dose if $s = 2$ and to a less power of dose if $s > 2$. The average kinetics will have contribution of each of different kind of dose-response terms. These considerations indicate that the majority of dicentric-forming sites would have to be multi-stranded and all ring-forming sites bi-stranded.

The presence of additional breaks in a site will affect the probability of asymmetrical rejoining with fast-neutron radiation if the probability that a bi-stranded site becomes effective is q; the probability that a tri-stranded site becomes effective is $3q$. The chances of reunion to take place will be still higher if the sites overlap and chromosomes are broken in overlapping sites. Thus, with the increased probability of rejoining at higher doses of fast neutron radiation, the yield for two-hit exchanges within a site will not be proportional to dose, but to less than one power of the dose. This component of dose response evidenced with densely ionizing radiation will also affect the kinetics with sparsely ionizing radiation.

The expressions for yields of aberrations in bi- and tri-stranded sites can be given as:

	Bi-stranded site	Tri-stranded site
Fast neutrons	$Y = k\,(D)\,q + c$	$Y = k\,(D)\,Q + c$
γ-rays	$Y = k\,(D^2)\,q + c$	$Y = k\,(2D^2)\,Q + c$

where k is the mean number of breaks in a chromosome in a site/rad, D is the radiation dose, Q the probability of asymmetrical rejoining at a site as affected by post-irradiation environment (q) and influence of additional breaks, and c number of spontaneous exchanges observed at zero dose.

The foregoing equations have been derived on theoretical as well as experimental grounds and provide an estimate of the expected yields of exchanges within a site as the dose changes. The expressions for yield of aberration in sites with higher number of strands can be similarly obtained. In deriving these equations it has been assumed that sites do not overlap, and that there is no effect of multiple breaks in a strand within a site. In the present investigation the influence of these two factors could not be evaluated. This investigation also suggests that a general formulation on the kinetics of two-break chromosome aberrations should take into consideration the size of chromosome complement, nuclear volume and nature of karyotypic symmetry.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Lightning, Novae and Quasars

Quasars 'or something' have been a desideratum of the electrical discharge theory of cosmic atmospheric phenomena and universal evolution since its inception in 1941, and the absence of any reference to galaxies which are radiating an inordinate amount of energy was one of the main difficulties facing the theory. As the years went on, I had to conclude that the energy must be largely in the unobservable range, for example, X-rays or γ-rays. However, in the original outline of these ideas published in 1944 (ref. 1*a*), I emphasized that galaxies exist which possess an emission line spectrum, and that in all probability they are those which the theory required. In a recent review article[2] on quasars, this is still their only optical characteristic mentioned.

On the theory, they are analogous to the naked eye phase of novae, that is, they represent the phase around peak current in the discharge[1*b*], during the neutralization of the electric field built up slowly during lifetime of a star or galaxy, probably by asymmetrical impacts between grains[1*c*], though the exact nature of these field-building processes is not yet agreed even in thunderclouds[1*d*].

In a paper presented to the second U.S. Air Force Conference on Atmospheric Electricity in 1958 (ref. 1*e*) I pointed out that this phase of the discharge lasts for about 10 μsec in the lightning discharge, from 1 to 10 min in a solar flare[1*f*], about 10 days in a nova[1*b*], and might be expected to last for 10^6 years in the corresponding galactic discharge. However, if, as seems reasonable in view of the geometrical similarity between the two cases, we assume that this phase will occupy a similar fraction of the total life of the outburst on the stellar and galactic scales, we obtain an estimate of 10^4–10^5 years for the peak current phase of the galactic discharge, which agrees well with Greenstein's range of estimates of 10^3–10^7 years for the duration of quasars[3].

Another characteristic which quasars and novae have in common is the extreme rapidity with which their radiation can vary during the outburst, due very probably to the pinching out of the discharge. In Nova Hercules 1934, for example, the brightness of the star fell abruptly by about nine magnitudes soon after its peak. Thereafter its brightness increased somewhat more slowly until it approached its pre-extinction value.

From the photographs[4] of the well-known 'jet' or discharge channel in the radio source Virgo *A*, *NGC*4486, it would appear that these pinches also occur on the galactic scale, since the jet is divided into three 'sausages' by the pinching of the channel in two places.

A corresponding phenomenon was recently photographed in the lightning flash[5], so these varied observations would appear to afford another interesting link-up between phenomena on these three very different scales[1*g*].

The characteristics of the phenomena will be different from those already considered in relation to the pinching of laboratory discharges, as we have now to consider the breakdown of an electrostatic field[1*h*,*j*]. As indicated in the recent article just referred to[1*g*], huge electrostatic as well as electromagnetic forces are involved.

It may also be noted that the discharge theory of necessity introduced circular magnetic fields in and around the spiral arms of the galaxies from its initiation in 1941. In 1953 magnetic fields along the arms were introduced by Chandrasekhar and Fermi[6], but as Cowling emphasized[7], without a proposed origin of these fields, that did not take us much farther. However, in 1961 it was proposed[8] to transform these last fields into circular fields around the arms in agreement with those originally introduced in 1941 by the electrical discharge theory.

Furthermore, in 1961 (ref. 1*k*) I estimated the total energy liberated by a radio galaxy as 10^{60} ergs when the value available for comparison, derived from Virgo *A*, was 10^{58} ergs (ref. 9). From a survey of all the available data, Heeschen[10] later arrived at a value of 10^{60} ergs in agreement with the earlier value deduced from the electrical discharge theory.

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GEOPHYSICS

Backscattering from the Upper Atmosphere (75–160 km) detected by Optical Radar

An optical radar system has recently been used to detect and study backscattering from the upper atmosphere (75–160 km). Although a much more detailed investigation is in progress, our preliminary results seem to indicate rough agreement with those of Fiocco and Smullin[1]. These authors suggested micrometeorites as a source of the echoes. When observations were made for brief time intervals (0·5 h or less), peaks were sometimes observed in the return-signal profiles. It should be noted, however, that when averaged over a period of several

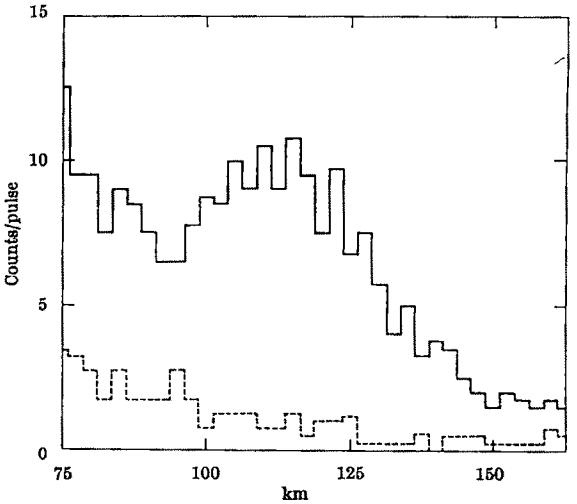


Fig.1. October 29, 1965, 07h 30m—08h 00m U.T. —, Observed return (average of ten laser pulses); – – –, background (average of ten laser pulses)

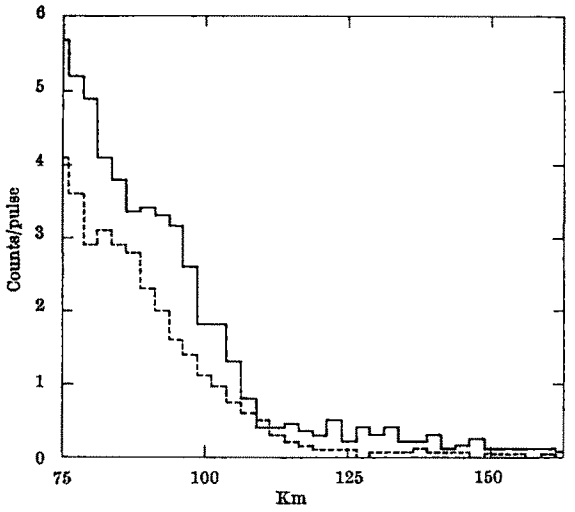


Fig. 2. October 31, 1965, 05h 30m - 09h 30m U.T. ——, Observed return (average of one hundred laser pulses); – – –, background (average of twenty laser pulses). (Note change of vertical scale from that of Fig. 1)

hours the return signal tends to become relatively smooth. It would seem, therefore, that the altitudes of the scattering layers, if they exist, fluctuate rapidly with time. The total scattering cross-section at 6943 Å observed by us is of the same order of magnitude as that reported by Fiocco and Colombo[2] ($\overline{N\sigma} \approx 10^{-12}$ cm^{-1}).

The optical radar consists of a Korad *K*-2*Q* ruby laser which is capable of producing single pulses containing 6–10 J at 6943 Å with a width of 10 nsec and a beam divergence of no more than 10 milliradians. The ruby is 8 in. long, 5/8 in. in diameter and is surrounded by a helical flash lamp. *Q*-spoiling is achieved by means of a Kerr cell. The laser pulse is brought to a focus at a 1/8 in. diameter aperture in a rotating mirror. The aperture is located in the focal plane of the University of Maryland's 20-in. reflector, which is a Broken Cassegrain with a 300-in. effective focal length. The beam divergence of the light leaving the telescope is less than 0·4 milliradians. In order to prevent air breakdown, the rotating mirror is installed in a chamber which is evacuated to a pressure of about 10μ mercury. An average energy of 1·0 J per pulse was sent into the atmosphere. Each laser pulse was monitored for proper firing time and for pulse amplitude. The return signal from the telescope is reflected by the rotating mirror to the cathode of a cooled E.M.I. 9558*A* photomultiplier tube (*S*-20 cathode) which is gated off during the actual laser firing. A 10 Å pass-band interference filter was used in the experiment.

Due to the small magnitude of the signal, the experiment consists of counting individual photoelectrons. The pulses are processed by Chronetics Nanologic Modules and sent through logic circuits[3] to an 'on-line' *CDC* 160 computer which is programmed[3] to store the counts from each altitude interval. At present the system is capable of obtaining an atmospheric scattering profile, for each laser pulse, with an altitude resolution of 2·5 km.

All measurements were made at College Park, Maryland (76° 57′ W. long., 39° N. lat.), while looking at the zenith. Figs. 1 and 2 give the results for two nights out of five for which we have obtained data. Fig. 1 shows the result of a series of shots taken during a short time interval. The scattering appears to be enhanced over the altitude ranges from 100 to 125 km. The total number of counts received (in 10 pulses) from 75 to 150 km was 2,240. The background was 350 counts. The number of photoelectrons due to backscattering is thus 1,890 with a mean error of 51 due to statistical uncertainty. Fig. 2 is the average of 100 laser pulses, over a period of 4 h, on a different night. The magnitude of the return signal is much lower than in Fig. 1 and no peaks are present. For the range 75–150 km we have: a total return of 5,070 counts, a background of 3,330 counts, a backscattering of 1,740 counts with a mean error of 147 counts. The high background is due to the fact that we use the same optical path for both transmitting and receiving. It is difficult to shield the phototube completely from the high light-levels present and from ruby fluorescence. Background measurements were made, during observations, by placing a black cloth over the end of the telescope tube.

We thank Prof. S. F. Singer for his encouragement and advice. This work was supported, in part, by the U.S. Weather Bureau under grant *WBG–4–FY–64*, A.R.P.A. grant *SD–101*, National Aeronautics and Space Administration grant *NGR* 21–002–022 and Bureau of Ships contract *NObsr* 72710.

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PHYSICS

Surface Entrainment of Air by a Water Jet

This communication reports an investigation of the conditions for air entrainment when a water jet impinges normally on an air–water surface. The calculated critical entrainment velocity of the jet is compared with experimental values.

The surface profile of a deep liquid into which a perpendicular jet of the same liquid is falling can be deduced by considering the surface tension and the pressure on the surface caused by air entrained by the descending jet. Surface tension will cause a meniscus to form between the liquid surface and the jet. The angles of contact will be zero, so the meniscus will have the usual form:

$$y^2 = \frac{2\sigma}{g\rho_L}(1 - \cos\psi)^* \qquad (1)$$

The equation is, however, more manageable in cylindrical co-ordinates about the axis of the jet. The transformation is straightforward but tedious, and yields:

$$r - r_0 = A\left[\tfrac{1}{2}\log_e\left\{\frac{1+\sqrt{1-\frac{y_2}{4A^2}}}{1-\sqrt{1-\frac{y^2}{4A^2}}}\right\} + 0{\cdot}5329 - 2\sqrt{1-\frac{y^2}{4A^2}}\right] \qquad (2)$$

where:

$$A^2 = \frac{\sigma}{g\rho_L}$$

The liquid jet, as it travels between the nozzle and the surface, will cause the air alongside to be accelerated. This air will strike the surface of the liquid causing a depression of the meniscus.

It is assumed that the viscous flow of air dragged by the jet is laminar, and no pressure variations occur. The velocity of the air parallel to the jet is U, and is a function of the radius of the jet and the height through which the jet has fallen.

Consider an infinitesimal annulus of air at radius r, radial increment δr, and height δy. The mass of the

annulus is $2\pi r\delta y\delta r\rho_A$ and the net viscous force acting on the annulus is:

$$-2\pi r\delta y\eta\frac{\partial U}{\partial r}\delta r,$$

$\frac{\partial U}{\partial r}$ being negative. Therefore, the acceleration of the annulus is given by:

$$\frac{\partial U}{\partial t}=\frac{\text{Force}}{\text{Mass}}=\frac{\eta}{\rho_A}\frac{\partial U}{\partial r} \qquad (3)$$

r and t are independent variables, so the partial differential equation is easily soluble.

$$\frac{\partial U}{\partial t}=-\frac{\eta}{\rho_A}\frac{\partial U}{\partial r}=K$$

Therefore:

$$U=Kt-\frac{\rho_A}{\eta}Kr+L \qquad (4)$$

where K and L are constants.

It is now assumed that the jet is a cylinder of radius r_0. The effect of this approximation on the air flow will be small if the flow is laminar.

The constants can be obtained from the boundary condition that at the surface of the cylinder $r=r_0$ and

$$U=V=V_0+gt$$

Substituting this condition into equation (4):

$$V_0+gt=Kt-\frac{\rho_A}{\eta}Kr_0+L$$

and, by equating the coefficients of powers of t,

$$K=g$$

and

$$L=V_0+\frac{\rho_A Kr_0}{\eta}=V_0+\frac{\rho_A gr_0}{\eta}$$

Therefore:

$$U=V_0+gt-\frac{\rho_A g}{\eta}(r-r_0) \qquad (5)$$

At the liquid surface:

$$V_0+gt=V_L$$

and so:

$$U=V_L-\frac{\rho_A g(r-r_0)}{\eta} \qquad (6)$$

The air incident on the surface causes a pressure equal to the rate of change of momentum per unit area

$$P=\rho_A U^2=\rho_A\left\{V_L-\frac{\rho_A g(r-r_0)}{\eta}\right\}^2$$

The air pressure P will cause a depression of the surface equal to:

$$y=\frac{\rho_A}{g\rho_L}\left\{V_L-\frac{g\rho_A}{\eta}(r-r_0)\right\}^2 \qquad (7)$$

Fig. 1 shows the surface tension curve for water and the air pressure curve for values of $V_L=200$, 300 and 400 cm/sec. The resultant surface profiles, the sums of these graphs, have been plotted. For $V_L=300$ or 400 cm/sec a maximum is present in the resultant surface which is unstable and will collapse to $y<0$, leaving a depression, with consequent air entrainment. A point of inflexion, in fact, occurs for $V_L=231$ cm/sec, and above this critical velocity air could be carried into the body of the liquid.

The formation of surface-entrained air bubbles was investigated experimentally, and it was found that a critical velocity for air entrainment did exist but varied with jet diameter. The results are shown in Fig. 2. As the jet diameter is reduced, the critical velocity approaches an asymptote at about 235 cm/sec. This asymptote agrees well with the predicted value.

It has been assumed in the analysis that the airflow is laminar. The Reynolds's number of the airflow will be approximately equal to that of a solid circular jet of air of the same velocity and diameter as the jet of water. This is because at any specific time the annulus of air

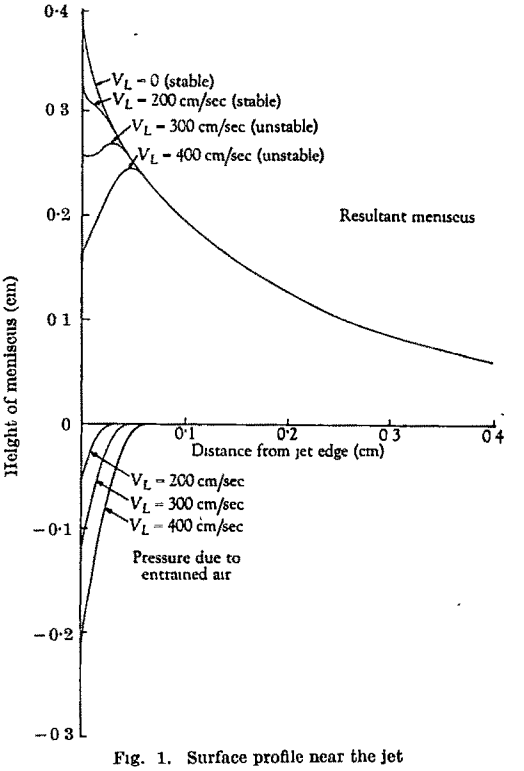


Fig. 1. Surface profile near the jet

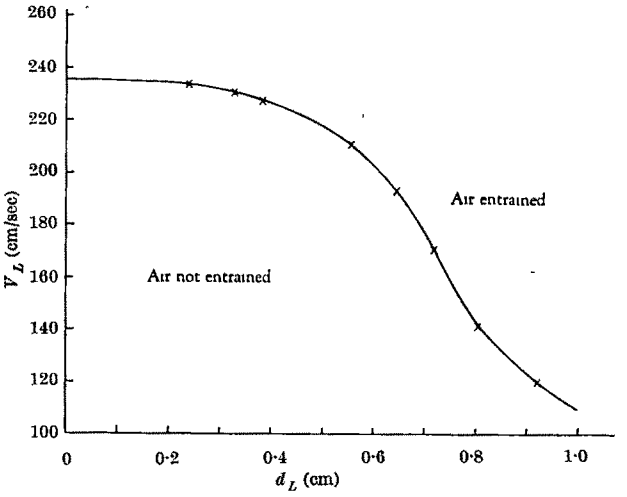


Fig. 2. Variation of critical entrainment velocity with jet diameter

will be influenced equally by an air or a water core. The Reynolds's number of the airflow when the critical velocity starts to fall (Fig. 2) is about 600 at $d_L=0{\cdot}4$ cm. The decrease of critical velocity as d_L increases is therefore probably due to the airflow becoming turbulent, as Streeter[1] quotes the transition region for a circular jet as being approximately defined by $300\leqslant N_R\leqslant 1{,}000$.

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* The following abbreviations are used in this communication. A, $\left\{\frac{\sigma}{g\rho_L}\right\}^{\frac{1}{2}}$ equals unit of meniscus height; d_L, diameter of jet at liquid surface; g, gravitational constant; K, constant of integration; L, constant of integration; N_R, Reynolds's number, r, radial distance from jet axis; r_0, radius of jet; t, time, time of fall of jet from the nozzle; U, air velocity at any point; V, jet velocity; V_0, jet velocity at the nozzle; V_L, jet velocity at the liquid surface; y, height above plane of liquid surface; η, viscosity of air; ρ_A, density of air, ρ_L, density of water; σ, surface tension of water; φ, angle of inclination of meniscus to the horizontal.

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Resolution in Low-angle Electron Diffraction

THE optical system of a high performance electron microscope can also be used for investigations other than simple transmission microscopy. One such 'unconventional' application is low-angle electron diffraction. This technique was introduced by Mahl and Weitsch[1] and has since been used in various investigations of structure[2–4]. Its advantage, as compared with X-ray low-angle diffraction, lies in the higher resolution which it offers, the much shorter exposure times required, and the possibility of making visible the area contributing to the diffraction pattern.

In the course of our investigations we became interested in the attainable resolution in connexion with various arrangements of the optical system. The resolution of the system can be defined by the number n given by the relation[5]:

$$n = \frac{r_F}{L} = \frac{r_F}{l_D M} \qquad (1)$$

where r_F is the radius of the primary spot on the photographic material, L is the effective camera length, l_D is the distance of the specimen from the object plane of the intermediate (eventually, projector) lens, and M is the magnification of the projector system.

The resolution of an optical system consisting of a Tesla *BS* 413 electron microscope equipped with a double condenser lens, an intermediate lens and a projector lens was investigated. The specimen was placed in its normal position and the objective lens was not energized. The first condenser lens was highly excited in order to produce a demagnified image of the cross-over and this image was focused by means of the second condenser lens, onto the object plane of the intermediate lens. The diffraction pattern in the object plane was then magnified and projected onto the photographic material by means of the projector system. The excitation of the intermediate lens could be chosen so as to operate it as a magnifying or reducing lens; eventually the intermediate lens could be switched off so that the diffraction pattern focused by the second condenser lens onto the object plane of the projector lens was magnified only in one stage.

The dependence of the resolution number n on the diffraction distance l_D (and hence on the way of operation) is given in Fig. 1 for three focal lengths f_E of the first condenser lens. The diameter of the cross-over is supposed to be 40μ. The left-hand side of the graphs (10 mm $\leqq l_D \leqq$ 180 mm) accounts for the intermediate lens functioning as a magnifying lens, the right-hand side (300 mm $\leqq l_D \leqq$ 1,500 mm) holds for the intermediate lens functioning as a reducing lens[6]. It is evident, from the optical point of view, that the resolution was higher for the intermediate lens operated as a reducing lens and, in fact, approached that of the best diffraction cameras. In practice, it is advantageous to use only the projector lens for magnification of the diffraction pattern (in Fig. 1, $l_D = 300$ mm) because the working parameters can be more easily reproduced and the resolution remains very high.

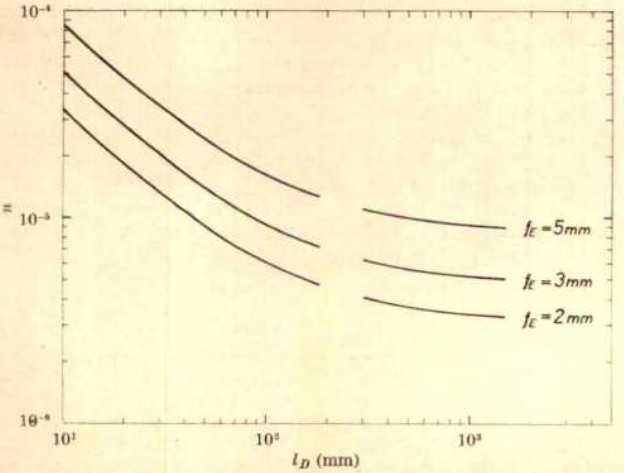


Fig. 1. Computed resolution number, n, for three focal lengths of the first condenser lens against diffraction length, l_D, characterizing different ways of operation of the projection system (excitation of the projector lens was kept constant)

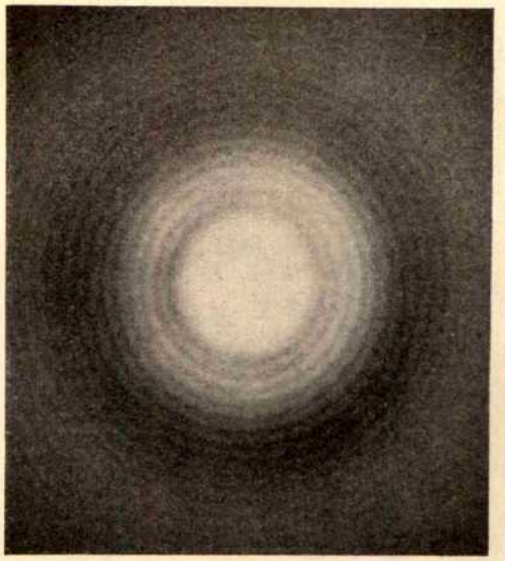


Fig. 2. Low-angle diffraction pattern of a random assembly of polystyrene latex spheres 4130 ± 80 Å

The largest observable spacing can be obtained from the Bragg equation:

$$2d \sin\theta = 2d\theta = \lambda \qquad (2)$$

where d is the spacing characterized by the Laue indices (h, k, l), θ is the Bragg angle, λ is the wave-length.

We can substitute $n = 2\theta$ in the equation (2) and thus obtain a relation for the theoretically largest observable spacing in form:

$$d_t = \frac{\lambda}{n} \qquad (3)$$

The introduction of the specimen results in an enlargement of the primary spot. If we suppose in our conditions that the real diameter of the primary spot is about twice the theoretical value, then we can write for the largest observable spacing:

$$d \approx \frac{\lambda}{2n} \qquad (4)$$

To test experimentally the validity of relation (4) we used spheres of monodisperse polystyrene latex. It can be shown[6] that the mean diameter of the spheres can be considered to be practically equivalent to the Bragg spacing d, because the difference of scattering angle belonging to successive secondary maxima corresponds closely to the angle 2θ in the equation (2).

An example of the pictures taken during the tests is given in Fig. 2. It shows the diffraction pattern of a random assembly of latex particles 4,180 ± 80 Å in diameter obtained under the following experimental conditions: $\lambda = 4{\cdot}33 \times 10^{-2}$ Å ($u^* = 75$ kV), $f_E = 2{\cdot}5$ mm, $l_D = 300$ mm (the intermediate lens was not energized), $L = 35{\cdot}7$ m, $2r_F = 3{\cdot}6 \times 10^{-1}$ mm. The resolution number, n, according to equation (1), was 5×10^{-6}, and the theoretically largest observable spacing, d_t, was 8·600 Å. If we consider the enlargement of the primary spot by the presence of the specimen, the observable spacing, d, from equation (4) is 4·300 Å. Clearly resolved higher secondary maxima in Fig. 2 show that, from the optical point of view, observation of spacing given by equation (4) is possible. The aforementioned calculations and experiments were performed with the assumption that a normal hairpin filament

was being used in the electron gun. The use of a point cathode (and hence a lower value of the primary spot diameter, $2r_F$) results in a still higher resolution than given in Fig. 1.

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GEOCHEMISTRY

Ion Separation in Bursting Air Bubbles: an Explanation for the Irregular Ion Ratios in Atmospheric Precipitations

It is well known that a considerable part of the soluble salts present in soil, lakes and rivers is derived from rainwater[1]. There is little doubt that the salt content in the rainwater comes mainly from ocean water spray, which is carried into the atmosphere from the surface of the sea, where tiny droplets are formed by air jets shooting out of the top of bursting air bubbles[2].

Table 1

Location	Date	Br^- (mg/l.)	$Cl^- + Br^-$ (mg/l.)	$(Cl^- + Br^-/Br^-)$
Jerusalem	16.2.1961*	0·105	5·65	53·8
Jerusalem	16.2.1961†	0·122	5·56	45·5
Tel-Aviv	October 1962	0·358	105·95	287·6
Haifa-Hadar	October 1962	0·073	8·88	121·6
Daphna	February 1963	0·061	4·61	75·8
Ein Harod	May 1963	0·201	5·43	27·0
Sde Boker	Winter 1962/63	0·261	3·48	13·3
Nicosia	October 1961	0·18	8·88	49·3
Prodromos	January 1962	0·17	1·78	10·5
Göttingen‡	1–3.11.1951	0·008	0·70	87·5
Bolschewo§ (Moscow District) in snow-water	24.12.1937	0·009	0·35	39·0

* After 12 hours rain.
† After 24 hours rain.
‡ Behne, W. (ref. 6).
§ Selivanoff, L. S. (ref. 7).

Table 2

Location		Na^+/K^+
Southwest Coast, Iceland*		18
Southwest Coast, Norway*		16·5
West Coast, Ireland†		6·3
Rain, Tel-Aviv sea shore‡		35·2
Rain, Jerusalem‡		6·7
Rain, Lodd‡		10·4
Rain, Beer-sheva‡		9·3
Rain, Daphna‡		14·6
Snow from Tasman Glacier	27.4.1958§	1·11
Snow from Mt. Egmont	3.5.1958§	2·83
Snow from Arthurs Pass	22.8.1958§	2·0

* Eriksson, E. (ref. 8).
† Gorham, E. (ref. 1).
‡ Rainy season in Israel 1960/61, analysed by the Geological Department of the Hebrew University.
§ Wilson, A. T. (ref. 9).

Note: All sodium–potassium ratios are given in terms of Na^+/K^+ values, although in the literature quoted they are given as K^+/Na^+.

The reason why the salts, derived from such action, found in rainwater and snow, have ion ratios incompatible with the ratios found in ocean water has remained obscure. Although the Na^+/K^+ ratio in sea water is 27, it is always lower in precipitation (rain and snow), sometimes being as low as 2. The Cl^-/Br^- ratio in sea water is 302, but is always much lower in rainwater or snow, often less than 50, sometimes less than 20. Examples are given in Tables 1 and 2.

Explanations of these facts have so far not been very satisfactory, including the suggestions that they are brought about by the influence of terrestrial dust, by oxidation of halogens by ozone, by biological absorption of potassium in the ocean surface, or by fractionation crystallization in the atmosphere.

We propose here a mechanism for the separation of ions which becomes effective at the moment and shortly before the bursting of ocean water bubbles: in part this follows the suggestion made by Ken Sugawara and Nobuko Kawasaki[3] for Sr^{++}/Ca^{++} and Na^+/Cl^- separation. Such a mechanism would lead to a spray of droplets with ion ratios different from those of the ocean water from which it is derived. As the bubbles become thinner and thinner, a solution runs down the wall between the concave inner surface and the convex outside surface. There will be a tendency for the larger and generally heavier ions to stay in the skin[4], the smaller ones will run off with the solution. When the jet atomizes the bursting skin, the small droplets formed will be relatively rich in the larger ions. It is clear that this mechanism will be influenced by a number of factors, and will probably depend on initial concentrations and the radius of the bubbles. The jet might also disperse solution from the bottom of the bubble, thus admitting varying quantities of original solution to the fine top spray: this may somewhat obscure the effect.

We have carried out some preliminary investigations to test the hypothesis. Distilled water was prepared by boiling sea water, tap water and lake water at atmospheric pressure with and without the conventional spray trap. It can be seen from Table 3 that the spray was reduced through the conventional spray trap by an order of magnitude or more: however, the ratio of the ions, contained in the spray, differed essentially in the same way from the ratio of the ions in the original solution, and was independent of the efficiency of the trap. The variation of ratios caused by artificial distillation was similar to the variation experienced with sea water and rainwater in Nature.

Another series of experiments was undertaken with sea water as well as with artificial salt solutions at ambient temperature (20° C). In these experiments, instead of carrying out distillations, air was bubbled through the various solutions (every bubble was about 5 mm diameter) in a conventional gas washing flask instead of distillation. The air was filtered through cotton wool before and after it was passed through the salt solution. The salts absorbed by the cotton wool from the air were extracted with water, and the resulting solution was analysed. The cotton wool itself contained certain salts, and thus deductions were made for the amounts of Cl^- and Br^- or Na^+ and K^+ respectively—values having been obtained by extraction of the cotton wool with water (see Table 4*a*).

Table 3. Ion Separation by Distillation

Sample	In 1 l. original sample			In 1 l. distillate			In 1 l. original sample			In 1 l. distillate		
	Cl^- (mg)	Br^- (mg)	Cl^-/Br^-	Cl^- (mg)	Br^- (mg)	Cl^-/Br^-	Na^+ (mg)	K^+ (mg)	Na^+/K^+	Na^+ (mg)	K^+ (mg)	Na^+/K^+
Ocean water	22,470	74	304	0·178	0·007	25						
				2·31*	0·191*	12·1*						
Lake of Kinereth	369	3·23	114	0·055	0·0067	8·2						
				1·017*	0·219*	4·6*						
Dead Sea water	203,000	4,600	44	0·145	0·013	11·2	39,900	6,600	6	0·204	0·055	3·7
				1·526*	0·137*	11·1*						
Tap water, Jerusalem	132	0·427	310	0·08	0·006	13	83·5	3·7	12·6	0·24	0·064	3·8

* Figures received without spray trap.

Table 4a. Ion-separation by air-bubble bursting (absorption on cotton wool)

	Bubbling time (h)	Original ion-ratio Cl^-/Br^-	Absorbed on 1 g cotton (after deduction of blank value) Cl^- (mg)	Br^- (mg)	Cl^-/Br^-
Sea water	54	300	0·484	0·0028	172
,,	125	300	0·398	0·0026	153
,,	150	300	0·951	0·0079	120
		Na^+/K^+	Na^+ (mg)	K^+ (mg)	Na^+/K^+
Sea water	312	27	0·230	0·040	5·7
KBr 0·44 g, KCl 3·0 g, NaCl 143·2 g, 1	50	33	0·362	0·024	15·0
		Mg^{++}/Pb^{++}	Mg^{++} (mg)	Pb^{++} (mg)	Mg^{++}/Pb^{++}
$Mg(NO_3)_2 \cdot 6H_2O$ 102·5 g, $Pb(NO_3)_2$ 0·132 g, 1	75	116	0·352	0·049	7·0

Table 4b. Ion-separation by air-bubble bursting (absorption in triple-distilled water)

	Bubbling time (h)	Original ion-ratio Cl^-/Br^-	Absorbed in 1 l. tri-distilled water Cl^- (mg)	Br^- (mg)	Cl^-/Br^-
Artificial sea water	316	364	1·775	0·018	98
,, ,, ,, + NaCl	312	400	3·292	0·0282	117
,, ,, ,, + NaCl	312	400	4·733	0·0468	101
		Na^+/K^+	Na^+ (mg)	K^+ (mg)	Na^+/K^+
Artificial sea water	312	27	3·94	0·55	7·2
		Mg^{++}/Pb^{++}	Mg^{++} (mg)	Pb^{++} (mg)	Mg^{++}/Pb^{++}
$Mg(NO_3)_2 \cdot 6H_2O$ 102·5 g, $Pb(NO_3)_2$ 0·132 g, 1	240	116	0·328	0·180	2·0
		Na^+/Ca^{++}	Na^+ (mg)	Ca^{++} (mg)	Na^+/Ca^{++}
NaCl 30 g, $CaCl_2$ 3·4 g, 1	312	10	0·243	0·0453	5·4
NaCl 30 g, $CaCl_2$ 30 g, 1	312	1·1	0·206	0·382	0·54

Another artificial solution, containing magnesium and lead nitrate, was treated in the same way and the spray analysed. The cotton wool used for absorbing the spray contained magnesium to the extent of less than 0·024 mg/g cotton and lead to the extent of less than 0·005 mg/g cotton (see Table 4*a*).

In another series of experiments, the salts carried over by the air were absorbed in water that had been distilled three times. The original solutions were also prepared by dissolving the various salts in thrice-distilled water (see Table 4*b*).

It can be seen that an air stream with single bubbles shows the effect almost as markedly as the distillation at 100° C. An air stream where many bubbles coalesce to form a foam has practically no effect of ion separation. The fact that the spray of boiling solutions shows the separation of ions clearly indicates that no solid-phase separations occur during evaporation from droplets as suggested as a 'post-wave breaking fractionation' (Sugawara and Kawasaki[3]); the separation seems to occur only in the bursting bubble.

The change of ion ratios by 'syn-bubble breaking fractionation', as Sugawara and Kawasaki propose to call it, is probably of considerable importance in the accumulation of the heavy or large ions, such as potassium, found in arid soils in which rainwater has been evaporated to dryness for a long time, without the residue being washed out. The way in which heavy metals accumulate in latterites may also be related to the ion separation in breaking bubbles.

The analytical procedures used in these investigations were as follows:

Cl^- (Br^-) —mercurimetric method.

Br^- —modified Van der Meulen method (Kaplan and Schnerb[5]).

K^+ and Na^+—flame-photometric method.

Mg^{++} —complexometric (EDTA) method.

Pb^{++} —colorimetric method, according to *British Pharmacopoeia*.

Ca^{++} —flame-photometric method.

We thank Miss C. Halevy for her help with the analytical work.

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CHEMISTRY

Collapse of Polyacrylamide Molecules on Electron Irradiation

Individual spherical molecules of glassy amorphous polymers, with molecular weights of more than about 5×10^5, can be deposited from a solvent-precipitant mixture, on to a thin substrate and observed in the electron microscope after shadowing with a heavy metal[1,2].

It has been reported that molecules of polyacrylamide behave in an unusual way when examined in the electron microscope[2]. The molecules appear to collapse into a shapeless mass, even in a weak electron beam, and even though the glass transition temperature of polyacrylamide is of the order of 130° C. The behaviour is similar to that of molecules of rubbery polymers, which are not self-supporting. Richardson[2] has suggested that this effect may represent some inherent weakness of this type of polymer structure to electron irradiation.

The observation has been confirmed and the effect investigated experimentally by subjecting the bulk polymer to electron irradiation. Results suggest that the phenomenon is due to intramolecular bond formation rather than to degradation of the polymer.

Particles of polyacrylamide weighing about 15×10^{-5} g were subjected to electron bombardment in an A.E.I. *E.M. 6G.* electron microscope, using electron energies of 30–100 kV, electron currents of 15–70 μamp and exposure times of 5–100 sec. The particles were then examined visually under a low-power optical microscope and a solubility test was carried out.

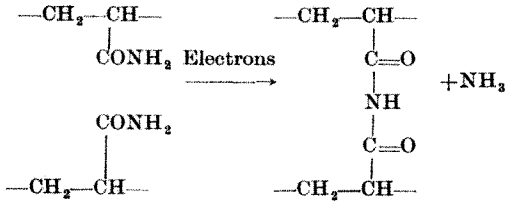
It was found that the polymer quickly became insoluble after even short exposures to a weak electron beam (for example, 10-sec exposure in an electron beam of 15 μamp). Water is the best solvent for polyacrylamide, and a 10^{-3} per cent solution of non-irradiated polymer can be obtained in about 3 h without agitation, while the irradiated polymer readily swells to several times its original volume, but remains quite insoluble after immersion in water for 10 days.

After short exposure times to weak electron beams, the polymer does not change visually, except for a decrease in volume of about 10 per cent, which was observed in the electron microscope. The polymer particles remain transparent, colourless, and identical with the non-irradiated polymer. After long exposures, the polymer becomes faintly yellow in colour, but still remains transparent. The yellowing becomes more pronounced as the exposure time and/or intensity is increased, and finally it becomes opaque. As the exposure conditions become more severe, an accompanying decrease in solvent swelling occurs.

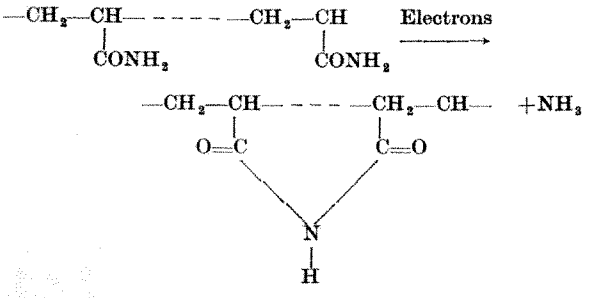
Using a simple indentation test, no difference in mechanical behaviour was detected between the irradiated and

non-irradiated polymer. Softening commenced at 130 ± 5° C in both cases.

These observations strongly indicate that intermolecular cross-links are formed, on electron irradiation, rather than that the polymer is degraded. It is proposed that the following imidization occurs:



The collapse of single molecules of polyacrylamide in an electron beam may be explained on the basis of intramolecular imidization. The molecules are sprayed from a solvent–precipitant mixture in which the solvent is more volatile than the precipitant. On evaporation, the solvent becomes progressively poor and conditions for polymer–polymer contacts become highly favourable. Spherical, self-supporting molecules can be isolated if the solution is sufficiently dilute. Each molecule consists of a tightly and randomly coiled chain. In this conformation, amide groups along the chain will be in close proximity to one another, and favourable conditions will exist for the condensation of neighbouring amide groups on electron irradiation, as follows:



Further experiments are planned, in an effort to confirm cross-linking. The relationship between irradiation conditions and degree of cross-linking will be investigated from solvent swelling measurements.

Thanks are due to the American Cyanamid Co. for a donation of polyacrylamide.

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Electron-transfer Reactions of Europium (II) in Aqueous Perchloric Acid Solutions

Electron-transfer reactions with europium (II) as the reducing ion have been studied in aqueous perchloric acid solution, $\mu = 2{\cdot}0$ M, using conventional spectrophotometric techniques. Such reactions are of interest in that little is known about the readiness with which electrons are transferred to and from *f*-orbitals[1,2]. Although europium (II) is the most stable of lanthanide (II) oxidation states, it reacts rapidly with oxygen and oxygen-free systems have therefore to be used. In preliminary experiments it was shown that europium (II) reduction of perchlorate ions is negligible (europium (II) concentrations of the order 0·04 M).

The reaction

$$Eu^{II} + V^{III} \rightarrow Eu^{III} + V^{II}$$

is first order in both the reactants

$$\text{Rate} = k_{obs}[Eu^{II}][V^{III}]$$

where k_{obs} is hydrogen-ion dependent

$$k_{obs} = k_1 + k'[H^+]^{-1}$$

The rate constant k_1 (0·0090 l.mole^{-1}sec^{-1} at 25° C) corresponds to the reaction of Eu^{2+} with V^{3+}, and it is assumed that k' is for the reaction of Eu^{2+} with VOH^{2+}. Thus k' may be written k_2K_H, where k_2 (2·00 l.mole^{-1}sec^{-1} at 25° C) is the true rate constant and K_H (0·002 l.$^{-1}$mole) (ref. 3) the hydrolysis constant for V^{3+}. From the temperature-dependence activation parameters corresponding to k_1 and k_2 are $\Delta H_1^{\ddagger} = 11{\cdot}4$ kcal mole^{-1}, $\Delta S_1^{\ddagger} = -30{\cdot}1$ e.u., $\Delta H_2^{\ddagger} = 6{\cdot}2$ kcal mole^{-1} and $\Delta S_1^{\ddagger} \sim -35$ e.u. Rates observed for the reaction of chromium (II) with vanadium (III) ($k_{obs} = 0{\cdot}85$ l.mole^{-1}sec^{-1} in 1·0 N, $HClO_4$ at 25° C, $\mu = 2{\cdot}0$ M) (ref. 4) are considerably faster than those for the europium (II) reaction ($k_{obs} = 0{\cdot}013$ l.mole^{-1}sec^{-1} under identical conditions) although the free energy change is slightly less favourable.

From the difference in standard oxidation potentials of 0·02 volts for the Eu^{II}/Eu^{III} and Cr^{II}/Cr^{III} couples[5] an equilibrium constant of 2·2 can be calculated for the reaction:

$$Eu^{II} + Cr^{III} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Eu^{III} + Cr^{II}$$

Since the exact nature of the solution medium is important, and the difference in standard oxidation potentials is so very small, such an equilibrium constant can be considered only approximate. Both the forward and reverse reactions have been studied and are extremely slow at 25° C ($k_3 \sim 1{\cdot}7 \times 10^{-5}$ l.mole^{-1}sec^{-1} and $k_4 \sim 1{\cdot}4 \times 10^{-5}$ l.mole^{-1} sec^{-1}). The addition of chloride ions does not affect the forward reaction, but in the reverse the rate is much faster and substitution inert $CrCl^{2+}$ is formed. With $CrCl^{2+}$ as the chromium (III) reactant the forward reaction is also much faster:

$$Eu^{2+} + CrCl^{2+} \xrightarrow{k_5} Eu^{3+} + Cr^{2+} + Cl^-$$

and at 25° C $k_5 = 2{\cdot}2 \times 10^{-3}$ l.mole^{-1}sec^{-1}. A comparison of rate constants for the reactions $Eu^{2+} - EuCl^{2+}$ (ref. 6), $Fe^{2+} - FeCl^{2+}$ (ref. 7), $Cr^{2+} - CrCl^{2+}$ (ref. 8), and $Eu^{2+} - CrCl^{2+}$ (which have little or no free energy change) and for the reactions $Cr^{2+} - Co(NH_3)_5\ Cl^{2+}$ and $Eu^{2+} - Co(NH_3)_5\ Cl^{2+}$ (ref. 2) (which have almost identical free energy change) supports the belief that it is more difficult to transfer electrons from *f*-orbitals.

The reaction

$$Eu^{II} + V^{IV} \xrightarrow{k_6} Eu^{III} + V^{III}$$

is too fast for a full kinetic study using conventional techniques. At 0° C with reactant concentrations $\sim 10^{-4}$ M ($\varepsilon = 530$ for Eu^{II} at 320 mμ) and $[H^+] = 0{\cdot}2$ N, k_6 is $\sim 10^3$ l.mole^{-1}sec^{-1}. In the reactions of europium (II) with chromium (III), vanadium (III), vanadium (IV) and iron (III) (from ref. 9) there is a rough linear correlation of rate constants with the standard free energy change.

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BIOCHEMISTRY

Action of Neuraminidase on Human Kidney Alkaline Phosphatase

STARCH-GEL electrophoresis and chromatography on substituted cellulose ion-exchangers have demonstrated that extracts of human kidney contain more than one protein possessing alkaline phosphatase activity. Similar patterns of distribution of enzymatically active zones after starch-gel electrophoresis have been obtained by several workers[1,2] and three distinct zones can often be demonstrated: a single zone migrating rapidly towards the anode (Fig. 1, zone 1), a second broad, heterogeneous component (Fig. 1, zone 2), and a third band which migrates very slowly and which may represent enzyme bound to lipid material (Fig. 1, zone 3). The first rapidly migrating component cannot be demonstrated in all extracts, but may sometimes appear after storage at −15° for some months. Elution patterns of kidney alkaline phosphatase from DEAE-'Sephadex' columns exhibit a broad similarity, but here also there is some variation from extract to extract. A common feature of all such chromatograms, however, is a sharp peak of activity appearing first in the effluent (Fig. 2). The elution pattern of the remaining enzyme is more varied, but often a second fairly broad peak is obtained[3]. The presence of sialic acid groups is known to increase the electrophoretic mobility of proteins[4], and Robinson and Pierce[5] have demonstrated that, after treatment with neuraminidase to remove sialic acid, the electrophoretic mobility of certain alkaline phosphatase components was reduced. To investigate whether the binding of sialic acid is in any way related to the heterogeneity of kidney alkaline phosphatase, this enzyme has been treated with neuraminidase.

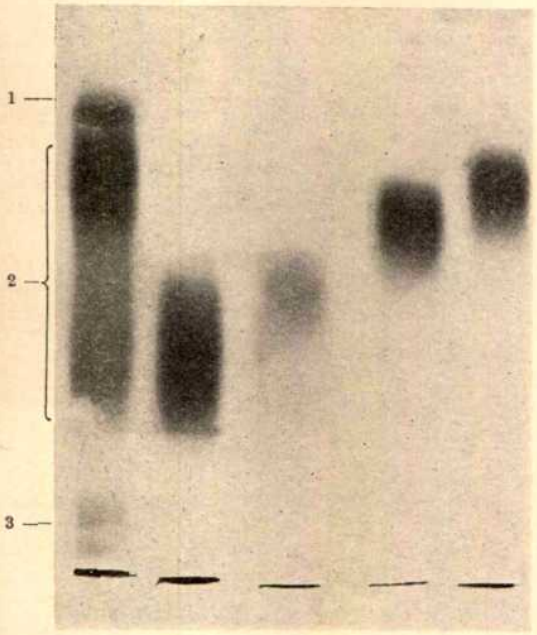


Fig. 1. Starch-gel electrophoresis of alkaline phosphatase of human kidney extract (*e*), and fractions (*A*–*D*) from DEAE-'Sephadex' column. Numbers indicate active zones in whole extract; letters correspond with chromatographic fractions of Fig. 2. (Anode at top, sample slots near bottom)

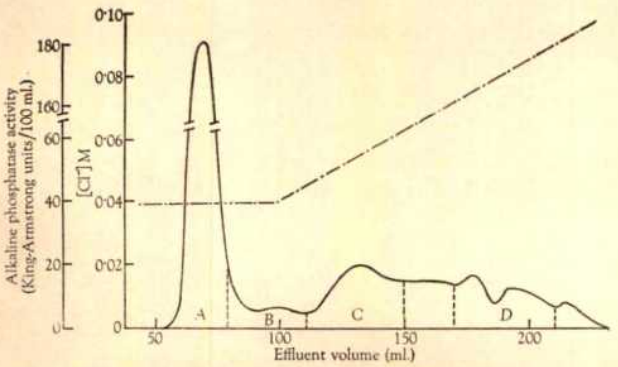


Fig. 2. Chromatogram of kidney extract on DEAE-'Sephadex' column. ———, Phosphatase activity; – – –, chloride concentration

Extracts of alkaline phosphatase were made by Morton's[6] butanol method from human kidneys obtained from the post-mortem room within a few hours of death. The crude extracts were concentrated by dialysis against a 30 per cent 'Carbowax' solution and partially purified by chromatography on DEAE-'Sephadex'. The enzyme was eluted by an increasing chloride ion concentration in *tris* buffer[5] and 5 ml. fractions were collected. Each fraction was assayed for phosphatase activity and chloride concentration, and fractions coinciding with peaks of enzyme activity were concentrated prior to electrophoresis on starch gel. Horizontal starch-gel electrophoresis was carried out in the discontinuous buffer system of Poulik[7] at *p*H 8·6 for 17 h at 10 V per cm and zones of enzyme activity were then located by the method of Estborn[8]. Kidney alkaline phosphatase extract (0·1 ml.) or concentrated column effluent fractions were incubated at 37° in 0·4 ml. of *tris* buffer (*p*H 7·7) with 50 U. neuraminidase (L. Light and Co.) for 20 h (ref. 5).

Fig. 2 shows a chromatogram of kidney alkaline phosphatase on DEAE-'Sephadex' and Fig. 1 the electrophoretic mobilities on starch gel of the various kidney phosphatase components eluted from the column. The first peak of the chromatogram corresponds with the most cathodal portion of the second electrophoretic band and it appears electrophoretically homogeneous. Alkaline phosphatase that elutes at progressively higher chloride ion concentrations consists of further portions of the second band of increasing electrophoretic mobility. Electrophoresis of all fractions demonstrated that the original pattern obtained with whole kidney extract could be built up. Re-chromatography of individual fractions followed by electrophoresis showed that their relative mobilities remained unchanged.

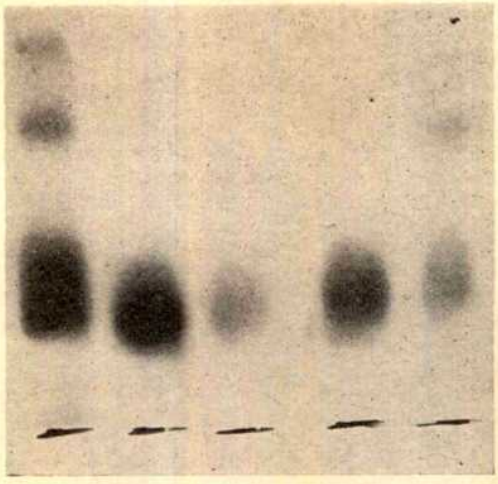


Fig. 3. Starch-gel electrophoresis of alkaline phosphatase of kidney extract (*e*) and column fractions (*A*–*D*) after neuraminidase treatment. (Anode at top, sample slots near bottom)

Fig. 3 shows the effect of treatment with neuraminidase on the electrophoretic distribution of whole kidney alkaline phosphatase and on the several chromatographic fractions. With both the whole extract and the chromatographic fractions, the main product of such treatment was a slowly migrating component, and a comparison of the

electropherograms showed that the slowly migrating enzyme species produced from each were electrophoretically identical. There was no significant loss in enzyme activity. Faster-migrating zones of low activity could be demonstrated in the kidney extract and in the last chromatographic fraction, *D*. These may indicate the presence of neuraminidase-resistant components in kidney alkaline phosphatase or they may arise from incomplete action of neuraminidase. Some evidence for the latter possibility was obtained by prolonging the time of incubation with neuraminidase when the remaining faster bands became more diffuse and weaker in activity.

The heterogeneity of the chromatograms and electropherograms of kidney alkaline phosphatase might suggest that there is in this organ more than one protein with alkaline phosphatase activity and that the several proteins are structurally different. However, as the results of chromatographic and electrophoretic separation procedures with different tissue preparations are not always identical, it is possible that there is only one kidney alkaline phosphatase, perhaps represented by the first chromatographic peak since this is the component which occurs most reproducibly, from which other enzymatically active forms may be derived. The present experiments would seem to indicate that the structurally different, but functionally similar, alkaline phosphatases of human kidney arise as a result of variations in the amount of bound sialic acid. Removal of varying amounts of sialic acid during the extraction and isolation procedure may give rise to the variation between chromatograms of different extracts. Boyer[1] has demonstrated that kidney alkaline phosphatase exists in two immunologically distinct forms, so that it would seem that the action of neuraminidase can alter the antigenic properties as well as the electrophoretic mobility of kidney alkaline phosphatase. The question of whether differences in the amount of sialic acid bound by the enzyme exist in the cell, so giving rise to several forms of the enzyme *in vivo*, or whether they are artefacts produced in the laboratory from a single enzyme species, has still to be answered.

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Separation of Serum 5′-Nucleotidase and Non-specific Alkaline Phosphatase Activities

Phosphatases which exhibit a high specificity for the hydrolysis of nucleoside-5′-phosphates (5′-nucleotidases[1]) usually occur in association with non-specific alkaline phosphatases, for example, in blood serum. Methods which have been used for the estimation of the relative activities of the two enzymes in serum have depended on comparison of the rates of hydrolysis of two different phosphate substrates[2], or on the specific inhibition of 5′-nucleotidase by nickel ions[3]; few attempts have been made to separate the two types of enzyme activity[5]. This communication describes the resolution of the 5′-nucleotidase and alkaline phosphatase activities of human blood serum by ion-exchange chromatography and gel-filtration.

Sera from patients with obstructive biliary disease, in which both types of enzyme activity are greatly increased, were used as the source of the enzymes. Chromatography on DEAE-cellulose was carried out in 0·05 M *tris*-HCl buffer (*p*H 7·7)—the elution being by means of a gradient of increasing chloride concentration; the serum sample was dialysed overnight against the starting buffer before application to the column. Gel-filtration was carried out in 0·9 per cent w/v sodium chloride solution on a column of 'Sephadex' *G*-200 (Pharmacia, *AB*, Uppsala, Sweden). In each case the effluent fractions (3 ml.) were tested for alkaline phosphatase activity by the method of Kind and King[4] and 5′-nucleotidase activity by the method of Campbell[2]. In both types of experiment the recoveries of 5′-nucleotidase and alkaline phosphatase were between 80 and 100 per cent. The results are shown in Figs. 1 and 2. Ion-exchange chromatography shows that the greater part of the 5′-nucleotidase activity elutes at a higher chloride concentration than the bulk of the alkaline phosphatase (Fig. 1). Both enzymes are to some extent heterogeneous, and each major peak contains residual amounts of the other type of activity. It is probable that these contaminants represent portions of the different enzymes eluting at the same chloride concentrations rather than a lack of specificity in the enzyme assays: 5′-nucleotidase from other sources has been shown to have negligible action on phenyl phosphate, the substrate used for alkaline phosphatase assays, while the nickel-inhibited hydrolysis of adenylic acid, the method used to estimate 5′-nucleotidase[3], is highly specific for this enzyme. On gel-filtration, most of the 5′-nucleotidase activity is completely excluded from the gel (Fig. 2), while alkaline phosphatase elutes as a fairly symmetrical peak in the 7*S* globulin region. As on ion-exchange chromatography, the 5′-nucleotidase is heterogeneous on gel-filtration.

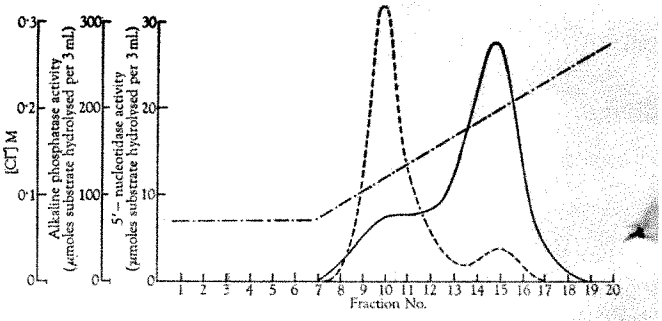


Fig. 1. Chromatogram of serum (1 ml.) on DEAE-cellulose column (28 × 1 cm). ———, 5′-Nucleotidase activity; – – –, alkaline phosphatase activity; ——·——, chloride concentration. Fraction volumes, 3 ml. each

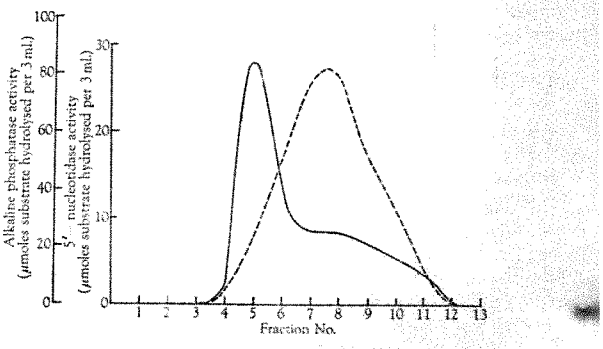


Fig. 2. Gel-filtration of serum (1 ml.) on 'Sephadex' *G*-200 column (32 × 1·3 cm). ———, 5′-Nucleotidase activity; – – –, alkaline phosphatase activity. Fraction volumes, 3 ml. each

The gel-filtration behaviour of the major part of the 5′-nucleotidase activity suggests that this enzyme may be of high molecular weight, an inference compatible with the observation of Kowlessar, Pert, Haeffner and Sleisenger[5] that 5′-nucleotidase is found in the β-lipoprotein and slow α_2-globulin regions after starch-gel electrophoresis of serum at *p*H 9. However, the combination of considerable negative charge at *p*H 7·7, as shown by the higher chloride ion concentrations required to elute 5′-nucleotidase from DEAE-cellulose, with apparently large molecular size may indicate the presence in the molecule of a carbohydrate component, since carbohydrate-containing proteins may behave anomalously on gel-filtration.

These methods for the separation of serum alkaline phosphatase and 5′-nucleotidase confirm the distinct identities of these enzymes, and provide a basis for the further purification and study of the latter.

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Isolation of γ-Hydroxynorvaline from *Lathyrus odoratus* Seed

In the course of an investigation of the metabolic pathways responsible for the incorporation of ^{14}C-cyanide into the amino-acids of young seedlings[1], paper chromatographic methods indicated that a previously unrecognized amino-acid was present in low concentration in extracts of *Lathyrus odoratus* (sweet pea) seedlings. This amino-acid has now been shown to be γ-hydroxynorvaline (I, α-amino-γ-hydroxypentanoic acid). The hydroxyamino acid first appears as a brown-coloured spot on chromatograms treated with ninhydrin, but the colour changes rapidly to the more usual blue-purple characteristic of most amino-acids.

γ-Hydroxynorvaline is present also in dry sweet pea seed and this material has provided a source for isolation. Ground seed (15 kg) was extracted twice with 75 per cent (v/v) ethanol (60 l.) during a period of 4 weeks. The extract obtained after pressing and filtering was applied to a column containing 8 kg of 'Zeo-Karb 225' resin (H^+ form) to absorb amino-acids. After non-cationic substances had been removed by thorough washing, the amino-acids were recovered by elution with 2 N ammonia. The eluate was evaporated to dryness and the residue was re-dissolved in water (1·5 l.) and adjusted to *p*H 4·5. This solution was applied to a column of 'Dowex-50 *W*' (× 8, mesh 100–200, length 75 cm, diam. 15·5 cm) and, after washing with 10 l. of water, the amino-acids were fractionated by elution with 0·25 N ammonia. Early fractions (each of 50 ml.) contained large amounts of γ-glutamyl-β-aminopropionitrile (*Lathyrus* factor)[2] in addition to small quantities of aspartic and glutamic acids: 40 g of pure γ-glutamyl-β-aminopropionitrile was obtained from these pooled fractions. γ-Hydroxynorvaline was present in two groups of fractions: first in fractions with alanine, serine and some other neutral amino-acids eluted after γ-glutamyl-β-aminopropionitrile, and then in the last ten of 104 fractions collected in all. In most of those later fractions the hydroxyamino acid was mixed with basic amino-acids, but the last three fractions contained only γ-hydroxynorvaline. After crystallization from aqueous-acetone, pure γ-hydroxynorvaline (200 mg) was obtained from the solid present in these three fractions.

The elution of γ-hydroxynorvaline at two points during the fractionation can be explained by lactonization of a proportion of the γ-hydroxyamino acid under the influence of H^+ ions liberated during amino-acid absorption on the cation-exchange resins. Molecules existing in the lactone form are basic and consequently are eluted together with, or later than, the basic amino-acids.

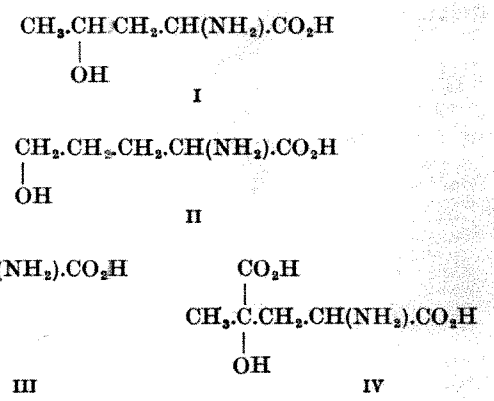
Table 1. The R_F values of γ-hydroxynorvaline and related compounds

(All values are expressed relative to leucine, 1·00)

Solvent	γ-Hydroxy-norvaline	γ-Hydroxynor-valine lactone	δ-Hydroxy-norvaline	γ-Hydroxy-valine
Butanol-acetic acid*	0·50	0·76	0·38	0·46
t-Amyl alcohol-acetic acid†	0·39	0·49	0·31	0·36

* Butan-1-ol–acetic acid–water mixture (90 : 10 : 29, by volume).
† Upper phase of 2-methylbutan-2-ol–acetic acid–water mixture) 10 : 1 : 10, by volume).

The isolated material gave the following elementary analysis (in per cent): C, 45·2; H, 8·32; N, 10·2 ($C_5H_{11}NO_3$ requires C, 45·1; H, 8·33; N, 10·5). When treated with N-hydrochloric acid at 120° C for 1 h, it was converted largely into its lactone, which appeared initially as a yellow spot moving ahead of the amino-acid on paper chromatograms. The R_F values determined for γ-hydroxynorvaline and its lactone are given in Table 1 together with those for δ-hydroxynorvaline (II) and γ-hydroxyvaline (III), two isomeric amino-acids isolated from soybean seeds[3] and *Kalanchoe diagremontiana*, respectively. In all solvents tested, the isolated γ-hydroxynorvaline behaved exactly as authentic synthetic material[5], kindly provided by Dr. A. J. MacLeod.



The identity of the isolate was confirmed further by electrophoretic comparison with synthetic γ-hydroxynorvaline. When paper electrophoresis was performed, essentially as described by Gross[6], at *p*H 2·0 (formic–acetic acid buffer) for 1·5 h (60 V/cm, 40 m.amp), the synthetic compound was separated into its two diastereoisomeric forms, which moved 26·2 and 28·7 cm, respectively, towards the cathode. Somewhat surprisingly, the natural hydroxynorvaline also gave two spots, moving identically with those obtained from the synthetic compound, but present in different relative proportions. It is unlikely that γ-hydroxynorvaline was present in the original seeds as a mixture of isomers, and so a small amount of the amino-acid was isolated directly from two-dimensional paper chromatograms. In this instance only the fast-moving diastereoisomer was observed. Presumably, during the large-scale isolation, partial epimerization had occurred at the γ-carbon atom as lactone was formed on the ion-exchange resin. Under identical electrophoretic conditions, threonine moved faster than *allo*threonine and, by analogy, natural γ-hydroxynorvaline probably possesses a *threo* configuration.

Additional confirmation of the γ-hydroxynorvaline structure was obtained by treating the hydroxyamino acid (50 mg) with 55 per cent (w/v) hydriodic acid and red phosphorus at 130° C for 12 h when norvaline was produced (compare Fischer and Leuchs[7]). This was isolated using

a small 'Zeo-Karb 225' column and analysed as follows (in per cent): C, 51·1; H, 9·58; N, 11·8 ($C_5H_{11}NO_2$ requires C, 51·3; H, 9·48; N, 12·0).

Unfortunately this hydriodic acid treatment also effected complete racemization and so no information was obtained concerning the configuration at the α-carbon atom in natural γ-hydroxynorvaline. Optical rotation measurements on isolated γ-hydroxynorvaline (mixed diastereoisomers) gave $[\alpha]_D^{20}$ values of +22° ($c = 5$ in water) and +32° ($c = 2·5$ in glacial acetic acid). When measurements were made using a 2·5 per cent solution in 5 N hydrochloric acid, the freshly prepared solution exhibited a dextro-rotation. As lactone was formed, $[\alpha]_D^{20}$ decreased: finally, after standing overnight, the $[\alpha]_D^{20}$ became constant at −16°. The higher positive value determined for $[\alpha]_D^{20}$ in glacial acetic acid compared with that in water tentatively indicates an L-configuration at the α-carbon atom but, in view of several recorded exceptions to the Clough–Lutz–Jirgensons rule[8], this conclusion will require confirmation.

Paper chromatography has shown that γ-hydroxynorvaline is present in several other legume seeds including species of *Astragalus*. At present nothing is known concerning the metabolic relationships of this hydroxyamino acid in legume seedlings, but it could arise by γ-decarboxylation of γ-hydroxy-γ-methylglutamic acid (IV) which is present in another legume (peanut seedlings[9]) and other plant species[10].

The sweet pea seed was provided by Unwin's Ltd., Histon, Cambridge. I thank Mrs. Jean Retallick and Mr. A. Smith for their assistance in this work.

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Effect of Oestradiol in Adrenal Corticoidogenesis: an Additional Step in Steroid Biosynthesis

Oophorectomy in the albino rat results in diminished pituitary adrenocorticotrophic hormone (ACTH) secretion and in diminished adrenal secretion of corticosterone[1]. The latter is demonstrable in peripheral plasma after injection of ACTH or following stress, in adrenal venous effluent, and in adrenal slices incubated *in vitro*. Administration of oestradiol-17β reverses these effects. Although the adrenal response to oestradiol may, in part, be mediated by changes in ACTH secretion, a direct effect is also suggested by the finding that oestradiol stimulates corticosteroid production when added to adrenal slices *in vitro*[2], and when administered to hypophysectomized, oophorectomized rats *in vivo*[3]. Additional investigations suggest an apparent inhibition of steroidogenesis in adrenal tissue after oophorectomy[4]. This communication reports observations giving a more specific definition of the relation of the ovary to adrenal function.

Female rats of the Sherman strain were oophorectomized when 30 days old and were not subjected to further manipulation for the next 6 weeks. Oestradiol-17β ('Estradurin', Ayerst) was then injected subcutaneously as a single depot dose of 100 μg/100 g body-weight. Investigations were carried out 2 weeks later. Adrenal homogenates were prepared and incubated for 60 min at 37° C under 95 per cent oxygen–5 per cent carbon dioxide. The reaction mixture contained: 0·154 M potassium chloride, 0·02 M sodium bicarbonate, $1·25 \times 10^{-3}$ M Ca^{++}, $3·3 \times 10^{-3}$ M glucose-6-phosphate, 10^{-3} M TPN, and 20 mg adrenal tissue/ml. 10 μg corticosterone was added to half the beakers before incubation. The incubates were extracted with chloroform and steroid production was measured in equal volumes by acid fluorescence[5], by ultra-violet absorption at 240 mμ, and by the blue tetrazolium (BT) reaction[6]. Fluorometry is the most specific of these methods for corticosterone, since other steroids which fluoresce are not produced in significant quantities by the rat adrenal cortex[7]. Ultra-violet absorption is based on the Δ-4,3-keto configuration in ring *A* which includes many other steroids, such as deoxycorticosterone, 18-OH-corticosterone and aldosterone, as well as corticosterone. The BT reaction is even less selective and requires only the C-20, 21 α-ketolic side chain.

The results of a typical experiment are summarized in Table 1. Corticosterone yields were significantly lower ($P < 0·01$) in adrenal homogenates from oophorectomized rats than those from intact or oestradiol-replaced animals when measured by fluorescence. Similar values were obtained with ultra-violet absorption. However, no difference between intact and oophorectomized rats was demonstrated with the BT reaction. Following addition of corticosterone at the outset of incubation, a 50 per cent loss of the added steroid occurred with the adrenal homogenates from oophorectomized rats when measured either by fluorescence or by ultra-violet absorption. No loss could be detected with the BT reaction.

Table 1. Steroid Production by Rat Adrenal Homogenates *in vitro**

Method	Direct incubation (μg/100 mg adrenal) Intact	Oophor.	Oophor. + oestradiol	Corticosterone added (μg/beaker remaining) Intact	Oophor.	Oophor. + oestradiol
Fluor.	35·0	20·3	41·0	9·5	5·4	9·2
Ultra-violet	44·6	20·2	53·4	10·2	5·4	11·0
BT	60·0	57·2	64·4	13·5	12·5	14·0

* Each value represents the mean of three observations.

These data indicate that total corticosteroid production by adrenal homogenates *in vitro* is not diminished by oophorectomy. However, the amount of corticosterone obtained is decreased, and this finding is consistent with previous observations[1]. The diminution in the yield of corticosterone may be explained by the capacity of adrenal tissue from oophorectomized rats to metabolize corticosterone further. The resulting metabolite(s) is characterized by a loss of the Δ-4,3-keto configuration in ring *A* but an unaltered side chain. I suggest that one effect of oestradiol is to inhibit the rate of the reaction responsible for the formation of this metabolite.

Additional data relating to this hypothesis were obtained by the addition of both cholesterol-26-^{14}C and cholesterol-4-^{14}C to adrenal homogenates prepared as described. Following incubation, isocaproic acid-^{14}C production was measured by the method of Ichii *et al.*[8], and corticosterone-4-^{14}C production by the addition of corticosterone-1,2-^{3}H with chromatography to constant ^{14}C : ^{3}H ratio on a column and four thin-layer systems with silica-gel *G*. Radio-isotopes were obtained from New England Nuclear Corporation. The chromatographic systems were: *cyclo*hexane–ethyl acetate, 2 : 8; ether; chloroform–acetone, 7 : 3; butyl acetate–methanol, 99 : 1. Measurements were made with a Packard Tri-Carb liquid scintillation spectrometer. The results are summarized in Table 2. Oophorectomy carried out in advance

Table 2. Products resulting from Incubation of Cholesterol-4-^{14}C and -26-^{14}C (0·2 μc. of Each/100 mg) with Rat Adrenal Homogenates *in vitro* (d.p.m./100 mg Adrenal Tissue/120 min)*

Product	Intact	Oophor.	Oophor. + oestradiol
Isocaproic acid-^{14}C	154,741	169,741	168,731
Corticosterone-^{14}C	48,639	30,432	48,206

*Each value represents the mean of three observations.

resulted in a significant reduction ($P < 0.01$) in the yield of corticosterone from labelled precursor, although the total amount of cholesterol converted to corticosteroids was not changed, as measured by the production of isocaproic acid-^{14}C. The observations based on unlabelled and labelled steroids are in accord.

The significance of a step (or steps) in the adrenal cortex for the metabolism of corticosteroid beyond corticosterone regulated by oestrogen is not clear. One possibility is that this mechanism provides a means of regulating the output of physiologically active steroid independent of that controlled by pituitary ACTH secretion.

These results will be discussed in greater detail in a subsequent publication. This investigation was supported in part by research grant (*AM*-03370) and research career development award (*AM*-K3-13960) from the U.S. Public Health Service. I thank Miss Lydia K. Brown and Miss Nancy L. Hough for their technical assistance.

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Effect of Deuterium Oxide on the Relaxation of Glycerol-extracted Muscle

DEUTERIUM oxide is known to retard the contraction of living muscle[1–3]. The site of its action, however, remains obscure; it affects significantly neither the membrane excitation[4–6] nor the contraction of the proteins in glycerol-extracted muscle[5]. Deuterium oxide has, however, a differential effect on the contractile response to a number of nucleotides; whereas the interactions with adenosine triphosphate (ATP) and cytodine triphosphate (CTP) are not affected, those with inosine triphosphate (ITP) and uridine triposphate (UTP) are retarded[5]. The ATPase of myofibrils[6] and myosin *B*[7] is reduced by deuterium oxide under certain conditions; at neutral *p*H the myosin *B* ATPase is, however, not significantly affected[7]. In the case of ITPase, the reaction is always slower on substitution of deuterium oxide for normal water (H_2O) (ref. 7).

This communication reports additional observations on glycerol-extracted muscle. Preparations which have the property of spontaneous relaxation after ATP-induced contraction are known to lose this property on pre-soaking in water–salt solution for variable periods of time[8,9]. It will be shown that in deuterium oxide the rate of this loss is reduced.

Rabbit psoas muscles were used which had been extracted in 50 per cent glycerol[10] for 3–16 weeks. Fibre bundles about 6 cm long and about 0·2 mm thick were dissected at room temperature and cut in half; one-half was observed in deuterium oxide and the other in normal water.

The bundles were pre-soaked in either deuterium oxide (99·8 per cent) or distilled water for periods of time varying from 15 min to 9 h and then mounted for 2 min in an experimental bath containing deuterium oxide– or water–salt solutions, respectively. The solutions contained 0·16 M potassium chloride, 0·01 magnesium chloride and *tris*-maleate buffer 0·02 M, *p*D 6·8, *p*H 7·2 (ref. 11); in some instances buffer was not used. ATP (final concentration 4 mM) was applied and, later, calcium chloride (final concentration 1 mM). The solutions were stirred continuously. The isometric tension was measured with a force-displacement transducer and direct-writing oscillograph (Grass).

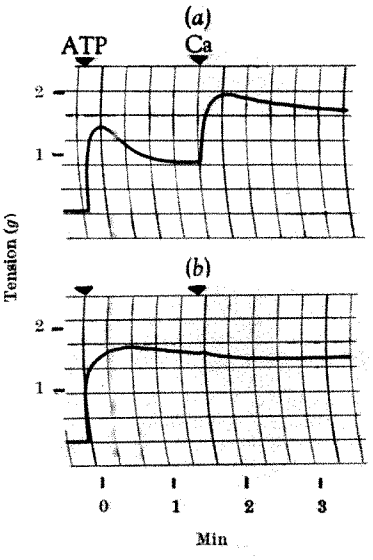


Fig. 1. (*a*) Isometric tension recording of psoas bundle extracted in 50 per cent glycerol at −20° C for 3 months. The bundle was soaked in deuterium oxide for 0·5 h and then placed in a deuterium oxide-salt solution. At 'ATP', 4 mM (final concentration) of ATP was added. This caused partial contraction followed by relaxation. At 'Ca', 1 mM (final concentration) of calcium chloride was added, resulting in recontraction. (*b*) Recording of the paired bundle which was soaked in normal water for 0·5 h and then placed in water-salt solution. ATP caused a full sustained contraction uninfluenced by calcium chloride

Muscle bundles, pre-soaked in water for 0·5–1 h, usually contracted maximally in response to ATP, did not relax spontaneously and were not influenced by subsequent addition of calcium chloride. In comparable experiments in deuterium oxide, on the other hand, there was usually spontaneous relaxation with recontraction occurring on addition of calcium chloride (Fig. 1). Table 1 summarizes the results and demonstrates that in general deuterium oxide, as compared with water, retarded the rate of loss of the relaxing property of the muscle. Deoxycholate, known to inactivate the relaxing factor[12,13], prevented the relaxation in deuterium oxide.

Table 1. SUMMARY OF RESULTS, COMPARING THE INFLUENCE OF DEUTERIUM OXIDE WITH THAT OF NORMAL WATER ON THE LOSS OF SPONTANEOUS RELAXATION IN GLYCEROL-EXTRACTED MUSCLE

No. of experiments	Period of soaking in water or deuterium oxide	Results	
		Deuterium oxide	Water
20	30 min—9 h	Spontaneous relaxation	No spontaneous relaxation
12	15 min—1 h	Relaxation velocity 2–3 times that in water	
10	15 min—1 h	Relaxation velocity equal to that in water	
5	30 min—1 h	No spontaneous relaxation	No spontaneous relaxation

The loss of spontaneous relaxation consequent on pre-soaking the preparation in water is due, presumably, either to the inactivation of the relaxing factor (sarcoplasmic reticulum) or to some soluble fraction diffusing out of the muscle. It has been suggested that water inactivates the relaxing factor and that this may be reversed by glycerol and sucrose[14].

It appears that deuterium oxide decreases the rate of the inactivation of the relaxing factor. Deuterium oxide will not, however, reverse the inactivation caused by water.

While deuterium oxide is known, of course, to retard reaction rates[15,16], there is no obvious suggestion as to the particular reactions affected in the present investigation.

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Effect of Kidney Beans (*Phaseolus vulgaris*) on Plasma Tocopherol-level and its Relation to Nutritional Muscular Dystrophy in the Chick

Dietary use of raw kidney beans (*Phaseolus vulgaris*) is known to increase the incidence of liver necrosis in weanling rats[1] and nutritional muscular dystrophy in chicks[2]. Although this effect has been attributed to some heat labile antagonist(s) to vitamin E, the nature of such antagonists and the mechanism of their action are unknown.

The purpose of this note is to report some findings which explain why raw kidney beans decrease the biological activity of vitamin E and what is the interrelationship of such process in the development of muscular dystrophy in chicks.

One-day-old White Plymouth Rock × Vantress chicks from vitamin E-depleted hens were used in this investigation. Each treatment consisted of replicated lots of ten male chicks housed in electrically heated battery brooders with wire-mesh floors. Feed and water were supplied *ad libitum*. The basal diet used was low-vitamin E casein-isolated soybean protein-torula yeast diet as described by Scott and Calvert[3], with modifications as follows: the diphenyl-*p*-phenylenediamine was omitted and the diet was supplemented with 1 per cent L-arginine, 0·0125 per cent ethoxyquin to prevent encephalomalacia and 0·1 p.p.m. selenium (as sodium selenite) to prevent exudative diathesis.

Chicks were killed at the end of the 4-week experimental period and scored for muscular dystrophy. The scoring scale ranged from zero, for absence of dystrophy, to 4, for maximal dystrophy. The average scores were determined by dividing the total scores for each group by the number of chicks in that group. Blood samples were taken from the chicks by heart puncture just before killing them. The heparinized blood was centrifuged at 2,500 r.p.m. for 10 min and the plasma was carefully pipetted off and frozen immediately for subsequent tocopherol analysis. Total plasma tocopherols were determined by the method of Quaife and Harris[4].

Results in Table 1 show the effect of raw and autoclaved kidney beans on plasma tocopherol-level and its relation to muscular dystrophy in chicks. Chicks on basal vitamin E low diet showed maximum symptoms of muscular dystrophy and a significantly low level of plasma tocopherol. Addition of 10 mg *d*-α-tocophenyl acetate to the basal diet increased the plasma tocopherol-level five-fold and reduced the muscular dystrophy to a minimum. Such direct correlation between the plasma tocopherol-level and the incidence of muscular dystrophy was reported earlier by Scott and Desai[5]. When 50 per cent raw kidney beans were included in the basal diet containing 10 mg *d*-α-tocophenyl acetate, the plasma tocopherol-level was significantly reduced and the muscular dystrophy was elevated to give a score of 3·6. On the other hand, when 50 per cent of raw kidney beans were substituted with an equivalent amount of autoclaved kidney beans, the plasma tocopherol-level was quite improved and the muscular dystrophy was correspondingly reduced.

Table 1. Effect of Raw and Autoclaved Kidney Beans on Plasma Tocopherol-level and Muscular Dystrophy in Chicks

Dietary treatment	Muscular dystrophy score (0–4)	Plasma tocopherol-level (μg/100 ml.)
Basal low vitamin E diet	4·0	159
Basal diet + 10 mg *d*-α-tocophenyl acetate/kg diet	0·8	887
Basal diet + 50 per cent raw kidney beans + 10 mg *d*-α-tocophenyl acetate/kg diet	3·6	227
Basal diet + 50 per cent autoclaved * kidney beans 10 mg *d*-α-tocophenyl acetate/kg diet	0·2	500

* Autoclaved at 15 lb. pressure for 30 min.

It appears from these results that at least one and the most important reason for this muscular dystrophy inducing effect of raw kidney beans is the interference in absorption and/or retention of vitamin E in the body. The interfering factor(s) could be specific heat-labile proteins or lipoproteins which bind vitamin E and make it unavailable to the body. The significance of effective absorption and retention of tocopherols for specific functions, such as prevention of muscular dystrophy, has already been discussed[6,7].

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PHYSIOLOGY

Cerebellar Activity, Apneustic Breathing, and the Neural Control of Respiration

Apneustic breathing, characterized by deep, sustained inspiratory activity, has figured prominently in theories on the neural control of respiratory rhythmicity[1]. This intriguing respiratory phenomenon has been described in the vagotomized, midpontile decerebrate animal[2–4]. In this preparation, the Hering–Breuer afferent system in the vagi and the pneumotaxic area in the rostral pons have been eliminated. Both the Hering–Breuer and the pneumotaxic influences appear to be predominantly inhibitory to the inspiratory mechanisms of the lower brain stem[4]. However, the apneustic area in the lower pons is not destroyed by midpontile decerebration and continues to provide a powerful facilitatory influence on the inspiratory neurones of the medulla[2–4]. In the presence of apneustic facilitation and the absence of Hering–Breuer and pneumotaxic inhibition, the medullary inspiratory neurones are excessively stimulated. The resultant excessive activity of the inspiratory neurones produces apneustic breathing[1–4].

In the vagotomized, midpontile decerebrate animal, the cerebellum as well as the caudal pons and the medulla remain intact. Thus the cerebellum may play a significant part in the phenomenon of apneustic breathing and, if such is the case, in the neural control of respiratory rhythmicity. The data obtained in the present work indicate that apneustic breathing and respiratory rhythmicity are indeed significantly affected by the cerebellum.

Thirty-five adult cats were used in this investigation. Anaesthesia was induced and maintained with ether until, in twenty-six experiments, the animals were decerebrated. Decerebration was effected at the midpontile level by a combination of arterial occlusion and brain stem transection procedures[5,6]. This decerebration technique normally minimized the problem of haemorrhage.

Cerebellar depression was effected by ischaemic, pharmacological, and surgical procedures. One or combinations of these procedures were utilized in each experiment. Ischaemic depression of cerebellar function was achieved by occlusion of the anterior and posterior cerebellar arteries with silver clips. Pharmacological depression was effected by application of procaine (1 per cent) or xylocaine (1 per cent) to the surface of the cerebellum or by injection of 0·05 ml. of these drugs subcortically. Surgical depression procedures consisted of ablation of part or all of the cerebellum by suction. The efficacy of the ischaemic technique was evaluated by post-mortem study of the brains after injection of vinyl acetate into the common carotid and vertebral arteries. The pharmacological application and injection areas were tagged with dyes. Ablation sites were determined by post-mortem examination of the brains after fixation.

Fifteen vagotomized, midpontile decerebrate cats exhibiting apneustic breathing and decerebrate rigidity were subjected to suppression of cerebellar function. In thirteen of these animals, significant enhancement of inspiratory activity was produced. This enhancement was expressed as an increase in the depth of inspiration, as an increase in the duration of inspiration, or as an increase in both inspiratory depth and duration. Respiratory rate, when altered, was decreased. Pulmonary ventilation per unit of time was increased, decreased, or unchanged depending on the relative alterations in respiratory depth and rate. Heart rate and arterial blood pressure tended to remain at normal or supranormal levels in these vagotomized, midpontile animals[7,8]. Decerebrate rigidity was often visibly augmented by cerebellar depression.

Eleven cats were subjected to midpontile decerebration and cerebellar depression prior to vagotomy. Midpontile decerebration typically produced a moderate increase in the depth of inspiration and, in some animals, a decrease in the rate of respiration. The long sustained inspiratory activity characteristic of apneustic breathing did not appear. Later, ischaemic, pharmacological, or surgical depression of cerebellar function in these midpontile animals markedly enhanced either or both the depth and the duration of inspiratory activity. In eight animals in this series, apneustic breathing patterns developed in spite of the integrity of the vagi (Fig. 1). Subsequent vagotomy produced further augmentation of inspiratory depth and duration.

Nine cats under ether anaesthesia were subjected to cerebellar depression without prior decerebration or vagotomy. Suppression of cerebellar activity in these animals typically produced significant augmentation of inspiratory depth. Respiratory rate, when altered, was almost invariably decreased. One animal exhibited a prolongation of inspiration that was suggestive of apneustic breathing. Moderate spasticity appeared in some of these animals; heart rate and arterial blood pressure tended to remain within normal ranges.

Analysis of the effects of depression of selective portions of the cerebellum revealed that augmentation of inspiratory activity was obtained from suppression of either the anterior or the posterior lobe of the corpus cerebelli. However, inspiratory enhancement was obtained more readily and was of greater magnitude when the anterior lobe was suppressed. Depression of function of both the anterior and the posterior lobes often produced greater augmentation of inspiratory activity than depression of either lobe alone.

Other investigators have revealed cerebellar effects on respiration. Stella[9] reported that ablation of the cerebellum in intercollicular dogs enhanced pulmonary ventilation. Moruzzi[10] found that faradic stimulation of the anterior lobe in precollicular cats suppressed respiratory activity, particularly the reflex respiratory responses elicited by occlusion of the common carotid arteries or by intracarotid injection of potassium cyanide. Zanchetti and Zoccolini[11] found that cerebellar stimulation in thalamic cats produced sham-rage outbursts which included marked hyperpnoea. More recently, Stella and Stevan[12] reported that cerebellar stimulation in intercollicular dogs may produce either facilitation or inhibition of respiratory activity.

In contrast with the investigations cited above in which cerebellar effects on respiration were studied in high decerebrate animals with the pontile pneumotaxic centre and the vagal Hering–Breuer system intact, the investigation described here explored cerebellar influences both in the presence and in the absence of pneumotaxic and Hering–Breuer activity. Consequently, the data from the present study can be related more directly to the basic neural mechanisms responsible for the phenomenon of apneustic breathing and for the neural control of respiratory rhythmicity. These data clearly indicate that the cerebellum in the cat provides a tonic, predominantly inhibitory influence on the inspiratory mechanisms of the lower brain stem. This cerebellar inhibition could be exerted on the excito-inspiratory neurones of the pontile apneustic centre, the inspiratory neurones of the medullary respiratory centre, or on both of these inspiratory mechanisms. The nature of the cerebellar influence places it in a position parallel to the inhibitory influences of the pneumotaxic centre and the Hering–Breuer system and antagonistic to the powerful facilitatory influence of the apneustic centre. Both the anterior and the posterior lobes of the corpus cerebelli, but particularly the anterior lobe, appear to give rise to this cerebellar respiratory activity.

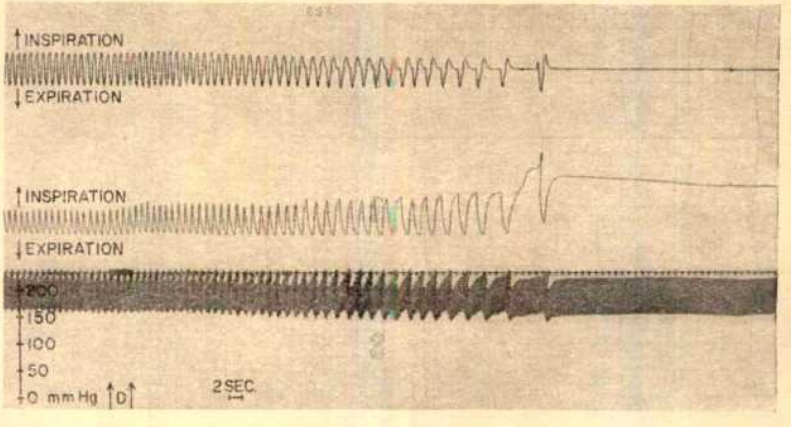


Fig. 1. Polygraph record of respiratory response to ischaemic depression (*D*) of cerebellar activity in a midpontile decerebrate cat, vagi intact. Sequence of tracings from the top: 1, respiration recorded with a Grass *PT* 5 transducer; 2, respiration recorded with a pneumograph; 3, arterial blood pressure recorded with a Statham *P* 23 transducer. Note the sustained inspiratory activity which developed approximately 1 min after *D*

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Heat Stress Degranulation of the Basophil Leucocyte in Man

Long an obscure but distinctive type of leucocyte, the basophil has in recent years been shown to play an active part in the immediate-type hypersensitivity reaction[1,2]. Evidence is accumulating that this cell releases its granules containing histamine and heparin in the presence of an antigen and a reactive antibody. These morphological changes of degranulation, however, are not specific nor limited to the allergic state. Certain drugs may induce similar degranulation in the complete absence of any sensitivity[3]. The investigation reported here extends our view of the role of this cell, since it shows that, in man, acute heat stress also induces the morphological changes of degranulation in the circulating basophil.

Ten normal, healthy, White and Negro male adults served as subjects. All medication was interdicted for 2 weeks before and during the entire investigation. Heat stress was achieved by exposing the men to an environment of 120° F with 50 per cent relative humidity for 30-min periods. To assess possible acclimatization, the 30-min stress was repeated daily for 7 days. Heparinized venous blood samples were obtained for absolute and morphological basophil counts[2] immediately before, immediately after, as well as 1 and 4 h after each daily heat stress. Control counts and morphological assessments were undertaken before and 1 week after the last heat stress. Cells were classified as normal or degranulated, that is, having fewer than normal granules, showing cytological evidence of extrusion of granules, or showing less staining. Table 1 summarizes representative data showing that, although the basophil count remained essentially normal, the subjects experienced a significant change in the morphology of the cell immediately after 30 min of heat stress. This was evident at 2 h, as well as at 4 h, and to a lesser degree at 24 h.

In nine of the ten subjects, 30 min of high temperature produced a dramatic increase in the number of basophils showing degranulation. The effect persisted for the entire 7 days, acclimatization not being evident. However, after a week of no exposure in the thermal chamber, the number of degranulated basophils returned to, or below, normal in each of the men.

The mechanism and significance of this heat-induced change in the basophil remain unknown, but it is interesting to speculate that perhaps the basophil releases the vasodilator histamine in response to elevated temperatures. This could thus serve as an adaptive mechanism since body temperatures did not rise appreciably during the 30 min of stress. It should be noted that the basophil is thermo-sensitive *in vitro*, remaining normal in chilled blood for longer than in blood kept at 37° C. Further investigation of the factors accounting for this stress-induced degranulation of the basophil would seem to be indicated.

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Table 1. Basophil Degranulation in Man immediately following Repetitive 30-min Heat Stress (120° F, 50 per cent Humidity)

Subject No.		Day 0 (before heat stress)	Days 1	2	3	4	5	6	7	Day 14 (no heat stress for 1 week)
1	Absolute basophil count (ABC)	69	49	59	46	41	53	49	71	65
	Degranulation (%)	13	79	42	80	85	81	59	42	5
2	ABC	36	39	20	28	39	41	24	25	35
	Degranulation (%)	25	51	20	32	27	39	54	64	14
3	ABC	25	43	34	35	46	55	55	55	60
	Degranulation (%)	32	79	76	65	67	74	61	65	18
4	ABC	33	34	35	43	33	44	26	34	63
	Degranulation (%)	39	76	45	69	54	63	76	85	10
5	ABC	15	38	21	19	28	23	15	20	50
	Degranulation (%)	26	41	66	73	71	69	86	75	12
6	ABC	66	78	65	66	75	86	80	70	45
	Degranulation (%)	21	29	24	57	35	51	56	87	22
7	ABC	34	36	45	41	31	35	25	28	46
	Degranulation (%)	38	52	51	24	42	45	32	30	30
8	ABC	71	45	33	36	38	51	39	45	73
	Degranulation (%)	29	88	84	86	81	76	84	75	27
9	ABC	49	56	48	40	26	54	34	54	54
	Degranulation (%)	31	14	27	75	92	94	44	75	27
10	ABC	30	41	41	40	39	34	21	21	46
	Degranulation (%)	13	82	82	82	79	88	76	76	19

Cholinesterase in the Mammalian Kidney

Cholinesterase activity has been demonstrated in portions of the renal tubule[1,2], but no one has determined which segment of the nephron contains the enzyme. This is surprising as cholinesterase activity is known to be associated with sodium transport in a number of tissues and might therefore be associated with sodium transport in the kidney. Koch[3] showed that cholinesterase is important for sodium transport through the gill epithelium of the freshwater crab and for sodium absorption from the anal papillae of the insect larva *Chironomus plumosus*. The gills of fish transport sodium and contain cholinesterase[4]. In frog skin Koblick *et al.*[5] found an excellent correlation of cholinesterase activity with average short circuit current and thus with sodium transport. Kamento[6] found cholin-

esterase in the gills and kidney of the crayfish, and his data suggest that at both sites it is involved in sodium regulation.

To demonstrate cholinesterase activity, I have used the method of Coupland and Holmes[7] on 20μ frozen sections of kidney, fixed overnight in 10 per cent formol saline. The optimal conditions for clearly defined staining of renal tubules are incubation for 5 h in a medium with acetylcholine as the substrate and an acetate buffer system to bring the final *p*H of the medium to five.

In the kidneys of twelve rats, four rabbits, four cats and two mice that had drunk freely before death, the only tubular cholinesterase activity was in short segments at the junction of the inner and outer strip of the outer medulla, beside the upper part of the vasa recta bundles. The exact segment could not be identified.

Six rats were given no water for 24 h before they were killed, so that they were producing a concentrated urine. The staining of their renal tubules was both more intense and more widespread than in the hydrated animals, extending from the inner part of the cortex to the junction of the outer and inner medulla (Fig. 1). These segments were identified as the thick ascending limbs of loops of Henle. The most intense staining was in tubules alongside the vasa recta and efferent arterioles which supplied them. By microdissection of portions of kidney in which the vessels had been injected[8], it was found that these tubules originate in juxtamedullary glomeruli and have long loops of Henle (Fig. 2).

The addition of 62*C*47 *h* di-iodide (10^{-5} M) to the incubating medium did not affect the cholinesterase activity of the tubules. It was completely inhibited by eserine (10^{-5} M) and 'Mipafox' (10^{-5} M). This is evidence that the enzyme present in the tubules is a pseudocholinesterase.

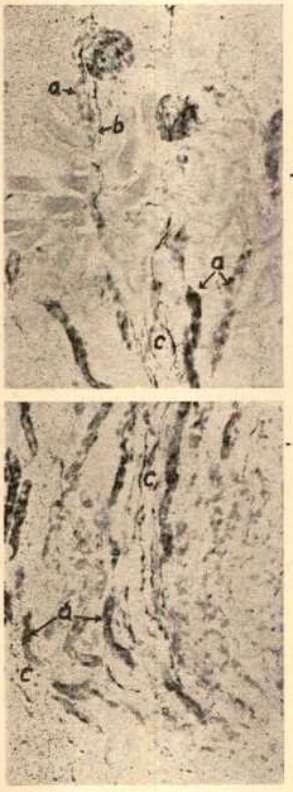


Fig. 1

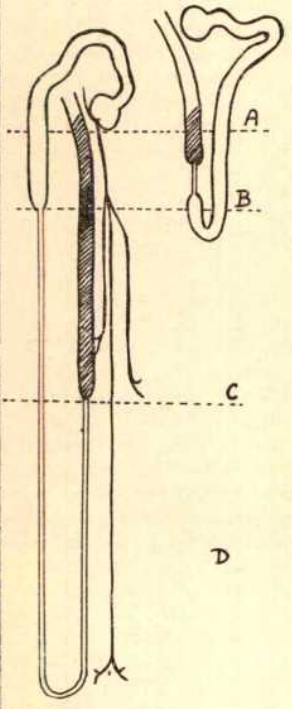


Fig. 2

Fig. 1. Sections from the medulla of a dehydrated rat showing (*a*) cholinesterase activity in the tubules, (*b*) efferent arteriole, and (*c*) bundle of vasa recta. (× 60)

Fig. 2. Diagram of the segments of tubule with cholinesterase activity. Black shading, hydrated rats. Cross-hatching, dehydrated rats. *A*, Cortex; *B*, outer strip; *C*, inner strip; *D*, inner medulla

It has been suggested that the counter-current mechanism for concentration of urine in the renal medulla depends on the active transport of sodium out of the ascending limb of the loop of Henle, and it is in the thick portion of this limb that I have found cholinesterase activity. It is increased in dehydrated animals producing a concentrated urine. It is therefore tempting to suggest that cholinesterase activity is linked with sodium transport in the mammalian nephron, and the work is being continued to see what further evidence there is for this.

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Stability of Adaptation of Critical Flicker Fusion Frequency to Intermittent Light

THE modification of critical flicker fusion frequency by intermittent light of varying frequency is unchanged by such physiological procedures as hyperventilation and rebreathing[1], and by drugs[2,3], which are known to alter mean critical flicker fusion frequency. Wave-length and the light–dark ratio of the light source[4] are important factors in determining binocular critical flicker fusion frequency, and the state of luminance of the opposite eye in determining monocular critical flicker fusion frequency[5]. Their possible influence on the adaptation phenomenon has, therefore, been investigated.

Light–dark ratio. Fifteen subjects were exposed on three days to light flickering at 20 and 50 c/s for 1 min after which ascending (*A*) or descending (*D*) thresholds of critical flicker fusion frequency were determined using Turner's method[6]. The order of measurement was randomized in a Latin square sequence. The light–dark ratio of the adapting light source varied on the 3 days between 9–1, 5–5, and 1–9, while that of the source used for determining critical flicker fusion frequency was maintained at 5–5.

The results were subjected to analysis of variance and are shown in Table 1. The difference (*d*) between mean values of critical flicker fusion frequency after adaptation to light at 20 and 50 c/s, with a light–dark ratio of 9–1, is not significantly different from that at light–dark ratios of 5–5 and 1–9. The mean thresholds after exposure to adapting frequencies with a light–dark ratio of 9–1 were significantly higher ($P < 0.01$) than with a light–dark ratio of 5–5 or 1–9. It may be that the first light produced a higher state of retinal light adaptation, which would be associated with higher critical flicker fusion frequency[4].

Wave-length. Eight subjects were exposed in random sequence to adapting lights at 20 or 50 c/s for 1 min, and *A* and *D* thresholds measured. The wave-length and luminance of the adapting light were either 565 mμ and 35 lumens/ft.² (yellow) or 620 mμ and 40 lumens/ft.² (red). The light source used to determine critical flicker fusion

Table 1. MEAN OF ASCENDING AND DESCENDING CRITICAL FLICKER FUSION FREQUENCY (C/S) AFTER EXPOSURE TO INTERMITTENT LIGHT AT 20 AND 50 C/S FOR 1 MIN WITH LIGHT–DARK RATIOS OF 9–1, 5–5, 1–9

		Frequency of adapting light (c/s) 20	50	*d*
Light–dark ratio of adapting light	9–1	31·30	32·10	0·80
	5–5	30·60	31·60	1·00
	1–9	30·75	31·60	0·85

$n = 30$ *S.E.* of difference = 0·102 c/s. 5 per cent critical difference = 0·204 c/s.

Table 2. Mean of Ascending and Descending Critical Flicker Fusion Frequency (c/s) after Exposure to Intermittent Light at 20 and 50 c/s for 1 min using a Red (*R*) or Yellow (*Y*) Light ($n = 16$)

	Frequency of adapting light (c/s) 20	50	d_1	Dd_1
R	31·66	32·50	0·84	0·10
Y	31·05	31·99	0·94	
d_2	0·61	0·51		
S.E. of difference		0·216		0·308

frequency was a neon lamp (wave-length 570–610 mμ, luminance 35 lumens/ft.²).

The results are shown in Table 2. The difference (d_1) between mean thresholds after adaptation to 20 and 50 c/s for 1 min with a red light was not significantly different from that with a yellow light. However, the mean thresholds after exposure to the red light were significantly greater ($P < 0·02$) than to a yellow light (d_2). Hecht and Schlaer[7] showed that colour of the light source was an important variable in determining critical flicker fusion frequency, although Landis[4] directed attention to the difficulty in controlling luminance and colour separately. This experiment demonstrates, however, the effect of previous adaptation to light of different wave-lengths on the subsequent determination of critical flicker fusion frequency. It is unlikely that the small difference in luminance between the adapting light sources contributed significantly to this effect.

Luminance of the opposite eye. Lipkin[5] showed that the critical flicker fusion frequency of one eye was related to the state of luminance of the contralateral eye. This was confirmed by a simple experiment in which ascending thresholds were measured in the left eyes of fourteen subjects after exposure for 1 min to light at 50 c/s with the right eye either adapted to the same background illumination as the left (1 lumen/ft.²) or dark adapted by being completely covered for 5 min. The mean critical flicker fusion frequency in the light-adapted state was 32·09 c/s and in the dark-adapted state 33·11 c/s (*S.E.* of difference = 0·30 c/s $P < 0·01$).

The experiment was now repeated in eight subjects with measurements of *A* and *D* thresholds in the left eye after exposure to 20 and 50 c/s adapting light, while the right eye was either light- or dark-adapted.

The results are shown in Table 3. They confirm a significant difference ($P < 0·01$) between the mean thresholds under conditions of light and dark adaptation (d_2), but the difference between mean thresholds after 20 and 50 c/s (d_1) with light adaptation was not significantly different from that with dark adaptation.

Table 3. Mean of Ascending and Descending Critical Flicker Fusion Frequency (c/s) of Left Eye after Exposure to Intermittent Light at 20 and 50 c/s for 1 min with the Right Eye Light (*L*)- or Dark (*D*)-adapted ($n = 16$)

		Frequency of adapting light (c/s) 20	50	d_1	Dd_1
State of adaptation	*L*	29·53	30·64	1·11	0·08
	D	30·46	31·49	1·03	
	d_2	0·93	0·85		
S.E. of difference			0·312		0·442

It appears, therefore, that the adapting effect of intermittent light of varying frequency on critical flicker fusion frequency is unaltered by changing the light–dark ratio or wave-length of the adapting light, or the state of light adaptation of the opposite eye. It has been suggested[1,6] that this phenomenon may have its counterpart in similar neurophysiological mechanisms of adaptation in other modalities where judgments have to be made and frames of reference established[8]. It is, perhaps, appropriate that such a phenomenon, so basic in the economy of the organism, should be stable and unaltered by physiological and pharmacological procedures which readily modify the actual threshold. Further investigation is indicated to determine the stability of intersensory adaptation[9].

We thank the staff of the electronics department of this hospital for help in construction of the apparatus, and the board of governors for financial assistance to D. S. P. One of us (P. T.) is in receipt of a Wellcome senior research fellowship in clinical science.

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PHARMACOLOGY

Water and Electrolyte Changes in Cardiac and Skeletal Muscle of Cats Late in Haemorrhagic Shock

Coleman and Glaviano[1] found, in hearts removed from dogs late in post-oligaemic irreversible shock, an increase in potassium, a decrease in sodium, an increase in water which was not significant statistically, and no change in chlorine content per kg of fat-free dry tissue. These changes in cardiac water and electrolyte content were different from those found in congestive heart failure in the dog by Benson *et al.*[2] and Yankopoulos[3] (increased sodium, chlorine, and water). Coleman and Glaviano concluded that "the results of these investigations imply that the gradual decline of blood pressure in haemorrhagic shock does not stem from myocardial failure". I feel that this conclusion is more general than can be justified, either by their data, or by my data or those of others which will be discussed here.

My data confirm and extend those of Coleman and Glaviano in another species, the cat. My investigations were performed in 1959 by the following method: The tissues were blotted, dried to constant weight, defatted with ethyl ether, and then powdered in a mortar prior to electrolyte determination. Sodium and potassium were measured by flame photometry after an aliquot of powder was ashed by heat, while chlorine was titrated potentiometrically in an aqueous solution of dried powder. My control values were similar to those reported by Robertson and Peyser[4] for the cat.

In hearts removed from cats late in post-oligaemic irreversible shock I have found a significant ($P < 0·05$) increase in potassium and in water content, a decrease in sodium which was not statistically significant, and no change in chlorine content (see Table 1). These results are essentially in agreement with those of Coleman and Glaviano and indicate that the heart of the shocked cat reacts in these respects as does the heart of the dog.

Included in my investigation were the diaphragm, which like the heart continued to contract during ischaemia,

Table 1. Water and Electrolyte Content of Various Muscles and of Plasma in the Shocked Cat

	Water (g)		Potassium (m.equiv.)		Sodium (m.equiv.)		Chlorine (m.equiv.)	
	C	*S*	*C*	*S*	*C*	*S*	*C*	*S*
Heart	3,715	4,103*	355	468*	186	173	148	161
Diaphragm	3,203	3,395	390	457*	124	103*	92	78*
Gracilis	3,242	3,433	423	441	95	102	69	66
Plasma	—	—	3·5	6·8*	151	151	121	119

* $P < 0·05$. Values are in grams or m.equiv. per kg fat-free dry tissue or per litre of plasma.
C, Control. *S*, Shock.

and the gracilis muscle, which did not. Water and electrolyte changes in the diaphragm were similar to those in the heart. There were no significant changes in the gracilis muscle. In the plasma, only potassium was significantly increased.

Changes in muscle water and electrolytes in shock, therefore, were not limited to the heart. Similar electrolyte changes were present in another muscle, the diaphragm, which continued to work during the development of shock. Likewise, in congestive heart failure in dogs, Benson *et al.*[2] found, in the psoas muscle, used as a control, electrolyte changes which were quite similar to those found in the heart. Finally, Robertson and Daniel[5] found no electrolyte changes in cat and dog hearts which had been made to fail by treatment with large doses of pentobarbital. It is worthy of note that although haemorrhagic hypotension is accompanied by prolonged elevation of blood-levels of catecholamines (Watts and Bragg[6]), the changes in cardiac water and electrolytes in shock are not the same as those following large doses of epinephrine in the cat (increased water, potassium, sodium, and chlorine), reported by Robertson and Peyser[4]. In view of these several observations, it seems unwarranted to draw any conclusions, either from the data of Coleman and Glaviano, or from my data, concerning the presence of myocardial failure in shock.

There is a considerable amount of evidence to support the statement of Robertson and Peyser[7] that "calculations of the absolute size of the extracellular volume of cardiac muscle from plasma and tissue electrolyte concentration appear liable to gross error". Nevertheless, because potassium content and calculated intracellular potassium concentration in cardiac muscle remained high, while odium content and calculated intracellular sodium concentration remained low, I believe that more specific conclusions can be drawn, which were also drawn by Coleman and Glaviano, that failure of the transport mechanism for potassium and sodium in the heart muscle, and therefore also a marked deficit in myocardial energy metabolism, are not primary factors in the pathogenesis of irreversible shock.

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IMMUNOLOGY

Sex Difference for Immune Depression and Runting in Neonatally Thymectomized Mice

AN amazing difference with regard to wasting following thymectomy has been observed between male and female hamsters; males showed severe runting even if thymectomized at up to 4 weeks of age, while females never runted at all[1]. No such differences have been reported for other species, though there may be such a difference for neonatally thymectomized rats (R. A. Good, personal communication).

In the course of our investigations concerned with carcinogen-induced tumours in relation to immune depression[2], the degree of wasting and the homograft reactivity of male and female mice thymectomized at birth had to be determined. The data, presented in Table 1, indicate also that in mice a sex difference may exist as far as runting is concerned as well as with regard to immune depression, the latter correlating well with the severity of the wasting syndrome.

C57BL and $(CBA \times C57BL)F_1$ mice were thymectomized within 24 h after birth according to Miller's technique[3]. When 8–10 weeks of age, all mice except the severely ill ones received a skin homograft (*CBA* skin on *C57BL* mice, $(RF \times C57BL)F_1$ skin on $(CBA \times C57BL)F_1$ mice; skin donors were female). In sham-operated controls, skin grafts in these combinations were always rejected within 2 weeks.

Table 1

No. of thymectomized mice	Runting: Fatal within 6 ms.	Runting: Not fatal within 6 ms.	Runting: % Runting*	Homograft reactivity: No. of mice grafted	Graft rejection: <2 wks.	Graft rejection: 2–3 wks.	Graft rejection: >3 wks.	% with depressed homograft reactivity*
Males 44[a]	21	8	66	32	7	7	18[c]	78
Females 35[b]	9	3	34	28	15	8	5[d]	46

* Differences between the groups are statistically significant at the 1 per cent level.
a, 24 *C57BL*, 20 $(CBA \times C57BL)F_1$; *b*, 18 *C57BL*, 17 $(CBA \times C57BL)F_1$; *c*, 14 died runted with intact skin grafts; *d*, all died runted with intact skin grafts.

Table 1 depicts the incidence of runting and the degree of the depression of homograft reactivity in these mice. Only mice without a trace of histologically demonstrable thymic remnants in serial sections of the upper mediastinum are included in the table. There was no strain difference within the sexes for the two parameters so that data for each sex could be pooled.

Apparently there was a significantly higher incidence of wasting and a stronger depression of homograft reactivity in the males. Early wasting, starting during the second and third months, was fatal in both sexes and the skin grafts were invariably kept intact until death. The mice with non-fatal runting, starting mostly after the third month, usually kept their skin graft from 3 to 10 weeks, while grafts on apparently healthy thymectomized mice rarely survived longer than 3 weeks. A parallel can therefore be drawn between the severity of wasting and the degree of immune depression.

Incidentally, if our figures are compared with data reported previously by others, one is struck by the comparatively low incidence of fatal runting and the moderate degree of immune depression especially in our female mice. Invariably fatal runting and a very high degree of immune depression following neonatal thymectomy have been reported by some authors for several mouse strains, including the *C57BL* that was also used in our experiments. This difference might, of course, be attributed to the possession of a more mature lymphatic system at birth by the particular sub-line used. It might be thought that the incapacity to reject foreign skin grafts should not in part be attributed to the physical deterioration of those animals due to infections rather than the deficient 'priming' of the lymphatic system *per se*. When infection was ruled out as a factor, as in the experiments by McIntire *et al.*[4] on germ-free mice, very little runting was seen and homograft reactivity was only slightly impaired.

To explain the lower incidence of runting and the lower degree of immune depression in female mice as compared to males, one might again assume that females are, at the time of birth, immunologically more mature than males. In that case neonatal thymectomy would less frequently lead to a wasting syndrome, whether this be the consequence of an auto-immune reaction subsequently leading to lymphatic atrophy and infections[5] or of a primarily deficient development of the lymphatic system followed by infections[3]. The strikingly dissimilar consequences of thymectomy in female and male hamsters, however, would implicate the sex hormones as a cause for this difference[1]. Oestrogens have in fact been shown to cause an increased mitotic index in lymphoid tissues[6] and evidence that female mice can be more reactive im-

munologically than males[7] would indeed suggest an influence of sex hormones on immune reactivity. Such an influence might also be operative in mice after neonatal thymectomy.

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Inter-breed, Intra-breed and Autoplastic Chimerae of the Domestic Fowl

In 1956 one of us[1] developed a technique of grafting the forebrain region on the chicken embryo, which then underwent more or less normal development as a chimera[2,3]. Only a few of the chimeric embryos hatched and lived (Table 1). In the work reported here, three random-bred breeds of chicken were used: Barred Rock, White Leghorn and Rhode Island Red. The majority of the chimeric birds which hatched developed from reciprocal graftings between the embryos of Rhode Island Red and White Leghorn breeds. 502 inter-breed, 19 intra-breed and 68 autoplastic operations were performed. Fourteen inter-breed, one intra-breed and five autoplastic birds hatched. As these figures show, the percentage of individuals able to hatch in the inter-breed chimeric embryos was very low, whereas in the autografted embryos, although the number of embryos operated on was small, this percentage was considerably higher. One of the reasons for this is the difficulty of matching the transection plane on the recipient with that of the donor of the graft. As the self-regulating capacity of the embryo at the operation stage is quite limited, the appearance of supernumerary parts is the result. Such embryos never hatch.

As shown in Table 1, ten between-breed compound birds survived for periods longer than 2 weeks. Of these, one White Leghorn–Rhode Island Red male and one Rhode Island Red–White Leghorn female lived more than 3 years, and another Rhode Island Red–White Leghorn female, still alive, is now more than 5 years old (Fig. 1). Most of these birds showed lowered viability, presumably caused by the operation. They also seemed highly susceptible to infectious diseases. The cause of death of some of them could not be diagnosed.

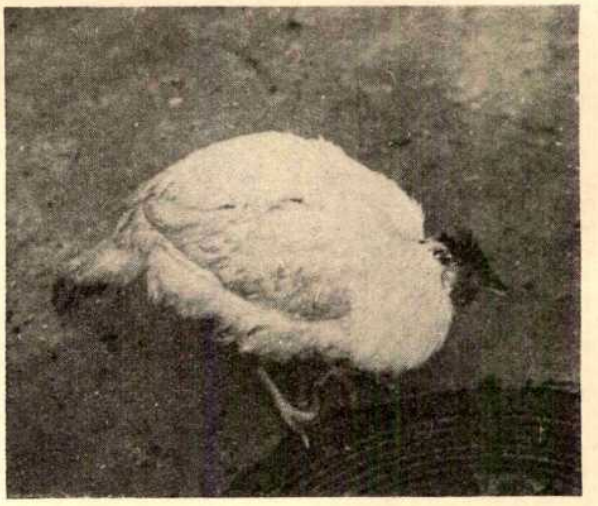


Fig. 1. Inter-breed compound ♀ No. 6. Body: White Leghorn; head: Rhode Island Red. Microphthalmia of left eye, crest underdeveloped. 5 years old

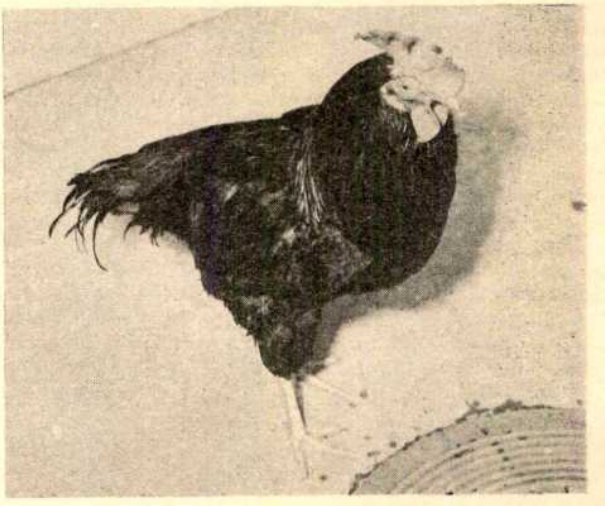


Fig. 2. Inter-breed compound ♂ No. 2. Body: Rhode Island Red; head: White Leghorn. Note: drooping crest; donor of graft a female. More than 3 years old at death

Three of them (Nos. 2, 4 and 7) died of chicken diphtheria.

All the inter-breed chimeric birds, males and females, had underdeveloped sex glands. They all failed to mate. The testes of one White Leghorn–Rhode Island Red male, a strong and hefty bird, which was killed when more than 3 years old, weighed 10·4 g (Fig. 2). When histologically examined, the testes contained a large number of spermatozoa. Motile spermatozoa were also found in the vasa deferentia. In the ovaries of female

Table 1

	Type of chimera	Sex	Length of survival in days	Body-weight (g)	Weight of sex glands (g)	Cause of death
(1)	Barred Rock→Rhode Island Red	Male	70	584 at 2 months		Sharp paroxysmal fits, cause unknown
(2)	White Leghorn→Rhode Island Red	,,	1,147	3,085 at 9 months 3,600 at 17 months	10·400	Diphtheria
(3)	,, ,, ,, ,,	,,	338		0·410	?
(4)	,, ,, ,, ,,	,,	180	1,695 at 6 months	0·530	Diphtheria
(5)	,, ,, ,, ,,	,,	15			Low viability
(6)	Rhode Island Red→White Leghorn	Female	1,826 1,826	2,300 at 22 months 1,900 at 58 months		Still alive
(7)	,, ,, ,, ,,	,,	1,240	1,985 at 11 months		Diphtheria
(8)	,, ,, ,, ,,	,,	249	1,350 at 6 months		?
(9)	,, ,, ,, ,,	,,	210	975 at 7 months		?
(10)	,, ,, ,, ,,	,,	22			Low viability

birds, numerous oocytes in the early stages of differentiation were always found. One White Leghorn female, which on 14 consecutive days was given intraorally 500 mg dry-weight calf anterior pituitary, laid one egg only. This demonstrated the capacity of the ovary to react to gonadotrophic hormones.

Three autoplastic birds—one Rhode Island Red male, one Rhode Island Red female, and one White Leghorn female—reached maturity. The Rhode Island Red female laid eggs which in due course hatched, but she also laid a number of very small yolkless eggs. The White Leghorn female laid her first egg when 13 months old. The autoplastic Rhode Island Red male is now 2 years old, but sterile.

Our cursory investigations on the pituitary have shown that this gland, although normal in general appearance, is smaller in chimeric birds than that of the normal bird of corresponding age. As the region from which the primordium which gives rise to the pituitary gland lies close to the plane of transection, it is reasonable to suppose that its development must have been affected by the operation.

Although a majority of the inter-breed chimeric birds which hatched died in the early months of life, there was no evidence that an immunity reaction was responsible for their death. Three inter-breed chimeras, two of which died when more than 3 years, and another, still alive at 5 years of age, prove conclusively that, barring accidental death and the consequences of imperfect operation, these animals are capable of living their normal life-span.

All the inter-breed compound birds failed to show mating instinct. In all of them the sex glands were underdeveloped to a greater or lesser degree. We think that in both the inter-breed and autoplastic animals the imperfect development of the pituitary gland is the primary cause of the hypofunctional state of the sex glands.

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Fixation of Parental Strain Lymphocytes in F_1 Hybrid Rats during Cross-circulation

IT is now well established that homologous disease follows the injection of immunologically competent cells into new-born animals[1–3], F_1 hybrids[4,5], irradiated animals[6] and adult animals rendered tolerant by neonatal injections of lymphoid cells[7], as well as after repeated injection of a large number of lymphoid cells into adults[8]. Peripheral blood lymphocytes have been shown to be immunologically competent, and homologous disease has been produced by the injection of whole blood[9], or buffy-coat layer cells[10]. To reduce the amount of damage suffered by lymphocytes during manipulation *in vitro*, a technique has been developed for cross-circulating the blood between two rats. Using this technique, homologous disease has been produced with the parent–F_1 hybrid combination, and the problem of whether transfused parental strain lymphocytes continue to recirculate in the host after transfusion, or whether they are rapidly fixed in the tissues, has been investigated.

Cross-circulation was established between a rat of a highly inbred 'Marshall' strain and a hybrid rat derived by crossing the 'Marshall' with a second highly inbred 'August' strain. The right common carotid artery and the right jugular vein of each partner were cannulated and the blood flow rate recorded by means of a drop counting device, the flow rate being regulated to about 2/3 ml. per min. After cross-circulation for 1 h, the 'Marshall' rat was taken off and within 15 min a second hybrid rat was cross-circulated with the first hybrid for another hour.

The effectiveness of the cross-circulation was assessed by labelling the red blood corpuscles of the 'Marshall' rat with chromium-51 and measuring the activity of blood samples taken from both rats at repeated intervals. The labelled cells were equally distributed between the 'Marshall' and first F_1 hybrid, and again between the first and second F_1 hybrid within 1 h of starting cross-circulation (Fig. 1).

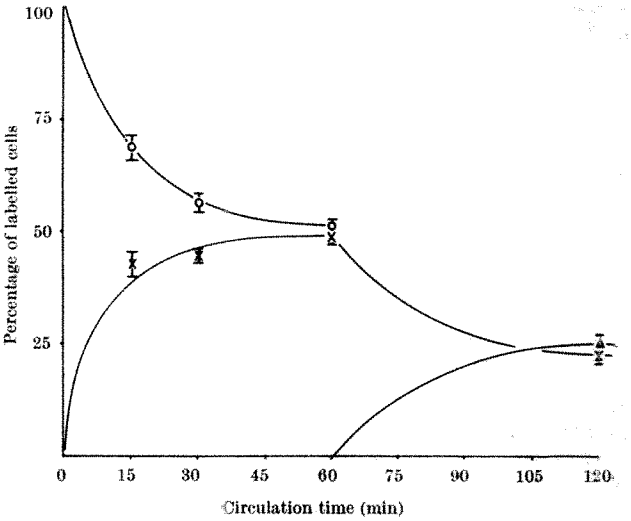


Fig. 1. Distribution of ^{51}Cr-labelled red cells between 'Marshall' (○), first F_1 hybrid (×) and second F_1 hybrid rats (△). The number of labelled cells in each animal as a proportion of the labelled cells initially administered to the 'Marshall' rat was calculated, assuming 6·3 ml. of blood per 100 g body-weight (ref. 11)

The spleen weight on the ninth day after cross-circulation was taken as a measure of the effectiveness of the peripheral blood in producing homologous disease[2]. Table 1 gives the results of such experiments. It is seen that cross-circulating a 'Marshall' with a hybrid rat for 1 h produces a splenic enlargement in the hybrid from the mean normal weight of 478 mg to 1,362 mg. When this procedure is repeated and the hybrid rat is then cross-circulated with a second hybrid for another hour, it is seen that in the first hybrid the spleen weighs 1,342 mg—which is not significantly different from the previous result—whereas in the second hybrid the spleen weighs only 842 mg. If increment in spleen weight is proportional to dose of 'Marshall' lymphocytes, it may be concluded that the 'Marshall' lymphocytes are distributed in the first and second hybrids in the ratio of 2·4 : 1, so that a large proportion of 'Marshall' lymphocytes are being fixed in the first hybrid during the periods of cross-circulation.

Table 1. SPLEEN WEIGHT FOLLOWING CROSS-CIRCULATION

Rat	No.	Cross-circulation	Spleen weight mg (mean ± S.E.)
Hybrid	6	None	478 ± 23
Hybrid	4	With 'Marshall' only	1,362 ± 32
Hybrid	4	With 'Marshall' followed by second hybrid	1,342 ± 20
Hybrid	4	With first hybrid only	842 ± 53
'Marshall'	6	None	597 ± 25
'Marshall'	8	With hybrid	778 ± 51

From Table 1 it is also seen that the 'Marshall' spleen is slightly, but consistently, enlarged. This is probably caused by an immunological response of the 'Marshall' rat to the foreign hybrid cells.

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PATHOLOGY

Genome Mutation and Carcinogenesis

It has not as yet been possible to establish a relationship between specific chromosome changes and the origin of malignancy. It is believed that the observations reported in this communication may offer an approach to this problem.

In their report on tumour chromosomes, Makino *et al.*[1] included tables giving the chromosome complements of single tumours. The chromosome complements of three female gastric carcinomata of the effusion type were recorded in this way.

Calculations on the heterogeneity of the complements of each tumour cell population showed that the individual data of these three tumours can be pooled[2]. After the complements had been arranged according to their total number of normal chromosomes, χ^2 values were calculated to test whether the total number of chromosomes of the complement influenced the distribution of the chromosomes over the nine groups of the Denver classification. The data presented in Table 1 show that it does not. The chromosome complements of the cells within the same tumour are characterized by a surprising homogeneity; χ^2 values have probabilities greater than 99 per cent.

Table 1. Homogeneity in the Distributions of Chromosomes over the Nine Groups of the Denver Classification, within Three Tumour Cell Populations*

Tumour	Total No. of chromosomes	Degrees of freedom	χ^2	P
Gs-53	3,592	56	25·58	$0{\cdot}995 < P < 1{\cdot}000$
Gs-54	1,880	24	10·05	$0{\cdot}990 < P < 0{\cdot}995$
Gs-42	763	8	1·54	$0{\cdot}990 < P < 0{\cdot}995$

* The cells were arranged according to increasing chromosome numbers and pooled in groups of six or seven.

When the pooled numbers for each group were compared with the numbers expected on the basis of the normal chromosome complement, striking differences were observed in some groups, while others showed perfect correspondence. The results are presented in Table 2. They indicate that in tumour *Gs*-53 five groups were normal and four abnormal. The results for tumours *Gs*-54 and *Gs*-42 reveal a similar phenomenon, that is, there are pronounced deviations in certain groups, while others show no significant difference. Each tumour is thus characterized by its deviating groups.

In tumour *Gs*-53 the deviations can be grouped in two parts. The number of chromosomes found in groups A_3 and G deviate by 54·8 and 47·4, respectively, from their expected values, and the number found in groups B and D deviate by 169·6 and 163·5.

On account of these characteristics of the tumour cell population—specific deviations of the tumour chromosome groups from the normal pattern, regularity in the extent of the deviations and the statistical homogeneity within the tumour—each of these tumours may be considered to be a clone, derived from a cell with an abnormal chromosome complement.

On this assumption, the number of chromosomes in each group of the abnormal chromosome complement can be estimated. As the modal number of tumour chromosomes usually approaches the diploid, triploid or tetraploid chromosome number, the stem cell from which the tumour originates should be about diploid, triploid or tetraploid. Since the 57 cells of tumour *Gs*-53 show that this tumour is near triploid (modal chromosome number of 62), it is believed that the stem cell of this tumour was triploid or nearly triploid. The figures estimated on this assumption for the nine chromosome groups are given in Table 3. Their total number, however, is 68 instead of 69. With a total of 68 chromosomes, a new estimate of the chromosome number per group was made. The data then showed a good fit with whole numbers.

A similar result is obtained when this kind of analysis is applied to the 27 cells of tumour *Gs*-54 (modal chromosome number(s) in the range of 68–72). Here the most likely number of chromosomes is 70 (see Table 3). As the total number of chromosomes of tumour *Gs*-42 is only 763, derived from thirteen metaphase plates, it is not possible to make a reasonable estimate of the underlying chromosome complement for this tumour. Of course it is not possible to be certain that the estimates of 68 chromosomes for tumour *Gs*-53 and of 70 for tumour *Gs*-54 are correct. When another number, for example the modal chromosome number, is taken as the original number of chromosomes, a slightly different chromosome complement is obtained. The figures in Table 4 show, however, that 68 and 70 are the most likely chromosome numbers, for these give the best fit with whole numbers, as indicated by the χ^2 values for the goodness of fit; these are smallest when the fit with whole numbers is best. Although this method permits the choice of a total

Table 2. Deviations of the Tumour Chromosome Groups from the Normal Chromosome Complement

Tumour		Chromosome groups									
		A_1	A_2	A_3	B	C	D	E	F	G	Total
Gs-53	Observed	169	159	211	482	1,268	305	439	294	265	3,592
	Expected	156·2	156·2	156·2	312·4	1,249·4	468·5	468·5	312·4	312·4	
	χ^2	1·05	0·05	19·23	92·07	0·28	57·06	1·86	1·08	7·19	
	P	n.s.*	n.s.	<0·001	<0·001	n.s.	<0·001	n.s.	n.s.	<0·01	
Gs-54	Observed	70	150	32	112	701	234	276	162	143	1,880
	Expected	81·7	81·7	81·7	163·5	653·9	245·2	245·2	163·5	163·5	
	χ^2	1·68	57·10	30·23	16·22	3·39	0·51	3·87	0·01	2·45	
	P	n.s.	<0·001	<0·001	<0·001	n.s.	n.s.	<0·05	n.s.	n.s.	
Gs-42	Observed	19	34	26	54	274	103	107	59	87	763
	Expected	33·2	33·2	33·2	66·4	265·4	99·5	99·5	66·4	66·4	
	χ^2	6·07	0·02	1·56	2·32	0·28	0·12	0·57	0·82	6·39	
	P	<0·025	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0·025	

*n.s., Not significant.

Table 3. Estimation of the Chromosome Complements of the Pseudotriploids, assumed to be the Origin of Tumours *Gs*-53 and *Gs*-54

	No. of chromosomes in the group									Total
	A_1	A_2	A_3	B	C	D	E	F	G	
Normal triploid	3	3	3	6	24	9	9	6	6	69
Tumour *Gs*-53										
Estimated Nos. when total is 69	3·2	3·1	4·1	9·2	24·4	5·9	8·4	5·6	5·1	69
Chromosome complement in whole Nos.	3	3	4	9	24	6	8	6	5	68
Estimated Nos. when total is 68	3·2	3·0	4·0	9·1	24·0	5·8	8·3	5·6	5·0	68
Tumour *Gs*-54										
Estimated Nos. when total is 69	2·6	5·5	1·2	4·1	25·7	8·6	10·1	6·0	5·3	69
Chromosome complement in whole Nos.	3	6	1	4	26	9	10	6	5	70
Estimated Nos. when total is 70	2·6	5·6	1·2	4·2	26·1	8·7	10·3	6·0	5·3	70

For an estimation of the No. of chromosomes in a group of the pseudotriploid, the total number of chromosomes found in this group is divided by the number, which is representative for one chromosome. For example, when the total No. of chromosomes of the pseudotriploid is 69, the estimation of the No. of chromosomes in group A_1 of tumour *Gs*-53 is $= 169 : \frac{3,592}{69} = 3{\cdot}2$ (3 in whole numbers).

Table 4. Goodness of Fit for Testing the Most Likely Total No. of the Abnormal Chromosome Complement

Total No. of chromosomes	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76
Tumour *Gs*-53	10·55	8·33	7·52	6·65	5·02	4·22	4·30	3·54	4·35	4·76	5·27	—	—	—	—	—
Tumour *Gs*-54	—	—	—	—	—	—	5·40	4·88	4·62	4·34	4·83	4·97	4·65	4·81	5·18	5·19

The expected No. for the goodness of fit has been calculated in the following way: $\frac{\text{Total No. of chromosomes}}{\text{Total No. of abnormal chromosome complement}} \times$ estimated No. in group. For example, when the total No. in the chromosome complement is 69, the expectation of group A_1 of tumour *Gs*-53, is $= 3,592/69 \times 3 = 156{\cdot}2$. With the aid of these expectations χ^2 values were calculated.

chromosome number, it is not known how far this choice is reliable.

The mechanism through which a cell with such an abnormal chromosome complement would arise still remains obscure. Possibly an abnormal mitosis with many simultaneous non-disjunctions can account for this.

Abnormal chromosome complements might also arise when the chromosomes of polyploid cells segregate, as indicated by experiments with fused hybrid cells[3]. Another mechanism with disturbing influence may be the endoreduplication of chromosomes[4]. Of course it cannot be ruled out that these tumours arose by gradual selection of many abnormal karyotypes or by gradual selection of clones within a tumour cell population[5].

The present findings, however, agree with Boveri's original hypothesis[6]: as early as 1914, Boveri proposed that tumours could originate by an abnormal chromosome complement. Multipolar divisions were assumed to be the origin of this kind of aberration. It is of interest that Boveri assumed that a large variation in chromosome counts in an otherwise uniform tumour should invalidate his hypothesis—it is precisely this variation that has concealed the underlying abnormal pattern.

The present results give support to the idea that a tumour cell population can sometimes develop out of a cell with a genome mutation. Such cells have occasionally been reported. Buckton *et al.*[7], for example, in their classification of aberrant cells, recorded such aberrations from the normal pattern in C_3 cells. As an explanation they suggested inversions, deletions and translocations. This kind of aberration is found in leucocytes after X-irradiation and these cells may persist for years. Such cells were also found by Visfeldt[8] in cultures of human skin fibroblasts after irradiation. Likewise, a diploid cell from bone marrow was recorded with one chromosome missing in groups 13–15 and one additional chromosome in groups 17–18.

Why these cells should develop into a clone of tumour cells is not clear. It is possible that the abnormal chromosome constitution disturbs the cell control mechanisms. It is possible that interference with cell differentiation results in cell proliferation.

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Chromosomes and Cancer

Ishihara *et al.*[1] have recorded detailed data on the chromosomal analysis of twenty human female tumours of the breast, ovary and colon. Further calculations on their data have revealed some interesting features concerning the distribution of the chromosomes over seven groups of the Denver classification. For this purpose we used the data given in Table 4 of the report by Ishihara *et al.*[1]. These data were converted into percentages, that is, the distributions of the chromosomes over the groups were expressed as a percentage of the total number of normal chromosomes per cell. This was the same method as was used by Galton *et al.*[2], except that, in order to facilitate a comparison between the normal human chromosome complement and that of the tumour cells, I excluded abnormal chromosomes from all calculations. (It should be noted that the exclusion of the abnormal acrocentric chromosomes[1,3] from all calculations does not affect the present conclusion.)

In a number of cases, Ishihara *et al.* gave two stemlines for one patient; when this was so, the lowest and highest number of chromosomes in each group were counted together and percentages computed for these numbers.

The results of these calculations are given in Table 1, and it can be seen that no tumour resembles another with respect to its chromosomal distribution. However, by

Table 1. Percentage of Chromosomes per Group for Various Tumours from Women
(After Ishihara *et al.*, Table 4, ref. 1)

Group	Breast patient 1	Breast patient 2	Breast patient 3	Breast patient 4	Breast patient 5	Breast patient 9	Breast patient 11
A	18·9	16·0	7·7	3·9	24·2	10·8	12·0
B	10·8	10·7	5·1	9·8	5·8	8·7	5·1
C	35·1	34·6	28·2	39·2	37·5	45·2	48·7
D	8·1	10·7	7·7	15·7	9·5	10·2	6·4
E	8·1	14·6	25·6	13·7	11·6	9·2	14·3
F	10·8	8·1	12·8	9·8	10·5	8·7	8·4
G	8·1	5·3	12·8	7·8	0·9	7·2	4·8
*n**	37	75	39	102	104	195	251

Group	Ovary patient 12	Ovary patient 13	Ovary patient 14	Colon patient 18	Colon patient 19	Normal
A	16·6	14·5	16·0	19·1	15·2	13·0
B	5·6	9·7	8·0	8·3	14·5	8·7
C	37·5	37·3	36·0	32·7	36·0	34·8
D	15·3	9·6	12·0	10·1	3·6	13·0
E	8·4	12·1	12·0	14·1	14·5	13·0
F	11·1	10·0	8·0	8·3	12·0	8·7
G	5·6	7·3	8·0	7·3	4·3	8·7
*n**	72	83	50	120	137	46

* *n*, Total No. of chromosomes.

Table 2. Gastric Tumours from Men
(After Makino *et al.*, Table 2, ref. 3)

Group	*Gs* 35	*Gs* 39	*Gs* 40	*Gs* 48	*Gs* 51	*Gs* 55	Normal
A	15·9	11·8	11·8	15·4	13·0	12·2	13·0
B	9·1	9·8	9·8	9·2	5·8	4·1	8·7
C	36·4	35·3	31·4	33·8	43·5	35·1	32·6
D	11·4	11·8	9·8	12·3	11·6	10·8	13·0
E	11·4	15·7	15·7	12·3	11·6	17·6	13·0
F	9·1	7·8	9·8	10·8	8·7	9·5	8·7
G	6·8	7·8	11·8	6·2	5·8	10·8	10·9
n	44	51	51	65	69	74	46

Gastric Tumours from Women

Group	*Gs* 41	*Gs* 42	*Gs* 44	*Gs* 50	*Gs* 53	*Gs* 54	*Gs* 56	*Gs* 59	Normal
A	12·5	10·4	13·1	10·6	15·2	13·3	9·3	14·8	13·0
B	10·7	7·1	8·2	10·6	13·2	5·8	9·3	4·5	8·7
C	35·7	36·2	42·6	40·9	35·5	37·8	40·0	44·3	34·8
D	10·7	13·7	11·5	6·1	8·8	12·4	14·7	6·8	13·0
E	16·1	13·3	11·5	13·6	12·1	14·6	14·7	12·5	13·0
F	7·1	7·8	8·2	15·2	8·1	8·5	2·7	8·0	8·7
G	7·1	11·5	4·9	3·0	7·3	7·6	9·3	9·1	8·7
n	56	58·8	61	66	63·2	69·5	75	88	46

comparing the chromosomal distributions of all tumours with those of normal cells, it can be seen that in the majority of tumours, group *C* has more chromosomes, whereas groups *D* and *G* both have fewer chromosomes than normal. These discrepancies can be interpreted if it is assumed that reciprocal translocations are formed between the acrocentric chromosomes of group *D* and those of group *G*, and this produces small fragments and submetacentric chromosomes, indistinguishable from the chromosomes of group *C*. This idea is supported by the finding that the nucleolus in human cells is formed by nucleolus organizers located on the short arms of the acrocentric chromosomes[4,5] and, according to Ohno *et al.*[6], these chromosomes are more liable to undergo breakage. Moreover, 'minutes' form a considerable proportion of the abnormal chromosomes of the tumours described by Ishihara *et al.* Further evidence is obtained by calculations on the data given by Makino *et al.*[3]. The data given by Makino *et al.* in their Table 2 (concerning gastric tumours of male and female origin) were converted in the same way as those of Ishihara *et al.* Abnormal chromosomes and tumours possessing a normal chromosome complement were excluded from all calculations. For the tumours *Gs* 42, *Gs* 53 and *Gs* 54 the mean percentages were calculated with the aid of Tables 3, 4 and 5 of Makino *et al.*, respectively. This was done after it had been demonstrated that the distribution of the chromosomes over the groups was the same, irrespective of the number of chromosomes present.

A comparison of the chromosomal distributions of all tumours with those of normal cells (Table 2, this communication) shows that in the majority of tumours, originating from both males and females, group *C* has more chromosomes than normal, but that both groups *D* and *G* have fewer. These observations are in good agreement with those made from the data of Ishihara *et al.* 'Minutes' also form a considerable proportion of the abnormal chromosomes described in the publication of Makino *et al.*

In addition, the data given here in Tables 1 and 2 have been tested by Cochran's test. For this purpose the percentages of each group of all tumours were compared with the normal percentages. The differences found were recorded as '+' if the percentage of the tumour group was higher than that of the normal, as '−' if the percentage of the tumour group was lower than that of the normal, and as 'O' if there was no difference between the two percentages.

The results of this test are shown in Table 3. It can be seen from these results that the signs are not randomly distributed over the groups. Thus group *C* has a preponderance of positive signs, while the opposite trend is observed for both groups *D* and *G*. This finding therefore supports the interpretation already given. Nevertheless it is possible that this trend does not come to light in a particular tumour as a consequence of other changes.

Table 3. Cochran's Test Applied to the Data of Tables 1 and 2. The O Signs had been Added Arbitrarily to the '+' Signs

Group	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Sign O	1	1	—	—	—	2	—
Sign +	14	12	22	4	14	12	5
Sign −	11	13	4	22	12	12	21

$Q \approx X_{(6)} = 32{\cdot}16$ $P \approx 0{\cdot}00002$

In conclusion, it can be stated that the formation of reciprocal translocations involving the acrocentric chromosomes (groups *D* and *G*) may help in interpreting the abnormal chromosome complement of widely different tumours.

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Regulation of Leucocyte Alkaline Phosphatase and the Philadelphia Chromosome

IN recent years there has been a number of reports concerning the relationship in the human cell between chromosome 21 and leucocyte alkaline phosphatase (LAP)[1-3]. It has been suggested that, since the LAP was elevated in trisomy 21 and lowered in chronic granulocytic leukaemia (CGL) in which the Philadelphia (Ph^1) chromosome was present, the structural gene for LAP was located on the distal one-third of chromosome 21. The changes of LAP in these diseases were in the proper direction and a gene-dose effect seemed plausible.

However, in a case of chronic granulocytic leukaemia with the Ph^1 chromosome[4], it was shown that elevated levels of leucocyte alkaline phosphatase could occur. In addition, Mellman *et al.*[5] demonstrated that in the presence of an extra chromosome 21 of mongolism, excessive amounts of protein (enzyme) were produced even of *X*-linked genes. These reports were strong evidence against location of the structural gene for leucocyte alkaline phosphatase on the distal one-third of chromosome 21.

An attempt was made[4] to interpret the results according to the operon concept of Jacob and Monod[6]. The proposal suggested a system involving two regulators, the second of which was called a 'modifier'[7]. The requirement of a series of regulators for the activity of an operon has been well established[8,9].

A formulation of the foregoing situation could be made as follows:

Normal $- 2R_2 + 2R_1 + 2OS \rightarrow$ LAP—normal
CGL with $Ph^1 - 1R_2 + 2R_1 + 2OS \rightarrow$ LAP – decreased

(R_2 is regulator 2 or modifier; R_1 is regulator 1; OS is operator-structural gene complex; LAP is leucocyte alkaline phosphatase).

If the formulation is useful, certain predictions may be made with respect to LAP-levels with changes in the number of chromosome 21 or the Ph^1 chromosome.

In CGL with two chromosomes 21 and a Ph^1 chromosome (47 chromosomes with four normal *G*-group chromosomes and one Ph^1 chromosome), the control mechanism may be written as follows: $2R_2 + 2R_1 + 2OS \rightarrow$ normal LAP. The LAP is expected to be at near normal levels because the regulation would have been returned toward normal, despite the Ph^1 chromosome. In CGL with 47 chromosomes, if the addition to the complement is a second Ph^1, the regulation may be written: $1R_2 + 2R_1 + 2OS \rightarrow$ decreased LAP. The increase of chromosome number has not increased the number of R_2, and thus the status is comparable with the usual case of CGL, 46 chromosomes, one of which is a Ph^1 chromosome.

Two cases of chronic granulocytic leukaemia with two Ph^1 chromosomes have been described recently[10]. One case showed 30 per cent cells with 46 chromosomes, two of which were Ph^1 chromosomes; 70 per cent of the cells had 47 chromosomes including two Ph^1 chromosomes and are suitable for testing the formulation. The LAP was low (0). In the 47 chromosome complement, the second Ph^1 might be considered the 'extra' one. However, as the 46 chromosome cells had two Ph^1 chromosomes, it is probably better considered the 'stem' line from which the 47 chromosome cells arose. If so, there would be no normal chromosome 21 from which the extra *G*-group chromosome might arise. Therefore, the additional chromosome is thought to be a number 22. Consistent with this deduction is the fact that the patient did not develop an elevated LAP despite the acute blastic crisis described by the authors. It is usual that in CGL (with the Ph^1 chromosome) for the LAP to be low except in complications such as acute blastic crisis, when it commonly rises. The failure of the increase in LAP in this case is interpreted as a complete lack of R_2 with uninhibited action of R_1. This would result in more or less complete block of the OS complex.

Corroborating these views is a recent case of CGL seen at the City of Hope Medical Center. The patient, a 39-year-old Caucasian female, had an initial LAP score of 31 (normal 45–90). Cytogenetic investigations were undertaken several months after initial diagnosis when she was hospitalized for an acute blastic crisis. Her marrow showed bimodal, equal distribution of 46 and 47 chromosome cells, each with one Ph^1 chromosome. In the 47 chromosome cell, the addition was in the *G*-group. At least one *G*-group chromosome must have been a 21, since the LAP, tested simultaneously with the marrow study, revealed a score of 290.

The manner in which the modifier might work has not been elucidated. One possibility may be described as follows: the second regulator (or modifier) codes for a protein which combines with an effector (a metabolite or some other small molecule) in the cytoplasm. The resultant molecule may act directly on the R_1 gene or on the product of the R_1 and its effector (R_1-effector). It is the R_1-effector substance which controls the operator. In this way the ratio between R_2-effector and R_1-effector would determine how much R_1-effector could be available for inactivation of the 'operator'. If the modifier gene R_2 were absent on one chromosome (the Ph^1 chromosome), less regulation of R_1 would occur. Therefore, more R_1-effector would be available to inhibit operator and lower LAP.

In mongolism extra quantities of modifier due to trisomy of 21 would provide a more marked inhibition of R_1, so that more of the product of operator-structural genes would be present. Therefore, LAP would be elevated. In trisomy 21, much more is happening than the simple increase of modifier (R_2). The extra chromosome is associated with profound disturbances of metabolism; however, increases of an enzyme may be logically traced to the presence of increased amounts of regulator in the cell. Regulators for other genes may be present on chromosome 21 and, for that reason, elevation of other enzymes would be expected. The report of Mellman *et al.*[5] suggests such an interpretation. The fact that regulator need not be on the chromosome near the structural genes or on the same chromosome has been demonstrated in bacterial systems[6]. If control works at the level of messenger RNA as suggested by Ames and Hartman[11] and others, it is conceivable that the location of the regulator gene may be on chromosomes other than those with the structural genes. Thus, the explanation used for the variation of LAP in the presence of the Ph^1 chromosome may be extended to the data of Mellman *et al.*[5].

Seeming exceptions to the findings predicted by the hypothesis may occur because of effectors which are common to several inductive or repressible pathways. This may explain the elevation of the LAP in CGL when acute inflammatory processes of widespread nature or acute transformation of the leukaemic process occurs. The complexity of the situation in mammalian cells makes it difficult to apply the theory rigidly. Nevertheless, we suggest that a beginning may be made in the understanding of regulatory mechanisms of protein synthesis in the propositions submitted. Some support for the hypothesis is its application to alkaline phosphatase levels in cultured heteroploid cells[12]. In this investigation, alkaline phosphatase was always lowered when the number of chromosomes in the *G*-group was decreased.

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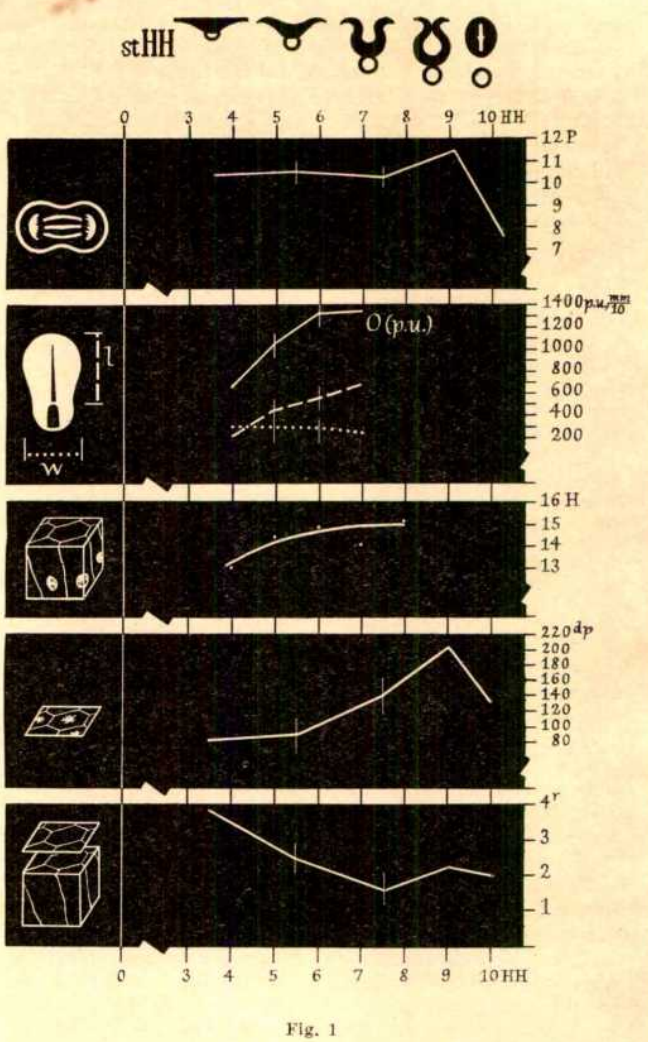


Fig. 1

ANATOMY

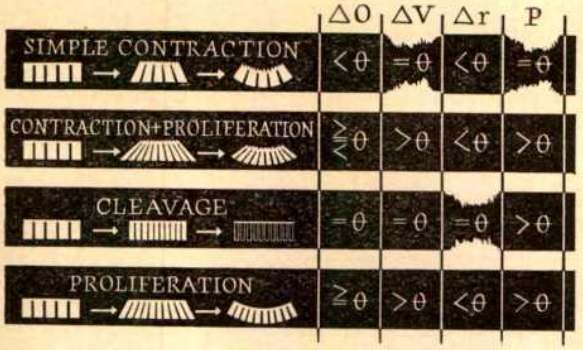
Influence of Mitotic Activity on Neurulation Movements

According to the present view, the forces responsible for the closure of the neural tube reside in the neural plate itself. On this view, the disproportion between the surface area and the volume of the neural plate is the real mechanical cause of neurulation movements. This disproportion can arise either by a diminution of the surface area, or by an increase in volume, or, occasionally, by a combination of both processes. In all these cases the neural plate cells must be firmly anchored in the developing internal limiting membrane system[1,2]. The first possibility has been generally conceded as the theory of active contraction[3-6], which presupposes the existence of a contractile system settled either extra- or intra-cellularly at the level of the internal limiting membrane. Volume increase of the neural plate may occur either as a result of cell multiplication (proliferation hypothesis), or by an augmentation in size of particular cells (hydration hypothesis[7]). The active part of proliferation has generally been overlooked, in spite of the fact that only one research worker[7] denies the existence of mitoses in the neural plate.

In an attempt to clear up this confusion we measured the quantitative parameters in 115 chick neurulae. A previous series of experiments, in which various noxious factors were placed on the embryo during neurulation, has demonstrated unambiguously that the phase of formation of the neural lips is extremely important for the development of dysraphic malformations of the central nervous system. Our attention was, therefore, focused on this phase. We attempted to discover: (1) Whether mitotic division does occur during neurulation. We found that proliferation does occur, and is higher than in any of the following stages[8] (Fig. 1, *P*). (2) What happens to the surface area of a neural plate. We found that it increases linearly (Fig. 1, 0), due to its lengthening (Fig. 1, *l*). The fact that the width, *w*, of a neural plate does not change at all is contrary to the theory of a mere contraction. As no diminution of the surface extension of a neural plate can be observed, an increase in volume is to be anticipated. Two facts give evidence of this process: (*a*) the steep decline in the surface-volume ratio (Fig. 1, *r*); (*b*) the mirror increase in the number of colchicine-arrested mitoses per square surface unit (Fig. 1, *dp*). On the basis of these data we reject the assumption[9] that, during neurulation, the cells do not attain their original size after being duplicated, and that this process would, therefore, suggest the cleavage of a miolecithal egg. The hypothesis of differential hydration is also endangered[10]. We have shown repeatedly that the number of nuclei per unit volume is increased during neurulation. This fact alone is incompatible with the idea of hydration (Fig. 1, *H*).

Table 1, summarizing our results, shows that both the theory of simple contraction and the 'cleavage' hypothesis cannot be applied to the chick. We thus attempted to determine whether proliferation may be considered an effectual factor in neurulation movements. The following objections to the active contraction, simultaneously taking place, can be raised:

Table 1



(1) On the electron micrographs we have not succeeded in finding a continuous 'contractile' layer in the cytoplasm beneath the cell surface[11]. The denser spots occur in the cytoplasm of cells only in the neighbourhood of, and as an integral part of, desmosomal contacts.

(2) It seems improbable that contraction occurs in harmony with the surface expansion of the neural plate, without its width being altered.

(3) The number of dividing cells increases up to the moment of closure of the neural tube. Obviously this would have a disturbing effect on the continuity of the contractile layer.

(4) Since the proliferation activity remains constant, the number of mitoses per square unit of surface (Fig. 1, *dp*) is proportional to the number of cells anchored in each surface unit of the internal limiting membrane system. Any simultaneous contraction would produce a dramatic change in the curves *dp* and *r*. However, as no phenomenon of this type occurs, the proliferation activity may be considered as the main dynamic factor of neurulation movements, the static factor being the firm anchoring of the neural plate cells in the developing internal limiting membrane system.

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RADIOBIOLOGY

Induction of Chromosome Injuries in Mouse Bone Marrow under the Distant Effect of Ionizing Radiation

DESTRUCTIVE processes are known to be absent from shielded regions of the haemopoietic system but present in irradiated regions[1,2]. Yet it has been shown that shortly after irradiation the concentration of deoxyribonucleotides and nucleosides decreases. This is followed by a decrease in DNA in the cells of both irradiated and shielded regions of the bone marrow[3,4]. DNA synthesis has also been found to be inhibited not only in irradiated but also, to a somewhat lesser degree, in shielded tumours[5,6].

The question of the distant effect of radiation on the appearance and character of chromosome injuries is still open. The distant effect of radiation of the chromosome apparatus has been described for plant cells, where true chromosome rearrangements were observed[7–9]. There are also some data indicating that shielded mammalian tissues, such as the corneal and skin epithelium, do not show significant increase in the number of chromosome aberrations when compared with normal ones[10–12].

In the present communication we present an attempt to study the data on the distant effect of ionizing radiation on the chromosome in bone marrow cells.

Experiments were carried out on male and female mice of the *C57BL* strain weighing 18–20 g. The irradiation factors were as follows: X-ray machine of *RUP*-1 type; 210 kVp., 15 m.amp, filtration 0·75 mm Al + 0·5 mm Cu, dose rate 50 r./min. The animals were divided into three groups In the first group only one hind limb was irradiated with 700 r., all other parts of the body being shielded; in groups 2 and 3 surgically exteriorized intestine was irradiated with 700 or 3,000 r., the body being shielded. A 5–8-mm lead plate was used as a screen. Under these conditions the shielded parts of the body received 0·2–0·5 per cent of the total dose.

The animals were killed 2, 6, 24, 48, 72, and 120 h after the exposure to radiation (4–7 mice for each point). The femurs of the shielded limbs were fixed in Carnoy's solution. Bone marrow pieces were totally stained using Feulgen and light green stains, and squashed. Cells with chromosome injuries were counted at the stages of late anaphase and early telophase. About 100 cells at these stages were counted in each animal.

The percentage of bone marrow cells with chromosome damage in unirradiated mice was equal to 8·1 ± 0·4 per cent, whereas the proportion of cells with chromosome bridges only was 6·40 ± 0·8 per cent.

When only one hind limb was exposed to radiation, a distinct increase in the number of cells with chromosome injuries was found in shielded regions of the bone marrow (Table 1). In directly irradiated limbs, the percentage of cells with chromosome aberrations was 97·3 ± 0·9, 83·7 ± 4·4, 50·3 ± 6·8 and 18·1 ± 0·7, 6, 24, 48, and 72 h after irradiation, respectively.

Chromosome damages in the shielded bone marrow cells were not a result of direct irradiation. These cells were given no more than 3·5 r., but the total body X-irradiation with 7 and 15 r. induced only 10·0 and 11·4 per cent respectively of cells with chromosome aberrations 6 h after treatment. The majority of injured cells had bridges only (7·5 and 10 per cent, respectively). Thus, alterations found in the cells of the shielded bone marrow are due to the distant effect of radiation.

The distant effect also becomes manifest when an irradiated limb is removed 2 h after exposure. When the irradiated limb is removed 8–15 min after exposure, the number of chromosome aberrations in bone marrow cells does not differ from normal (Table 2). Therefore, humoral factors appearing in the irradiated limb start affecting the cells of the shielded bone marrow some 2 h after irradiation. The question arises whether such chromosome injuries in shielded bone marrow cells can be induced by the influences from other irradiated tissues. The percentage of cells with chromosome injuries in unirradiated bone marrow was about the same 3 days after local treatment of the exteriorized intestine with 700 and 3,000 r. and after local exposure of a hind limb to irradiation with 700 r. (Table 1).

Evidence in support of the humoral mechanism of this phenomenon was provided by experiments using injections of the extracts from irradiated tissues into intact mice. Bone marrow cells were destroyed by freezing three times in liquid nitrogen, then thawed and centrifuged at 6,000 r.p.m. The extracts from the intestine were prepared in the following manner. A part of the small intestine was carefully washed with saline solution with the addition of penicillin and streptomycin and then with pure saline solution; thereafter it was homogenized and centrifuged

Table 1. Percentage of Cells with Chromosome Injuries in Shielded Bone Marrow

Time after exposure (h)	Kind of treatment					
	Irradiation of one hind limb with 700 r.		Irradiation of the exteriorized intestine with 700 r.		Irradiation of the exteriorized intestine with 3,000 r.	
	Total No. of cells with chromosome injuries	Cells with bridges only	Total No. of cells with chromosome injuries	Cells with bridges only	Total No. of cells with chromosome injuries	Cells with bridges only
2	—	—	24·9 ± 0·9	22·6 ± 1·4	27·6 ± 0·8	25·3 ± 0·7
6	14·3 ± 1·9	13·1 ± 2·4	—	—	—	—
24	20·8 ± 2·1	18·6 ± 2·9	25·7 ± 3·8	23·2 ± 5·0	24·3 ± 5·2	19·2 ± 5·4
48	27·9 ± 1·3	26·8 ± 1·0	22·3 ± 1·1	21·1 ± 1·2	19·7 ± 2·6	17·2 ± 3·2
72	19·4 ± 4·6	18·7 ± 4·3	20·7 ± 0·9	19·4 ± 1·0	24·8 ± 4·1	24·2 ± 4·0
120	13·0 ± 2·0	13·0 ± 2·0	—	—	—	—

at 6,000 r.p.m. All these procedures were performed on ice and took 2 h. There were no whole cells or nuclei in the extracts obtained with this method. For the preparation of extracts, bone marrow and intestine were taken 2 h after total body irradiation. The extracts, 1 ml. per mouse, were injected into intact animals either intravenously or intraperitoneally, and bone marrow was fixed 2 h after the injection.

Table 3 shows that after an injection of the extracts from normal unirradiated tissues, the percentage of bone marrow cells with chromosome injuries does not differ from the normal one. When extracts from irradiated tissues are injected, the number of injured cells is 18·3 per cent and 21·9 per cent for the intestine and bone marrow extracts, respectively.

A cytological analysis showed that chromosome injuries observed in the cells of both the shielded and intact bone marrow (after an injection of the extracts from irradiated tissues) were represented mostly by chromosome and chromatid bridges (80–90 per cent) (Table 1) apparently formed as a result of stickiness.

The stickiness of irradiated chromosomes is known to be caused either by the depolymerization of DNA[13,14] or by a change in the structural organization of nucleoproteids[15,16]. Irrespective of which hypothesis is valid, the stickiness observed in our experiments may be associated with the changes in DNA concentration[3,4] and with a depression of DNA synthesis[5,6].

The data presented lead us to the conclusion that chromosome injuries appear not only in directly irradiated but also in shielded regions (mostly bridges) of the haemopoietic system. They are induced by humoral influences (observed not earlier than 2 h after exposure) from irradiated tissues. This is proved by the appearance of chromosome damage in the bone marrow cells in the same numbers and at the same time both with local irradiation of the small intestine and with injection of the extracts from the bone marrow or the intestine of irradiated mice into intact unirradiated animals.

Table 2. Percentage of Cells with Chromosome Injuries in Unirradiated Bone Marrow 6 h after Irradiation

Kind of treatment	6 h after irradiation	
	Total No. of cells with chromosome injuries	Cells with bridges only
Body shielded, one hind limb locally irradiated with 700 r. and amputated 2 h after irradiation	22·6 ± 0·5	21·8 ± 0·5
Body shielded, one hind limb locally irradiated with 700 r. and amputated 8–15 min after irradiation	8·4 ± 1·1	7·4 ± 0·8

Table 3. Percentage of Cells with Chromosome Injuries in Bone Marrow of Mice 2 h after Injection of Extracts

Injection of extracts	Total No. of cells with chromosome injuries	Cells with bridges only
Intact intestine	9·3 ± 0·5	9·2 ± 0·5
Intestine irradiated with 3,000 r.	18·3 ± 2·1	17·0 ± 1·5
Intact bone marrow	9·6 ± 0·6	9·6 ± 0·6
Bone marrow irradiated with 700 r.	21·9 ± 1·3	20·7 ± 1·0

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Influence of Sodium Salicylate on Radioactive Strontium Retention in Rat and Mouse

This communication reports a programme of test procedures to determine a satisfactory therapy for elimination of radioactive strontium from experimental animals. During the first hour following intraperitoneal administration, the isotope is still amenable to attack and we believe that intravenous sodium citrate is the most effective therapeutic agent[1]. At later times, as the isotope becomes progressively associated with skeletal tissue, citrate is ineffective and the problem of reducing body burden becomes one of improving the excretion over prolonged periods. Sodium salicylate was tested in this connexion in view of its evident pharmacological activity, its possible chelating properties and its low toxicity. Doses were calculated on the maximum recommended human dose.

Fourteen adult Sprague–Dawley rats, female, weight 200 g, were pre-medicated to establish an effective therapeutic concentration by intraperitoneal injection of sodium salicylate (200 mg/kg) for 4 days prior to administration of strontium-85. Each animal was then injected intraperitoneally with 1 μc. strontium-85 and daily salicylate treatment continued thereafter. Fig. 1 shows the cumulative urine and total excreta levels for the 23-day period following injection of the radioactive strontium compared with a control group of animals.

Ten adult *ASL* mice, female, weight 20 g, were also treated with salicylate (200 mg/kg) by oral administra-

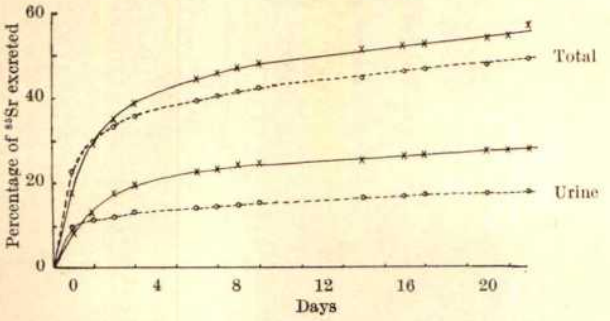


Fig. 1. Cumulative urinary and total excretion of strontium-85 from control (○) and salicylate-treated (×) rats

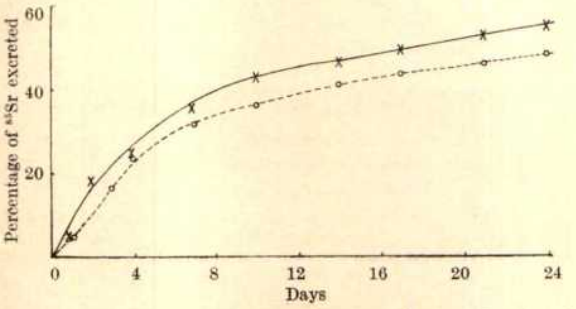


Fig. 2. Cumulative excretion of strontium-85 from control (○) and salicylate-treated (×) mice

tion in drinking water. After 7 days each animal was given an intraperitoneal injection of 1 μc. strontium-85. Salicylate treatment was continued for a further 24 days. Fig. 2 shows the combined excreta levels compared with control animals.

Table 1. Effect of Sodium Salicylate on Excretion of Strontium-85 from Female Rats expressed as Ratios of Amounts from Treated Animals to Amounts from Controls at Periods following Administration of the Isotope

Period (days)	Urine ratio	Faeces ratio
0–1	0·89	0·69
1–2	3·26	1·08
2–3	3·75	1·03
3–4	3·51	0·88
4–7	2·20	1·13
7–8	2·01	0·96
8–9	1·57	1·10
9–10	1·66	0·99
10–15	1·37	1·20
	Rest period	
15–17	0·74	0·68
17–18	1·38	0·96
18–21	1·60	0·80
	Rest period	
21–22	1·12	0·61

The effectiveness of salicylate in improving excretion of radioactive strontium is thus established in rats and mice. The salicylate does not appear to be maximally effective until 48 h have elapsed, which would indicate that salicylate is not playing the part of a chelating agent. Since a diuresis was observed, it is suggested that renal tubular resorption processes are affected. Renal output of strontium-85 from treated animals is increased after 24 h. The ratio of the levels of activity in excreta from treated rats relative to those from controls (Table 1) indicates an improvement in renal output by a factor greater than 3, while the faecal output is unchanged. Furthermore, a preferential excretion is maintained for several days, is lost after withdrawal of salicylate treatment and is restored again after further treatment.

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BIOLOGY

Degradation of Arci in a Fossil *Alnus* Pollen Grain

Recognizable biological degradation of fossil Tertiary palynomorphs has generally been limited to or most evident on structurally simple or smooth-walled pollen or spores[1]. Moore[2–5] described the effects of microbiological attack on Palaeozoic fossils and also noted that "some degree of selectivity of the saprophyte upon the host is demonstrated, and the structural features of the various spores are shown to exert considerable control over the distribution and manner of attack; the organism follows these features to a great extent. In spores where there is no marked structural differentiation the ornament of the exine, particularly in thick-walled forms such as *Convolutispora* (p. 364), plays an important part in controlling the distribution of the attack and the manner in which it is carried out"[6].

Goldstein[7], using extant pollen and spores as bait, observed that saprophytic phycomycetes attack different plant groups unequally. The difference in susceptibility may be due to variations in the chemistry of pollen and spore exines from one plant group to another. Various exine layers within a plant group may have different chemical composition[8]. It follows that layers of exine restricted in distribution over the pollen or spore, that is, ornamentation, might reflect differential chemical characteristics.

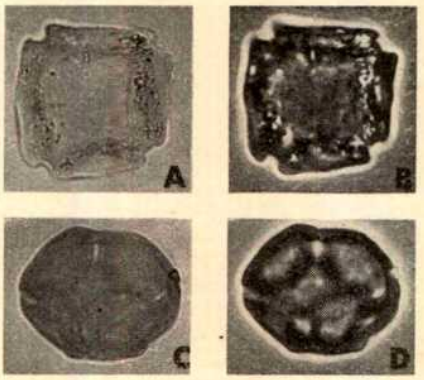


Fig. 1. Photomicrographs of fossil *Alnus* spp. pollen. *A–B*, Exhibiting biological or physico-chemical degradation of the arci; *A*, median focus under bright field; *B*, median focus, phase [contrast (× 1,000). *C–D*, Well-preserved grain with no apparent degradation; *C*, bright field; *D*, phase contrast (× 1,000)

A Tertiary *Alnus* pollen grain exhibiting marked degradation along the arci is further evidence of selective attack on the host (Fig. 1*A–B*). The causative nature of the degradation, whether biological or physico-chemical, remains unknown. It is believed, however, that the presence of the arci controlled the distribution of the process.

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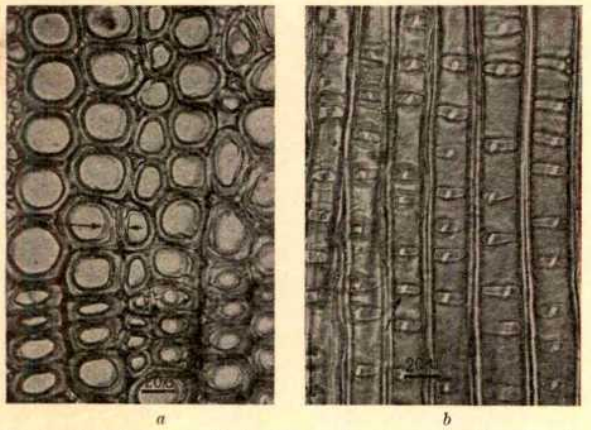
Wall Thickening in *Callitris glauca* R. BR.

Some species of the genus *Callitris* exhibit in varying degrees a special type of thickening of the cell wall in the vicinity of the bordered pits of tracheids. This 'callitroid thickening' (Fig. 1) is diagnostic, and was noted by Patton[1] and was also described by Jane[2].

Cronshaw[3] examined these thickenings in the electron microscope, and was able to show that the 'awns' which appear to project into the cell lumen, were in fact attached along their length to the radial walls, the thickenings usually tapering off in thickness towards the tangential wall.

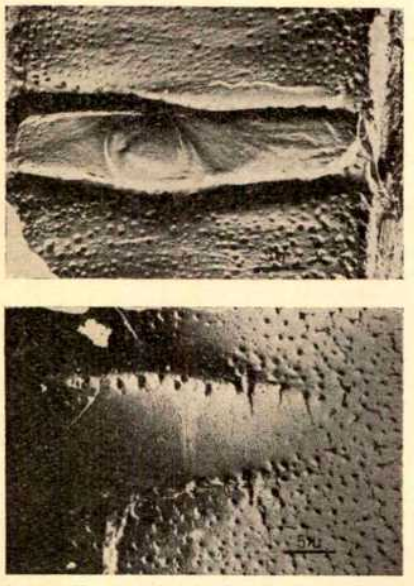
Carbon replicas of the inner surface of tracheids show that the wart structure[4,5] extends across the thickenings towards the pit aperture, but while the warty membrane persists the warts themselves tend not to occur within the confines of the area delimited by the thickenings. This area is more or less rectangular in shape (Fig. 2), but with a tendency for the thickenings to be closer to one another where they merge with the tangential wall. In tangential longitudinal sections, the thickenings can be seen to arch out over the pit aperture (Fig. 3), giving the appearance of a double border.

Ultra-thin cross-sections have recently been prepared from the xylem of *Callitris glauca* (Fig. 4) and it can be seen that the thickenings are caused by extensive eccentric



a *b*

Fig. 1. *Callitris glauca*. Photo-micrographs of a cross-section (*a*) and a radial longitudinal section (*b*) showing the thickenings (arrowed) in the vicinity of bordered pits



a

b

Fig. 2. *C. glauca*. Electron-micrographs of carbon replicas of the lumen sides of a radial wall (*a*) and a tangential wall (*b*), showing the extent of the thickenings. Note the wart structure which tends to disappear within the confines of the thickenings

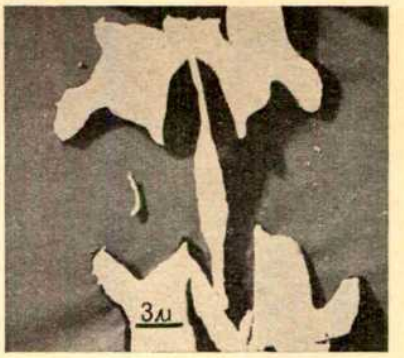


Fig. 3. *C. glauca*. Electron-micrograph of a thick tangential longitudinal section shadowed with platinum–palladium, showing the thickenings arching over a pit and giving the appearance of a double border

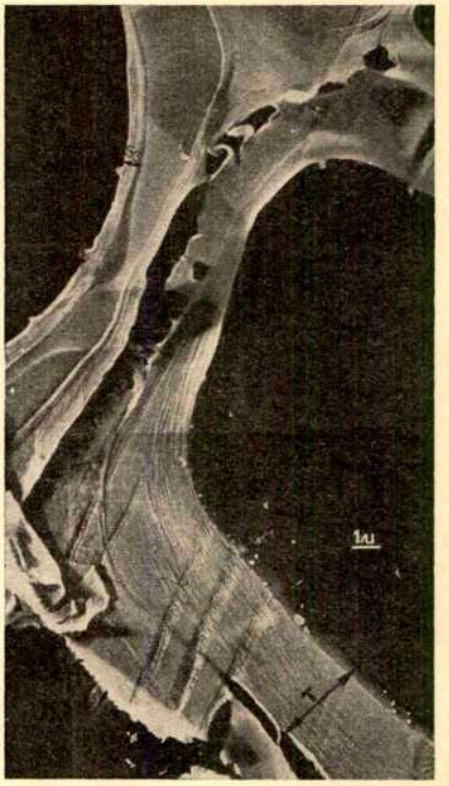


Fig. 4. *C. glauca*. Composite picture of electron-micrographs of a cross-section shadowed with platinum–palladium, showing the extremely thick S_2 layer (*T*) on the radial wall, compared with the normal thickness of S_2

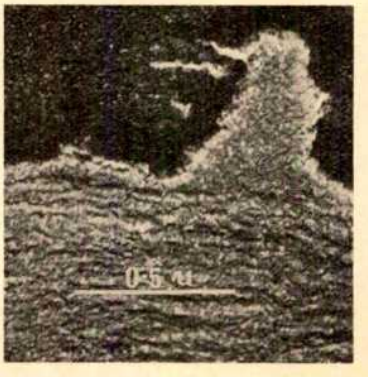


Fig. 5. *C. glauca*. Electron-micrograph of a cross-section through part of the thickened S_2 layer and including a wart

growth of the innermost layer of the secondary wall, generally known as the S_3 layer, as was suggested by Wardrop[6]. The thickest layer of the secondary wall is normally the S_2 layer, consisting of 30–150 lamellae[7], whereas the S_3 layer is normally much less than 30 lamellae. In callitroid thickenings, however, the S_3 layer is about twice the thickness of the S_2 layer.

A highly magnified section through the wart structure is illustrated in Fig. 5. The wart structure has been described by Wardrop and Davies[8] as a localized thickening of the cell wall in the form of a papilla. While this description is in general verified by Fig. 5, it can be seen that the wart itself has a different structure from the S_3 layer. It can be concluded, therefore, that the wart itself is not simply an extension of the S_3 layer. Examinations of other *Callitris* spp. are being carried out.

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Genetics of Cuckoo Egg Polymorphism

Recent work on avian chromosomes has opened new vistas in the investigation of egg polymorphism in cuckoos. The question of how some of the parasitic cuckoos can consistently parasitize several unrelated host species in one area, usually with eggs well-matched to those of each host[1–3], has long puzzled biologists. Speculations on the genetics involved have been undertaken by Miller[4] and Punnett (in Chance[2]) among others; these need reappraisal in the light of newer ideas and discoveries.

Punnett (*loc. cit.* and ref. 5) discussed among other possibilities that of *Y*-inheritance, and remarked that it would fail to account for the marked deviations from perfect mimicry known to occur in the case of the common cuckoo (*Cuculus canorus*)[3]. With due regard for Occam's Razor and modern cytogenetic knowledge, it is here proposed that the simple *W*-chromosome (to use the modern *Z*–*W* terminology for avian sex chromosomes) inheritance hypothesis is probably correct. The first positive identification of a *W*-chromosome in birds was reported in 1963 for the budgerigar (*Melopsittacus undulatus*)[6]. Recent work at the City of Hope Medical Center, Duarte, California (unpublished), has disclosed the presence of a large *W* in a Passerine species. Ohno *et al.* have shown that macrochromosomes are remarkably uniform in the avian sub-class Carinatae[7]. Hence it is likely that cuckoos will be found to possess a *W*-chromosome in the heterogametic (female) sex, a highly conjectural possibility prior to 1960 (when all the speculations on cuckoo inheritance occurred).

It is suggested therefore that *W*-inheritance can explain all observed polymorphic phenomena in *C. canorus* specifically, and in other cuckoo species with similar breeding biologies also. Briefly, some of the arguments involved are as follows:

A female cuckoo of a certain host-specific *gens* (egg-monomorphic tribe within a species)[3] would transmit to all her daughters the same egg-colour gene complex that she herself has. According to the *gens* theory, these daughters would be 'imprinted' to their foster-parent species[2,3]. Each daughter would then continue to lay host-matching eggs in nests of that species regardless of the *gens* of her father. Occasional deviations from this pattern can be explained by (1) crossing over (or translocation) between *Z* and *W*; (2) breakdown of imprinting; (3) lack of proper host nests, and other accidental situations. Any of these could explain the occasional cases of mis-matching cited by Southern[3]. It is possible that cases of more frequent misplacement which apparently occur in some areas[1,3] could be ascribed to breakdown in absolute *W*-linkage. For example, a translocation (or cross-over) between *Z* and *W* involving egg-colour genes could become established and spread through a local population because of imperfect discrimination by the host(s) in that area.

The foregoing seems to apply well to the didric cuckoo (*Chrysococcyx caprius*) in South Africa[8–10], in which species, however, *gentes* have not yet been demonstrated. This common bird should prove very suitable for the interesting task which remains: testing the *W* hypothesis cytologically and genetically.

I thank Drs. U. K. Abbott and W. J. Hamilton III, University of California, Davis, and M. B. Markus of Pretoria University, South Africa, for their advice.

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Localization of Ribonucleic Acid in the Cuticle of Nematodes

The demonstration of esterase enzymes in the cuticle of *Ascaris lumbricoides*[1] indicated that this complex structure was metabolically active. The increase in size after the final moult in many nematodes with consequent growth of the cuticle would imply that some biosynthesis takes place during this period. It has been assumed, however, that such biosynthetic activities occurred in the hypodermis[2]. Here I report the demonstration of RNA in the cortical layer of three parasitic nematodes, *Aspiculuris tetraptera*, *Syphacia obvelata* and *Ascaris lumbricoides*.

Two methods for the localization of RNA have been used, namely the acridine orange method of Bertalanffy and Bickis and Kurnick's methyl green-pyronin *Y* method, both methods as given by Pearse[3]. With the former method, fresh frozen 8μ sections were used, and for *A. tetraptera* and *S. obvelata* it was necessary to embed in 2·5 per cent gelatin before sectioning. Sections were cut on a Pearse–Slee cryostat at −20° C. With Kurnick's method, 6μ paraffin sections of worms fixed in Carnoy's fixative (6 : 3 : 1) for 2 h were used. With both methods, controls were pre-incubated in a solution of ribonuclease type IIA (Sigma Chemical Co.) containing 0·5 mg of enzyme per ml.[3]. The substitution of Fenwick's saline[4], at the same *p*H, for Krebs's Ringer solution had no detectable effect on the intensity of staining with acridine orange. In all the three species examined, only the inner cortex of the cuticle was positive; the reaction was absent in all controls including controls of ascarid material pre-incubated in a solution of RNase previously heated at 90° C for 10 min.

The demonstration of RNA in the inner cortex of the cuticle of these nematodes by two different methods precludes the possibility of artefacts and points to the synthesis of proteins in this layer. Chemical analyses of

the cuticle of *Ascaris*[2,5] and histochemical observations on *Aspiculuris* and *Syphacia*[6] have indicated that a large proportion of the cuticular solids are proteins.

The outer cortex in all the species examined is stabilized by disulphide linkages while the inner cortex stains intensely for sulphydryl groups[6,7]. These observations would suggest that a keratin-like protein is present in the cortex, but the X-ray diagram of this region of *Ascaris* is of the typical collagen type[8]. The matrix layer which lies below the cortex shows close similarities to collagenous structures[6,9], but the interface between the two layers constitutes the so-called fibrillar layer which may not necessarily be a morphological entity[5,6].

Investigations of collagen fibrogenesis have shown that ascorbic acid is required for the process and it has been suggested that this carbohydrate is necessary for the hydroxylation of proline residues[10]. By means of the method of Bacchus[3] for the detection of ascorbic acid, it has been demonstrated that in *Aspiculuris* and in *Ascaris* ascorbic acid is distributed diffusely in the inner cortex. Both ATPase (using Wachstein and Meisel's method) and acid phosphatase (using both the azo-dye coupling technique and Gomori's lead nitrate method)[3] have been located in the inner cortex of the three nematodes. These enzymes are known to participate in the formation of one or other of the structural proteins discussed here. It seems reasonable, therefore, to suggest that the cuticle of nematodes, as distinct from the hypodermis, participates in the synthesis of some of the proteins of which it is constituted. Presumably amino-acids and other smaller molecules necessary for such synthesis of protein macromolecules are supplied from the hypodermis. A fuller report of these investigations, now in preparation, will be published elsewhere.

I thank Dr. D. L. Lee for his interest and critical discussions during this work.

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Adhesion of Lymphocytes to Large Motile Cells in Cultures of Rabbit Thymus

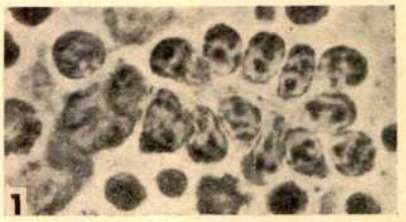
McFarland and Heilman[1] have described the attachment of lymphocytes to macrophages and to transforming blast cells in cultures of leucocytes derived from the mixed venous blood of two unrelated donors. The purpose of this communication is to direct attention to a similar type of association between small lymphocytes and large motile cells, which has been observed repeatedly in cultures of rabbit thymus.

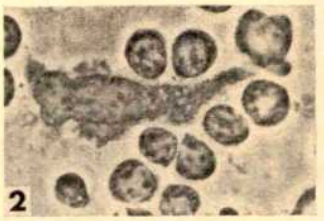
Thymic tissue was taken from healthy rabbits of either sex, their ages ranging from 21 days of foetal life to mature adults, and explanted under 'Cellophane' strips in a modified Rose culture chamber[2]. The chambers were filled with a fluid medium composed of equal parts of pooled rabbit serum and *TC* Medium 199 (Glaxo). After 24 h *in vitro* the explants showed profuse migration of small lymphocytes, together with occasional typical macrophages. In addition, numbers of large motile cells (LMC) were constantly found in the outgrowth, differing in morphology and pattern of movement from the macrophages.

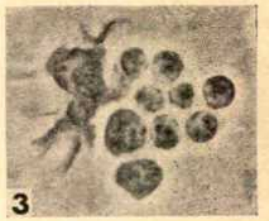
The behaviour of the LMC was recorded by time-lapse cinemicrography on 16 mm film, with the culture chamber on the stage of a phase-contrast microscope enclosed in an incubator maintained at 37° C, and also by a series of still photographs on 35 mm film.

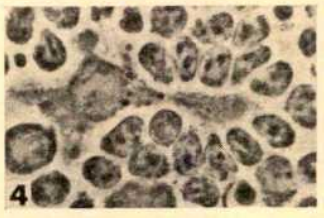
The appearance of a typical LMC is illustrated in Fig. 1; it is an elongated cell, with a broad veil of thin cytoplasm at the advancing edge, behind which lies an irregularly indented nucleus containing one or more nucleoli. Behind the nucleus there is an area of cytoplasm containing granules and small vacuoles, tapering off into a narrow cytoplasmic 'tail'. The time-lapse films reveal that the pattern of movement of LMC is characteristic, with the cytoplasmic veil at the anterior end undergoing rapid undulation; the remainder of the cell preserves a constant shape, with little or no surface activity. The most striking feature of these cells, however, is their ability to carry small lymphocytes which, having become attached to the 'tail' of the LMC, are then dragged along as the motile cell proceeds through the culture medium (Figs. 1–5). Throughout their association with the LMC (which may continue for a period of time varying from several minutes up to 1 h or more) the lymphocytes display a more or less complete cessation of their own normally active movements, remaining entirely passive and adhering to the same point on the surface of the LMC; occasionally a lymphocyte is seen to become detached and is left behind as the LMC moves on. The ultimate fate of such detached lymphocytes is not known, but during the first few minutes following their separation they can be seen to have some surface movement. They do not appear to regain the usual rapid motility of the small lymphocyte, but neither do they show any morphological evidence of impaired viability.

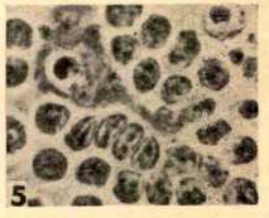
The number of lymphocytes attached to an individual LMC varies from 0 to 10 or more. In one culture chamber, containing 8 explants, 120 LMC were counted, to which were adhering a total of 271 lymphocytes. The frequency of occurrence of the association does not appear to vary with the age of the donor of the thymic explants; it is



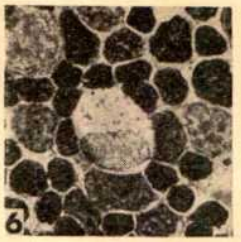








Figs. 1–5. Typical examples of the adhesion of small lymphocytes to large motile cells in living cultures of thymus taken from rabbits aged 13 days (Fig. 1) and 10 weeks (Figs. 4, 5) and from a 23-day foetus (Figs. 2, 3). In each case the LMC was moving from right to left. That in Fig. 5 has ingested the pyknotic nucleus of a dead lymphocyte. Phase contrast. (× 740)





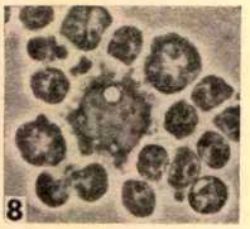


Fig. 6. Close contact between lymphocytes and a large cell with pale, slightly granular cytoplasm. Imprint of thymus, stained May-Grünwald and Giemsa. (× 410)

Figs. 7, 8. Grouping of lymphocytes round cells with irregular nuclei and vacuolated cytoplasm. Fresh, unfixed suspension of cells teased from thymus. Phase contrast. (× 700)

seen as commonly in cultures of thymus from foetal animals as in those from neonatal or adult rabbits, with the qualification that, in mature adults, the thymus tends to be replaced by adipose tissue, with consequent reduction in the numbers of motile cells in the cultures.

If an association of this kind occurs in the intact thymus *in vivo*, and is not merely a result of the abnormal environment of the living cells *in vitro*, it might be anticipated that some evidence of it would be visible in histological preparations; in fact, the density of the cell population in the thymus makes it extremely difficult to recognize such a relationship in stained sections, although Ishidate and Metcalf[3] have reported the congregation of dividing lymphocytes round PAS positive cells in sections of regenerating mouse thymus after cortisone-induced atrophy. However, the examination of stained imprints, or fresh unfixed suspensions, of thymus, in which there is some degree of separation of the cells, indicates that the close association of small lymphocytes with larger cells can be observed quite frequently (Figs. 6–8), suggesting that the events seen in the cultures may indeed take place in the intact thymus *in vivo*.

There is a strong resemblance between the behaviour of the thymic lymphocytes in the cultures described here and the attachment of peripheral blood lymphocytes to other cells depicted by McFarland and Heilman[1]. As these authors have pointed out, this type of contact differs from the active participation of lymphocytes seen in 'emperipolesis'[4] and in 'peripolesis'[5], where they move rapidly around either within the cytoplasm or over the external surface of other types of cell. The lymphocytes, both in the cultures of blood leucocytes and in those of thymus, remain immobile during their period of contact. McFarland and Heilman have suggested that such attachments may permit the transfer of RNA or other substances from cell to cell. The functional interpretation of the contacts between lymphocytes and LMC in the thymic cultures must take into account the nature and origin of the LMC. Judging by their morphology and pattern of movement they are not typical macrophages; furthermore, on the evidence at present available, they do not appear to possess highly developed phagocytic powers; Fig. 5 shows the only example so far encountered of phagocytosis by a large motile cell. The appearance and activity of these cells suggest, if anything, that they may be related to the large lymphocyte. The general appearance, in phase contrast, of the cytoplasm immediately behind the nucleus is compatible with the possession of an active Golgi apparatus; this, coupled with the existence of well-developed nucleoli, leads to the tentative hypothesis that the LMC may be actively synthesizing and secreting a substance, either into the culture medium or directly into the cytoplasm of the attached lymphocytes.

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Hydrodynamic Simulation of Small, Passively-sinking Biological Objects

Sinking velocity is an important ecological factor for many small biological objects such as pollen grains, airborne fruits and microplankton. Elaborate structures are often developed to increase drag and reduce sinking velocity, but their effectiveness cannot be directly investigated because of the difficulties involved in manipulation. The hydrodynamics of such small objects can be investigated, however, by using models of convenient size in highly viscous fluids. We have recently used this method in considering the feasibility of a planktonic mode of life for an extinct bivalve mollusc[1] and think the method should interest planktologists, palynologists and others who do not read palaeontological literature.

The flow pattern around bodies sinking steadily in a fluid depends on the Reynolds number $R = \frac{\rho v l}{\mu}$, where ρ = the density of the fluid, μ its viscosity, v the sinking velocity and l (length) a typical dimension. When the Reynolds numbers of the flows around geometrically similar prototype (suffix_p) and model (suffix_m) are the same the flow patterns are also similar. For example, a bivalve mollusc of l_p = 1 mm sinking in water at 1 mm/sec will have a similar flow pattern to a model of l_m = 80 mm sinking in glycerol at about 10 mm/sec.

To find the effect of different drag-producing structures on the prototype they may be simulated on the model and the sinking velocities and immersed weights of the variously modified models found experimentally over a range of viscosities. The equivalent settling velocity of the prototypes in water or air can then be calculated.

When sinking steadily a body experiences a drag force (D) equal and opposite to its immersed weight. When the Reynolds number is less than about 10^3 the drag force may be expressed:

$$D = \alpha \mu v l = \frac{\alpha R \mu^2}{\rho}$$

where α is a function only of R, the Reynolds number. From the experiments it is possible to construct a curve of $\alpha R : R$. Knowing the immersed weight of the modified prototype ($= D$) and the viscosity and density of the fluid αR_p can be calculated. Reference to the experimental curve produces R_p whence:

$$v_p = \frac{\mu_p R_p}{\rho_p l_p}$$

The only apparatus required is a series of U-tube viscometers and a hydrometer and a wide dropping tank deep enough for the model to travel at terminal velocity for a time sufficient such that it can be measured with a stop watch. A suitable range of Reynolds numbers can be obtained by mixing liquids of different viscosity such as glycerol and water. Thixotropic liquids (for example, methyl cellulose and water) must not be used.

Models are widely used to investigate the hydrodynamics of prototypes larger than themselves, but their use with very small prototypes is relatively unexplored.

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Continuous Recording of the Hydrostatic Pressure in a Sea Anemone

Investigation of the hydrostatic skeleton in the invertebrates has generally been limited to the use of manometric techniques to determine the hydrostatic pressures involved both at rest and during activity[1]. Some workers, notably Batham and Pantin[2], have based their results on manometer readings taken at frequent intervals, but there seem to be few reports of work using continuous recording of pressure changes.

Some recordings have been made of the hydrostatic pressure of the coelenteron of the actinian polyp *Tealia felina* with the view of making some comparison with the results previously obtained on *Calliactis* and *Metridium*[1,2] before investigating other animals. A four-channel pen recorder (manufactured by E. and M. Instrument Co., Texas) was coupled by d.c. amplifiers to a myograph to record activity, and a Bourdon gauge, by way of a photoelectric transducer, to record, simultaneously, pressure. The Bourdon gauge used had the advantage of low fluid displacement, about 15 mm³ per 130 cm water pressure applied, and a sensitivity of 1 cm pen deflexion for 6 cm water pressure at maximum gain. It was not convenient, however, to work at maximum amplification for long periods, but the instrument proved stable enough at lower gain to register constant pressures steadily over periods of at least several hours.

A glass cannula of 3 mm diameter with the mouth cut obliquely to minimize blockage was inserted through the lower part of the wall of the column of an anemone and connected to the gauge. A thread from the margin of the disk and a light isotonic lever were used to record simultaneously the movement of the *Tealia*. While the anemone remained fairly constant in shape the pressure recorded from the enteron gave values of up to 3·5 cm of water, comparable with those observed by Chapman for *Calliactis*[3]. Some considerable movement was recorded at night, including what appeared to be sharp retractions, but with no corresponding changes in pressure. A gradual increase in pressure of 2·8 cm of water was recorded, however, during 20 min at the commencement of activity and this was sustained during activity. This is in general agreement with the findings of Batham and Pantin[2], who point out that an increase in muscle activity will raise the average hydrostatic pressure. A typical recording of a naturally occurring contraction (Fig. 1*a*) shows a rapid build-up in pressure and a slow subsequent drop. The maximum pressure recorded from a natural contraction was 10 cm of water, the anemone in this case taking 1·75 h before returning to the initial pressure. Mechanical stimulation by poking hard at the base of the column gave maximum pressures (15 cm of water) when the anenome was half retracted before stimulation (Fig. 1*b*), but pressures of only 2 or 3 cm of water when the polyp was originally fully expanded. Long periods of relaxation were again experienced. These values of pressure recordings are again comparable with those observed in *Calliactis*[3] by use of manometric techniques. Although the Bourdon gauge in its present form is not so sensitive as the more refined manometric techniques[2], its use over longer periods and for continuous pressure readings without observational fatigue is clearly of value. Investigations are proceeding into the continuous recording of the coelomic pressure during burrowing in annelids and of the pressure relationships of heart and haemocoele in molluscs.

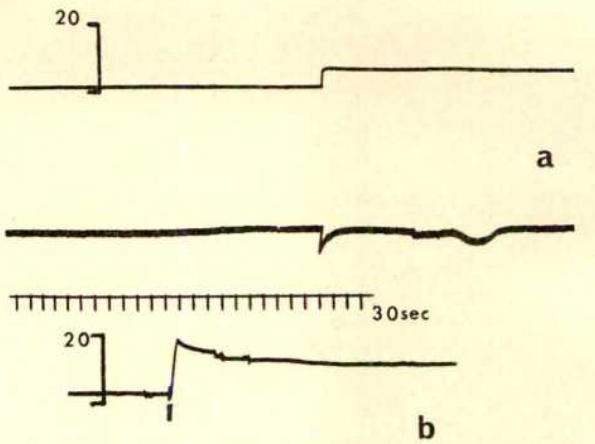


Fig. 1. Recordings of hydrostatic pressure changes in *Tealia*. Upward movement of the pressure traces indicates an increase in pressure, the scales representing a pressure of 20 cm of water and a 30-sec time mark. *a*, The sharp increase and slow relaxation of pressure (upper trace) of a naturally occurring contraction (lower trace, downward deflexion); *b*, rapid retraction of the anemone with mechanical stimulation at the point indicated

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MICROBIOLOGY

Effects of the Diethyl Ester of Pyrocarbonic Acid on Bacteriophage and Transforming DNA

The synthesis of the diethyl-ester of pyrocarbonic acid (DEP) was first reported by Boehm and Metha[1] in 1938. Later, G. Hecht[2] observed that this compound had a strong bactericidal effect. It is also known that DEP rapidly decomposes in water into toxicologically indifferent products; carbon dioxide and alcohol:

$$C_2H_5—O—CO—O—CO—O—C_2H_5 + H_2O \rightarrow 2\,C_2H_5OH + 2\,CO_2$$

These two characteristics—its bactericidal effect and its decomposition in water—make it useful as a sterilizing agent. In recent years a number of publications recommended it as an advantageous preservative for use in the food industry[3]. The mechanism of its bactericidal effect is not yet known exactly.

We performed experiments aimed at testing the effect of DEP on bacteriophage and on DNA capable of genetical transformation.

The phage material and the bacterial strains used in our experiments originated from the collection of the Microbiological Institute of the Medical University of Szeged (Hungary) and the DEP was a commercial product of Farbenfabriken Bayer *A.G.*

The virulent *Bacillus subtilis* phage referred to as 4*L* was propagated in yeast extract peptone media[4] on the sensitive strain of *Bacillus subtilis*, Marburg. The lysate we received contained 5×10^7 plaque-forming units (p.f.u.) per ml. 1 ml. of the lysate was incubated at room temperature with 0·02 ml. DEP (dissolved in 96 per cent ethanol in concentrations of 50–0·5 per cent) and after 24 h (after the decomposition of DEP) the number of infective phage particles was tested as described by

Table 1. INHIBITORY ACTION OF DEP ON 4L PHAGE

Initial P.F.U. per ml.	Concentration of DEP at the beginning of incubation (mg DEP per ml.)	P.F.U. per ml. after 24 h incubation	Inhibition (per cent)
5×10^7	0	5×10^7	0
5×10^7	0·1	5×10^7	0
5×10^7	1·0	5×10^6	90
5×10^7	10·0	0	100

Ivanovics and Csiszár[4]. The results of the experiment are shown in Table 1.

In another experiment we studied the effect of DEP on DNA capable of genetical transformation. The DNA was prepared from *Bacillus subtilis* 168 prototrophic strain according to the method of Marmur[5]. 1 ml. of DNA (0·8 mg DNA per ml., dissolved in 0·015 M sodium chloride plus 0·0015 M trisodium citrate, *p*H 7·0) was incubated at 0° C with 0·02 ml. DEP (alcoholic solution in concentrations of 50–0·5 per cent) and after 4 days the transforming activity of 0·1 ml. DNA solution was tested on the tryptophan auxotrophic strains of *Bacillus subtilis* 168 according to the method of Anagnostopoulos and Spizizen[6]. Table 2 summarizes the results of these experiments.

Table 2. EFFECT OF DEP ON DNA CAPABLE OF GENETICAL TRANSFORMATION

Initial concentration of DEP in a 0·8 mg DNA per ml. solution (mg DEP per ml.)	Percentage transformation to prototrophy by 0·1 ml. DNA after incubation for 4 days
0	0·10
0·1	0·18
1·0	0·10
10·0	0·12

These data show that DEP possesses a phage-inhibiting action in addition to its well-known bactericidal effect. On the other hand, the fact that the transforming activity of DNA practically was not altered by DEP indicates that its phage-inhibiting action probably takes place through the protein part of the phage particle.

At present, in our other experiments we routinely use DEP for phage elimination in bacterial filtrates and for sterilizing different DNA solutions.

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CYTOLOGY

Electron Micrography of Human Chromosomes

As a field of research, human cytogenetics is only 9 years old, but outstanding results have already been reported in numerous diseases. Further advances depend now essentially on the resolution of the microscope; as the limits of the optical system seem to have been reached, important new developments in cytogenetics should not be expected.

We therefore approached the present problem by electron microscopy, as several authors have recently done for animal cells of different origins[1–4].

Mitoses were obtained from human blood cultures and submitted to hypotonic solutions containing trypsin and RNase. Either of the following techniques was then used:

(1) The cell sediment was fixed in acetic acid–methanol and spread on collodion films. The best mitoses were selected by phase-contrast microscopy and their supporting film transferred on grids which were floated on uranyl acetate solution. Fig. 1 shows one chromosome from such a mitose. After DNase treatment, chromosomes appear empty, with an electron-dense envelope.

(2) The cell suspension was digested with trypsin–RNase–EDTA, mixed with a cytochrome solution, and spread on the surface of a Langmuir trough. The material was picked up on formvar–carbon-coated grids, fixed, dehydrated and conically shadowed with platinum. Depending on the strength and duration of enzyme action, chromosomes and nuclei appeared more or less fragmented, giving rise to many fibres of different sizes, most of which were parallel and showed some periodicity (Figs. 2–4). Fairly frequently, loose bundles of individual

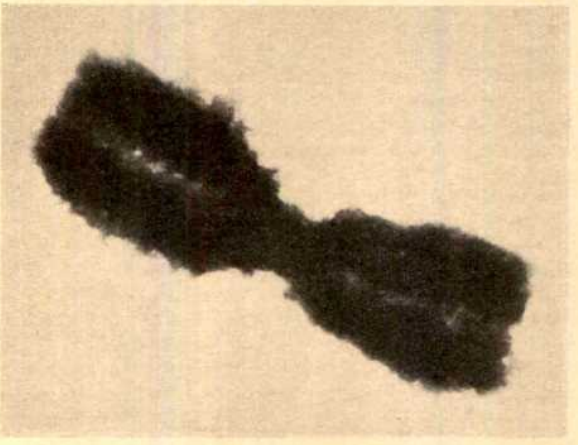
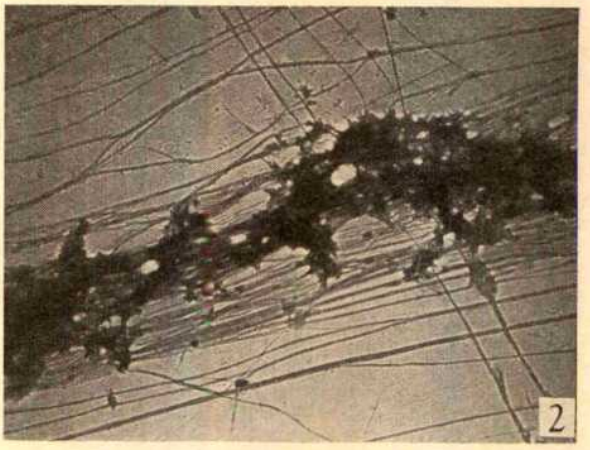
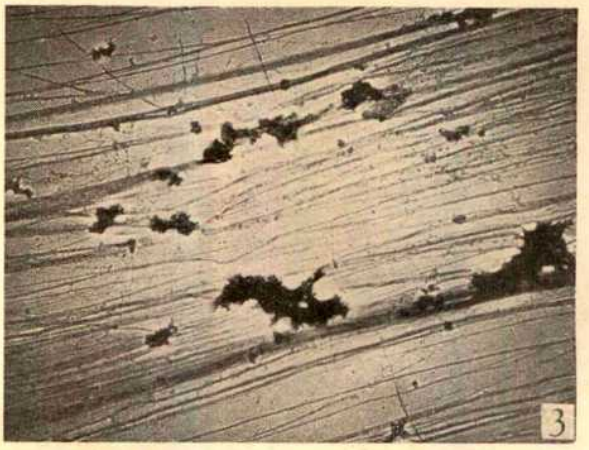


Fig. 1. A chromosome No. 1 (Denver classification) (× 18,000)





Figs. 2 and 3. Bundles of packed DNA fibres which can be followed over a length of at least 30μ (× 8,000)

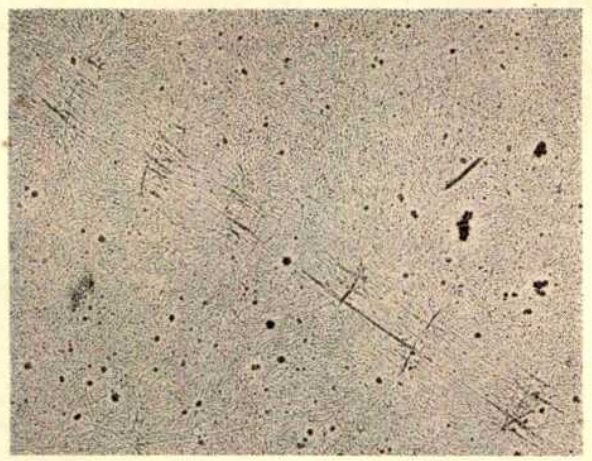


Fig. 4. Loose bundles of individual DNA threads which can be followed over a length of at least 40μ. They present cross-links at rather regular intervals. From these points, loops of individual DNA fibres spread on. No free ends of threads can be seen (× 24,000)

DNA threads presenting cross-links at somewhat regular intervals were found.

These methods enable investigation of human chromosomes to be made at much higher magnifications than previously; they show DNA fibres and their gross arrangement and structure.

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Cytoplasmic Inclusions in Neurones of Cercopithecus Monkeys

During the examination of spinal dorsal root ganglia from a large number of South African vervet monkeys (*Cercopithecus aethiops pygerythrus*) we have observed that eosinophilic cytoplasmic bodies are usually present in a high proportion of ganglion neurones at all levels of the cord. These bodies may be single or multiple, are commonly round but may appear discoid when seen on edge (Fig. 1) and may have basophilic internal structures (Fig. 2). Inoculation of ganglion suspensions into monkey kidney tissue cultures has not produced changes in these cultures.

Similar bodies have only very occasionally been observed in the anterior horn cells of the spinal cord. Cytoplasmic inclusions had not been noted elsewhere in the central nervous system until they were seen in the thalami of several monkeys used in the transmission of a non-viral toxicosis from ticks fed on these animals. A careful search of sections of the brains and cords of vervet monkeys used in a variety of other experiments has revealed identical inclusions, chiefly in the thalami, in over half the animals examined, including monkeys not inoculated in the central nervous system. In the brain the bodies are

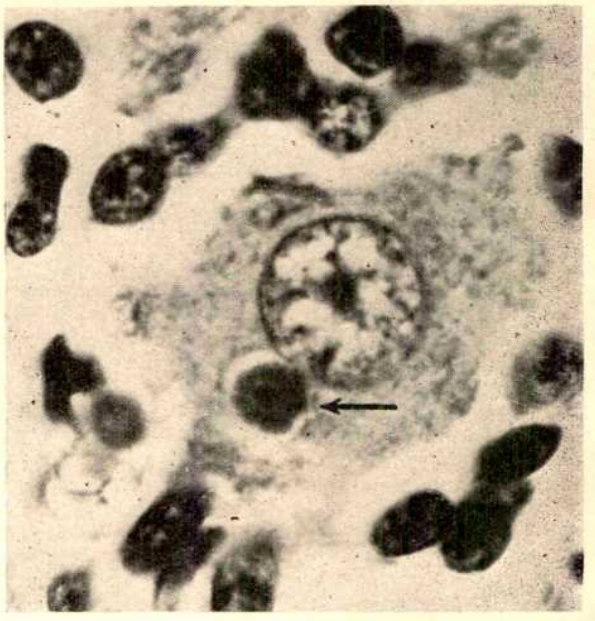


Fig. 2. Inclusion body in spinal ganglion neurone. Lendrum's stain (× *c.* 1,450)

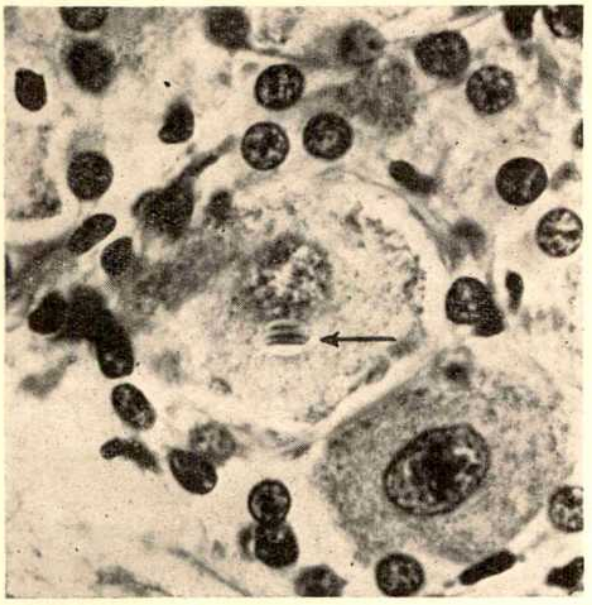


Fig. 1. Inclusion body in spinal ganglion neurone. Shorr's stain (× *c.* 900)

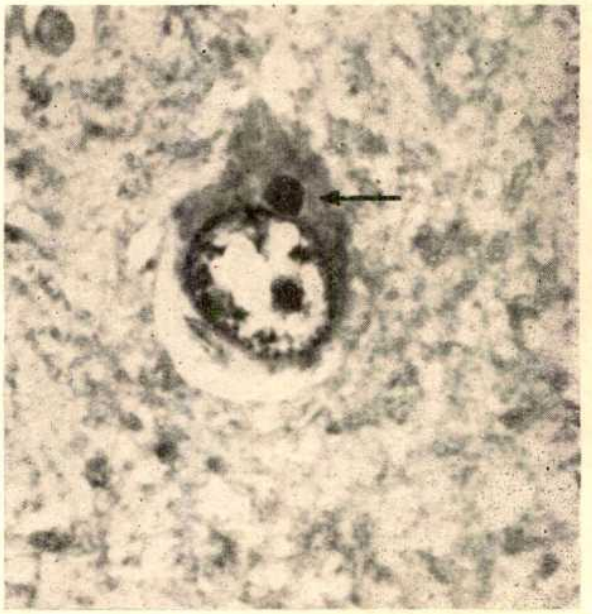


Fig. 3. Inclusion body in thalamus neurone. Haematoxylin and eosin stain (× *c.* 1,450)

usually round and regular in outline, measuring 4–5μ in diameter (Fig. 3). They are eosinophilic, but may have faintly discernible unstained internal structures. The bodies usually occur singly within neurones; but a large number of cells, especially in groups, may be affected, and in one section of thalamus measuring 8 mm by 10 mm more than ninety inclusions were seen. No clinical signs are associated with their presence.

These bodies were also observed in the thalami of monkeys inoculated intracerebrally with human brain biopsy material from cases of subacute inclusion encephalitis, and wrong conclusions about their presence may have been drawn if they had not been noted in the brains of other monkeys.

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VIROLOGY

Susceptibility of the Rhesus Monkey (*Macaca mulatta*) to *H*-1 Virus

THE host range of the *H*-viruses, a new group of small agents approximately 200 Å in diameter, is as yet unknown. *H*-1, *H*-3 and *HB* have been isolated from tumours, embryos, and placentae of human beings[1,2], while *RV* and *X*-14 have been obtained from tumour tissue of rats[3,4]. All these viruses are distinguished by their ability to produce an infection in new-born hamsters that results in death or a 'mongoloid-type' deformity, depending on the concentration of virus given. The present communication indicates that the rhesus monkey (*Macaca mulatta*) is also susceptible to an *H*-virus.

Two adult and healthy female monkeys, approximately 3 years old, were injected subcutaneously with 0·5 ml. of *H*-1 virus that had a haemagglutination (HA) titre of 1 : 2,560 with guinea-pig red cells. This was the same virus suspension previously described and used[5] for one of two human cancer patients (No. 2) who developed an *H*-1 virus viraemia and afterwards high antibody titres to *H*-1 after injection of this agent. The two monkeys injected with *H*-1 also developed a viraemia. Whole blood, drawn from the animals 4 days after the injection of virus, was inoculated subcutaneously into 2 litters each of new-born hamsters[6], all of which died within 13 days. *H*-1 virus was isolated from the livers and kidneys of the dead hamsters. The pre-injection blood, used as a control, caused no harm to two other new-born hamster litters. Sera from the latter animals, bled at 21 days of age, were tested for the presence of antibodies to *H*-1 and were negative.

Both pre-injection and post-injection sera from the monkeys were examined for the presence of antibodies to *H*-1. After treatment with kaolin to remove non-specific inhibitors[7,8], the sera were tested in various dilutions for their ability to inhibit the haemagglutination of guinea-pig red cells by 8 units of *H*-1 virus. The results were expressed as the highest serum dilution that completely inhibited the haemagglutination. HA-I antibodies appeared by the twelfth day in one monkey and were present in both animals by the nineteenth day (Table 1, Monkeys No. 1 and No. 2). The antibodies had disappeared by day 33, at which time the monkeys received a second injection of the same virus preparation. An anamnestic-type response occurred. The antibodies were specific for *H*-1 since similar tests with *HB*, *H*-3 and *RV* viruses were negative. Sera with HA-I titres also neutralized *H*-1. When undiluted and 10^{-1} dilutions of these sera were mixed with an equal volume of the *H*-1 virus used for injection of the monkeys, they afforded new-born hamsters complete protection, while a mixture of pre-injection sera plus virus killed control hamsters in 5 days. It should be noted that the monkeys did not appear ill at any time.

Table 1. HAEMAGGLUTINATION-INHIBITION TITRES TO *H*-1 VIRUS OF SERA FROM ADULT RHESUS MONKEYS PREVIOUSLY INJECTED WITH *H*-1

Day of treatment	Monkey No. 1	Monkey No. 2	Juvenile monkey with induced tumours
Pre-inj.	0	0	0
Day 7	0	0	0
12	40	0	80
19	80	80	1,280 (died)
33	0	0	—
1 week after re-inj.	80	80	—
1 month after re-inj.	0	0	—

* Guinea-pig cells were used for these procedures. Results are expressed as the highest serum dilution that completely inhibited haemagglutination of the cells by 8 units of *H*-1 virus.

The HA-I titres produced in the two normal monkeys were rather low compared with the high HA-I titres of 1 : 2,560 or more, previously observed in the human cancer patients. Since *H*-1 has been isolated from proliferating tissues such as human tumours, embryos, and placentae, it was thought that this agent might possibly multiply best where rapidly growing tissues were present. Accordingly an attempt was made to find a rhesus monkey with a spontaneous tumour. None was obtained, but through the courtesy of Dr. S. Munroe, a 1-year-old male rhesus monkey carrying three induced Rous-virus tumours[9] was made available. This animal received 0·5 ml. of the same suspension of *H*-1 given to adult monkeys No. 1 and No. 2. It developed an HA-I antibody titre of 1 : 1,280 to *H*-1 by day 19 (Table 1) at which time it died of complications due to the induced Rous-virus tumours. It is certainly not possible to ascribe the high antibody titre observed in this single animal to the presence of rapidly growing tissues. On the other hand, it is noteworthy that no impairment of antibody production to *H*-1 was observed in this monkey because tumours were present and the animal was in a terminal state.

In addition to the adult monkeys, six new-born full-term rhesus babies, housed at the Bionetics Research Laboratory, Falls Church, Virginia, were injected subcutaneously on the day of birth with 0·5 ml. of *H*-1 virus (Table 2). Three different strains of *H*-1 virus with high HA titres of 1 : 5,120 were used for these six animals. Monkeys No. 536 and No. 538 were injected subcutaneously with 0·5 ml. of the first hamster passage of *H*-1 isolated from the blood of human patient No. 2 noted previously, 4 days after the injection of virus. Monkeys No. 577 and No. 578 received another first hamster passage from this same 4-day blood, while monkeys No. 565 and No. 566 were injected with virus derived from the third hamster passage of *H*-1 isolated from a human embryo. Five of the six animals developed HA-I and neutralizing antibodies to *H*-1. The sixth animal (No. 538) died at 2 weeks, possibly before antibodies could be detected. No antibodies to *HB*, *H*-3 or *RV* were found in any of the sera. All the monkeys died with an autopsy diagnosis of pneumonia. The attendant nursery-room staff recorded as noteworthy that all six monkeys had a 'rough coat' and that 'painful' skin rashes occurred in four of the animals (Nos. 536, 566, 577 and 578). These observations were considered unusual in a large nursery with a daily capacity at any one time of approximately 45 individually isolated rhesus babies. The average expected death rate for such full-term infants is about 25 per cent.

Table 2. HAEMAGGLUTINATION-INHIBITION TITRES TO *H*-1 VIRUS OF SERA FROM RHESUS MONKEYS INJECTED AT BIRTH WITH *H*-1*

Time of treatment†	Monkey No. 536 (♀)	538 (♀)	577 (♂)	578 (♀)	565 (♂)	566 (♀)
Pre-inj.	0	0	0	0	0	0
2 weeks	320	0 Died	320	320	40 Died	0
3 weeks	—		1,280	1,280 Died		80 Died
2 months	160 Died		2,560			
3 months			320 Died			

* Same procedures used for titration as outlined, Table 1.
† Three different strains of *H*-1 virus were used for injections, two monkeys were injected with each strain.

On the basis of this preliminary work, death of the baby monkeys cannot be attributed to the virus injection. It can be concluded, however, that both adult and new-born rhesus monkeys are susceptible to infection with *H*-1 virus. A related observation is the recent report[10] that *H*-1 and *H*-3 viruses can be grown and passaged in monkey as well as human tissue cultures with the production of a cytopathic effect (CPE).

The work on new-born monkeys was carried out in collaboration with the staff of the Bionetics Research Laboratory, Inc., Falls Church, Virginia, under a contract to the National Cancer Institute, Bethesda, Maryland. I thank Dr. A. Pallotta, Dr. R. Miller and the nursery room staff for their help, and I also thank Dr. S. Munroe for supplying the juvenile monkey with Rous-virus-induced tumours.

This work was supported by grants *CA*–02042–11, *CA*–07826–01 and *CA*–07826–02 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, and grants *E*–110*E* and *E*–343 from the American Cancer Society.

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VETERINARY SCIENCE

Hypocuprosis of Cattle in the Argentine

Hypocuprosis in cattle is defined by low concentrations of copper in the liver[1–3] or in the blood[2,4,5] or other tissues[6] and is usually associated with some general external symptoms such as unthriftiness, diarrhoea and depigmentation of the hair[4]. Anaemia is sometimes, but not always, present. The levels of copper in the tissues which have come to be regarded as diagnostic of hypocuprosis are for the blood less than 0·70 γ per ml.[4,6] and for the liver less than about 30 (refs. 1 and 6) p.p.m. dry-weight; the severity of the condition increases as the tissue concentrations of copper fall below these thresholds, until at levels of 6 p.p.m. dry-weight of copper in the liver, sudden death in cattle has been recorded[2].

Hypocuprosis has been reported from a number of areas of the world[7,8]. Of these reports, only a few have been concerned with a deficiency induced by the presence of unusually low concentrations of copper (less than 3 p.p.m.) in the feed[4]. The majority of the reports[7] refer to a 'conditioned' copper deficiency in which there is a blocking of the utilization of normal amounts of copper in the forage (6–16 p.p.m.) by other constituents present, such as molybdenum and sulphate. These two components of the forage have been amply demonstrated to limit the copper storage in sheep[9], and have been reported to be involved in the same phenomenon in cattle[10].

Up to the present no information has been available regarding the copper status of cattle in the Argentine. Now, however, as part of a project initiated by the Special Fund of the United Nations with the assistance of the Food and Agriculture Organization, and the co-operation of the Instituto Nacional de Tecnologia Agropecuaria, this work has been undertaken. The report presented here indicates, for the first time, that conditions exist in parts of the Province of Buenos Aires which induce low levels of copper in the liver and blood of cattle pastured there. The values given in Table 1 refer to Aberdeen Angus cattle of the breeding herds during the period of active growth and located mainly in the eastern half of the Province.

From time to time reports had been received from different areas of the Province that some of the animals in the herds were unthrifty, were affected with diarrhoea and had rough coats, the hair of which showed depigmentation. On investigation these conditions were confirmed; however, the depigmentation was found to range from a mild browning about the ears in some animals to a more general-

Table 1

(*a*) Liver and Blood Copper* Status of some Cattle in Parts of the Province of Buenos Aires—1964

Zone 1—near Ayacucho								Zone 1 (*a*) Animals moved to Experiment Station Balcarce—high ground		Zone 2 9 de Julio		Zone 3 Los Toldos		Zone 4 Experiment Station Balcarce—low ground	
Affected area (*a*)						Non-affected area (*b*)									
Liver Cu (p.p.m.)†			Blood Cu (γ/ml.)			Liver Cu (p.p.m.)	Blood Cu (γ/ml.)	Liver Cu (p.p.m.)	Blood Cu (γ/ml.)	Liver Cu (p.p.m.)	Blood Cu (γ/ml.)	Liver Cu (p.p.m.)	Blood Cu (γ/ml.)	Liver Cu (p p.m.)	Blood Cu (γ/ml.)
21/2	30/3	22/4	21/2	30/3	22/4	10/3	10/3	21/4	21/4	9/4	9/4	22/3	22/3	5/3	5/3
6 5	7·4	6·3	0 37	0 52	0·47	9·7	0·64	93	0·84	7·1	0·50	7·4	0·29	6·5	0·33
6·8	7·7	7·4	0·40	0·52	0 43	20·4	0·73	131	0·78	7·1	0·58	7·8	0·32	8·0	0·45
7·2	8 5	6·6	0 38	0·55	0·53	46·9	0·76	136	0·77	10·6	0·88	17·8	0·26	9·0	0·52
7·9	8·6	8·5	0·32	0·40	0·33	50·1	0·74	162	0·95	12·9	0 55	18·5	0·26	9·0	0·41
8·4	12·3	9·9	0·40	0·62	0·60	55·8	0·89	169	0·96	13·1	0·64	21·5	0·32	12·0	0 33
9·8	23·0	9·4	0 36	0·53	0·47	84·8	0·79	170	0·92	19·4	0·72				
10·0	10·8	9·5	0·41	0·58	0·56	92·5	0·77	176	0·84	24·6	0·79				
10·8	12·6	9·3	0·68	0·65	0·72			184	0·87	25·8	0·86				
11·9	12·5	10·9	0·57	0·74	0·63			206	0·93	43·8	0·76				
13·2	15·6	11·8	0·55	0·75	0·53			263	0·84	52·6	0 91				
Mean 9·2	11·9	8·9	0·44	0·59	0·52	51·4	0·76	179	0·87	21·7	0·72	14 6	0·29	8·7	0·41
±*S.D.*2·2	4·5	1·7	0·10	0·10	0·11	21·2	0·07	44·5	0·11	14·8	0·14	5·8	0 026	1·7	0·080

(*b*) Copper, Molybdenum and Sulphate Concentrations of Grazed Pasture

Cu (p.p.m. d.w.)	7·0	8·4	7·6	8·7	8·9	6·6 7·5 9·6	6·4
Mo (p p.m. d.w.)	6·2	5·3	2·0	1·7	3·2	14·5 26·9 18·9	3·0
Cu : Mo	1·1	1·6	3·8	5·2	2·8	0·45 0·28 0·52	2·1
$SO_4^{=}$ (%)	0·63	0 62	0·41	—	0·52	0·31 0·27 0·53	0·43

* Copper determined from optical density at 435 mμ of diethyldithiocarbamate complex extracted into iso-amyl acetate
† On dry-weight basis.

ized lightening of the colour of the hair along the back and the flanks in others, to a frank greying of the entire coat in still others.

The pastures which the animals were grazing in these areas were mixtures of grasses, trefoils and herbs. Samples of these pastures were collected by random plucking across the sward, bulked and analysed for copper, molybdenum[11] and inorganic sulphate[12]. The values found for the copper contents showed that these were within the normal range, being from 6 to 11 p.p.m. dry-weight. The molybdenum concentrations were, however, as high as 27 p.p.m. dry-weight and not lower than 3 p.p.m. dry-weight. The sulphate contents ranged from 0·27 to 0·63 per cent dry-weight.

The possibility of the existence of a conditioned copper deficiency in the animals grazing these pastures and showing these symptoms was therefore explored. Samples of blood were taken by venepuncture with a stainless steel needle and, at the same time, samples of liver tissue were secured by biopsy[6]. The samples were then analysed for their copper content after the organic matter present had been destroyed by wet digestion with mixed sulphuric, perchloric and nitric acids[13].

Comparative data of the blood and liver copper concentrations from cattle in four zones investigated, together with some information on the copper, molybdenum and sulphate content of the pastures in the same areas, are presented in Table 1. Data from Zone 1 are divided into three parts. On the affected area (*a*) where the animals showed rough and visibly depigmented coat, diarrhoea and retarded growth, blood and liver samples were taken three times at approximately three-week intervals. On each occasion the same low values were found indicating a condition of chronic hypocuprosis. On another area (*b*) where the animals were obviously in better condition, showing little sign of depigmented hair and no diarrhoea, the copper-levels of the blood and liver were significantly higher than those encountered on the affected area (*a*). A sub-group of animals of the same strain as those grazing area (*a*) of Zone 1 were transferred following weaning to improved pasture on the high land of the Balcarce Experiment Station. These animals, which presented prime appearance with normal lustrous coats and no diarrhoea, were sampled at the same time as those on Zone 1 (*a*). The animals on Zone 3 showed extreme depigmentation of the hair.

While more extensive information will be necessary before a causal relationship can be drawn between the hypocuprosis of these cattle and the copper, molybdenum and sulphate contents of the forage which they graze, the data presented strongly suggest that these factors may be involved.

Where the copper : molybdenum ratio is less than 2·8 : 1 the incidence of a hypocuprosis in the animals is evident; where the ratio is greater than 2·8 : 1 the copper-levels of the liver and blood of the animals are significantly higher. The effect of copper supplementation on the copper status of animals in the affected areas is now under investigation.

The description of the soils where this hypocuprosis occurs will be reported elsewhere; but in general they are alkaline rather than saline—a condition which would lead to greater availability of molybdenum to plants. The waters lying on these soils as well as those of subterranean origin have been found to contain up to 1,000 p.p.m. of sulphate. This high sulphate intake in the water, superimposed on that found in the pastures, could raise the total sulphate intake to a level where relatively small amounts of molybdenum might be potentiated[9] to reduce the copper reserves of cattle exposed to this environment. An investigation is being carried out of the efficacy of treatment with copper (as Cu-EDTA given subcutaneously) in preventing this hypocuprosis.

We thank Dr. J. P. Culot for some of the molybdenum analyses of the pastures and for comments regarding the soil conditions in the area, and Dr. G. K. Davis for valuable discussions.

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AGRICULTURE

Blood Copper-levels in Sheep in Relation to Genetic Factors, Parity and Previous Swayback History

Blood copper-levels are known to be associated with the incidence of swayback in lambs (see Barlow *et al.*)[1]. A sample of fifty-five ewes was bled some 6 weeks prior to lambing and almost a year after a 15 per cent incidence of swayback had occurred among the lambs in the flock from which these ewes were drawn. The flock consists of three breeds (Scottish Blackface, South Country Cheviot and Welsh Mountain) and the crosses between these breeds. The ewes belong to two breeding classes, outbred (F_2) and 25 per cent inbred (I_1), and to four parities. The choice of animals for bleeding took account of these subdivisions within this flock and of swayback history of the lambs born to ewes of parity 2 and more in 1964. Blood copper values were estimated by the method of Butler and Newman[2].

The blood copper values (obtained by least squares) for the effects of breed and differences due to inbreeding, parity (in-lamb for first time versus later parities), swayback history and cross-breeding, are shown in Table 1. Breed had an apparent effect on total variation in blood copper ($0{\cdot}1 > P > 0{\cdot}05$), although not all the six breed types differed significantly from each other. On average, Blackfaces had the lowest blood copper value. Heterosis was suggested by the crosses having on average higher

Table 1. Blood Copper Values (μg/100 ml. Whole Blood) for Six Breed Types of Ewe and Differences due to Cross-breeding, Inbreeding, Parity and Swayback History

Class	Copper value*	*S.E.*
Blackface	27·79	4·80
Cheviot	35 35	4·78
Welsh	40·50	4·70
Cheviot–Welsh	43·15	4·83
Blackface–Welsh	37·89	4·67
Blackface–Cheviot	40·75	4·67
Cross-bred mean–purebred mean†	6·05	3 35
I_1–F_2	−3·63	3·32
Deviation, from later parity ewes with no previous swayback lambs, of		
First parity ewes	−10·08	4·26
Later parity ewes with previous swayback lambs	−15·68	3·98

* The breed effects shown refer to the F_2 class and to the later parity with no previous swayback lambs. The average of the six fitted breed values (37·57) may be compared with the actual average of 28·55 for the blood copper values determined for the 55 ewes.

† Difference derived from the fitted breed values.

values than the average of the purebreds ($0{\cdot}1 > P > 0{\cdot}05$). The difference between inbreds and outbreds was not significant. Most clearly, ewes which nearly a year ago produced lambs with swayback had much lower blood copper values than contemporaries which had previously had normal lambs ($P < 0{\cdot}001$). Females in-lamb for the first time had significantly lower values than ewes which had lambed previously ($P < 0{\cdot}05$).

For about 6 weeks prior to bleeding, the ewes, which ran together as one grassland flock, had access to a mineral lick containing 718 p.p.m. copper. It is possible, therefore, that sheep may have ingested different quantities of mineral, which could complicate interpretation of the results. However, the special management circumstances of the experimental flock are such that there was no segregation of the flock by breed or by the other subdivisions in the analysis. In spite of the access to a mineral lick containing copper, only two of the fifty-five sheep had blood copper values greater than the 60 μg/100 ml. whole blood, which has been accepted as a 'normal' level in the literature (for references see Barlow *et al.*[1]).

It has recently been found[3] in the same flock that the incidence of swayback in lambs in 1964 was clearly associated with breed and breed cross. The mean blood copper values of the three pure breeds ranked inversely to the percentage of lambs with swayback among these breeds in the previous year. The overall correlation between the present blood copper levels of the six breed types of ewe and the percentile incidence of swayback among the lambs of the same six breed types in the same flock a year ago was $-0{\cdot}82$ ($P < 0{\cdot}05$). The genetic implications of these findings merit further investigation in relation to the swayback syndrome.

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PSYCHOLOGY

Age, Cardiac Output and Choice Reaction Time

In an earlier communication to *Nature*[1] it was argued that the attempt to resolve the discrepancy in data on choice reaction time in relation to age in terms of the effective duration of signals[2] must be regarded as inadequate. The observations were based on a small sample ($N = 48$) of data from a psycho-physiological study of the possible effects of ageing on professional (that is, airline, military and test) pilots. The present communication is a brief account of some of the further results obtained to date in a larger sample ($N = 201$).

The design of the psychological experiments is intended to reflect the fact that flying requires, *inter alia*, making high-speed decisions and detecting low probability signals, as well as an ability to receive and retain significant amounts of information in the course of routine control procedures. Consequently in the measurement of choice reaction times, latencies of responses are recorded not only under normal ('basic') conditions but also under those of 'information overload'—in an attempt to estimate 'reserve capacity'. For a more detailed description of the methods used in this investigation the reader is referred to the earlier reports[1,3,4]. The evidence summarized in Table 1 makes it clear that, as before, the principal age differences in our sample of some two hundred active pilots are to be found in the intercept constant of the formula for choice reaction ($RT = a + b \log_2 n$) under conditions of information overload, not in the slope constant. The b constant, however, appears to be directly related to cardiac output, as assessed on the basis of ballistocardiogram stroke volume and of heart rate, recorded after exercise. Furthermore, the results of the pulmonary physiology studies under conditions of maximum effort are also in line with this trend.

Table 1

(Response time $= a + b \log_2 n$)

Correlation r^* = rate of gain of information with age and cardiac output (ballistocardiogram stroke volume and heart rate)

	Serial choice task alone		Serial choice task with overload		Difference (basic-overload)	
	r	p	r	p	r	p
Rate of gain of information						
× Age	−0 13	NS†	−0 27	0 001	0 25	0·001
× Cardiac output (pre-exercise)	0 06	NS	0 01	NS	−0·06	NS
× Cardiac output (post-exercise)	0 05	NS	0 08	NS	0 04	NS
Constant a (intercept)						
× Age	0·03	NS	0 22	0·01	0·18	0·02
× Cardiac output (pre-exercise)	−0 04	NS	0 01	NS	0·03	NS
× Cardiac output (post-exercise)	0·02	NS	−0·02	NS	−0·03	NS
Constant b (slope)						
× Age	0 02	NS	0 07	NS	0·07	NS
× Cardiac output (pre-exercise)	0 01	NS	−0·05	NS	−0 05	NS
× Cardiac output (post-exercise)	−0·08	NS	−0·26	0·01	0·23	0·01

		r	p
Cardiac output	(Pre-exercise) × age	0 15	NS
	(Post-exercise) × age	−0·17	0 05
Oxygen pulse	(Maximum effort) × age	−0·12	NS
	× Difference in rates	−0 20	0 01

* Quantitative analysis carried out with the aid of a Burroughs 5500 computer
† NS: not significant.

It is felt that these findings raise important questions of fact and of analysis. In the first place, they confirm the general impression that age differences in physiological and psychological tests are more revealing when observed under conditions of stress than under baseline or resting conditions[5]. In the second place, they add to the slowly accumulating evidence[1,4,6–8] that the population samples in those studies of ageing in which the occupational and health status of the subjects was not clearly defined may have to be regarded in some sense as non-normal. In particular, with reference to data on choice reaction times, the present investigation suggests that those earlier investigations which reported slowing of response with age as an increase in the slope constant must have included, no doubt inadvertently, sub-clinical cases of cardiovascular disorder. Of course, studies of ageing which would be entirely free of pathology are to some extent imaginary; however, the difficulties which arise about 'unscrambling' the effects of ageing and those of minimal pathology are not the most convincing confirmation of the supposed vulnerability of function in later adulthood.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 21

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Lt.-Col. J. A. Simson, M.C., and Mr. C. J. Sweeney, O.B.E.: "The Ordnance Survey and Land Registration".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Conference on "Delay Devices for Pulse Compression Radar".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. C. Wood: "Developments in the Control of Ectoparasites of Livestock".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. Bourlière (University of Paris): "The Ecology of Herbivores in the African Savanna and Forest" (Further lecture on February 23).*

Tuesday, February 22

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH-EASTERN BRANCH (at the Royal Aeronautical Society, Hamilton Place, London, W.1), at 2 p.m.—Symposium on "Some Practical Aspects of Process Control by Computer".

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (in the Lecture Theatre, Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "Special Techniques in Gas Analysis".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. MEDICAL ELECTRONICS GROUP (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. P. A. Marton: "Muscular Control".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. G. E. H. Reuter: "Some Mathematical Problems in the Theory of Random Processes" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. F. Gale: "Microbes, Macromolecules and Mayhem". (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Jean Hiernaux (Brussels): "Biological Studies on Some Human Populations of Central Africa. I. The Populations of Ruanda. Methods of Analysis".*

Wednesday, February 23

SOCIETY OF CHEMICAL INDUSTRY (joint meeting of the Food and Microbiology Groups, at the School of Pharmacy, Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "Microbiological Flora in Relation to Quality".

GEOLOGICAL SOCIETY (at the Royal Institution, Albemarle Street, London, W.1), at 5 p.m.—Prof. V. Beloussov (Academy of Sciences of the U.S.S.R.): "The Earth's Crust and the Upper Mantle"; Dr. M. H. Hey, Mr. E. A. Jobbins and Dr. A. J. Meadows: "The Barwell (Leicestershire) Meteorite, 24 December 1965" (demonstration).

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in the Physics Department, Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Dr. J. W. S. Cassels, F.R.S.: "What are p-Adic Numbers?"*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. L. Bliss: "Remote Synchronization of Television Waveform Generators".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. F. Bentham: "Theatre Lighting in Britain To-day".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "Mental Stresses Induced by Audio and Visual Hazards" opened by Mr. B. Shackel.

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. F. Bailey: "Climatic Protection of Packaged Goods?".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Dr. Betty Hobbs: "Modern Techniques in the Bacteriological Examination of Food".

Thursday, February 24

PARLIAMENTARY AND SCIENTIFIC COMMITTEE (in the River Room, Savoy Hotel, London, W.C.2), at 11.30 a.m.—Annual General Meeting.

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. Henry Harris: "Hybrid Cells from Mouse and Man—a Study in Genetic Regulation".

ST. MARY'S HOSPITAL MEDICAL SCHOOL (in the Lecture Theatre of the Wright Fleming Institute, London, W.2), at 5 p.m.—Prof. R. A. Gregory, F.R.S.: "The Gastric Antral Hormone: Past, Present and Future" (Augustus Desiré Waller Memorial Lecture).*

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 5 p.m.—Prof. F. W. Spiers: "Biological and Physical Aspects of the Irradiation of Bone".*

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. S. V. Perry: "Biochemistry of Muscle".*

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Automatic Data Processing" introduced by Mr. C. W. Pugh.

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31-34 Gordon Square, London, W.C.1), at 5.30 p.m.—Prof. G. L. Huxley: "Excavations and Studies in Kythera 1965".*

UNIVERSITY OF LONDON (at the Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5), at 5.30 p.m.—Prof. D. Ploog (Munich): "Social Behaviour and Group Interaction in Squirrel Monkeys".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. S. J. G. Semple: "Respiration and the Cerebrospinal Fluid." (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).*

UNIVERSITY OF LONDON (in the Engineering Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Jean Hiernaux (Brussels): "Biological Studies on Some Human Populations of Central Africa. II. Ecological Factors: The Effects of Heredity and Environment".*

CHEMICAL SOCIETY (in the Large Physics Lecture Theatre, Imperial College, London, S.W.7), at 6 p.m.—Dr. E. J. Bowen, F.R.S.: "Light Emission from Organic Molecules" (Liversidge Lecture).

Friday, February 25

INSTITUTE OF RURAL LIFE AT HOME AND OVERSEAS (at Friends International Centre, Courtauld House, Byng Place, London, W.C.1), at 1.15 p.m.—Dr. Roy C. Heather: "Water in Rural and Urban Life".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. A. M. Dawson: "Fat Absorption".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. O. Scanlan and Mr. R. Levy: "Distributed Network Synthesis".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. D. J. Parkyn: "The Use of Test Line Signals for Remote Monitoring of Picture Quality at Unattended Transmitters".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. W. H. G. Armytage: "History of the Future".

Saturday, February 26

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE (at the British Museum (Natural History), Cromwell Road, London, S.W.7), from 10.30 a.m. to 6 p.m.—Symposium on "Living in the Dark".

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Rex Wailes: "The Industrial Monuments Survey".*

Monday, February 28

INSTITUTE OF ACTUARIES (at Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. S. Benjamin: "Putting Computers on to Actuarial Work".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. C. J. F. Böttchler (Leiden): "Pathology and Chemistry".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. J. W. Tukey (Princeton University): "Numerical Spectrum Analysis". (Further lectures on March 2 and 4).*

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. I. Scollay and Mr. G. Caldwell: "Cold Press Moulding".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Michael Ward: "On the Highland Frontier of North Bhutan".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with research experience in the field of algology, microbiology or biochemistry) IN THE DEPARTMENT OF MARINE BIOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (February 25).

LECTURER IN THE SCHOOL OF EDUCATION—The Registrar, The University, Liverpool, quoting Ref. CV/418 (February 26).

LECTURER OR ASSISTANT LECTURER IN THE SUB-DEPARTMENT OF MATHEMATICAL STATISTICS—The Registrar, The University, Liverpool, quoting Ref. CV/427 (February 26).

UNIVERSITY LECTURERS (2) IN METALLURGY—The Secretary of Faculties, University Registry, Oxford (February 26).

LECTURER (honours graduate in science, pharmacy or medicine) IN PHARMACOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (February 28).

SENIOR RESEARCH ASSOCIATE (with a Ph.D. degree in chemistry or equivalent experience in research) to work on the chemistry of boron in collaboration with Prof. N. N. Greenwood—Prof. N. N. Greenwood, Department of Inorganic Chemistry, The University, Newcastle upon Tyne (February 28).

A.R.C. RESEARCH ASSISTANT (graduate in the biological sciences or in chemistry) IN THE DEPARTMENT OF BOTANY to work with Dr. J. S. Pate on the metabolic functioning of leaves—The Secretary, Botany Department, The Queen's University, Belfast, Northern Ireland (March 1).

LECTURER (with good honours degree and some postgraduate experience) IN MECHANICAL ENGINEERING—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (March 1).

SENIOR LECTURER OR LECTURER IN PHYSIOLOGY OR ZOOLOGY; and an ASSISTANT LECTURER OR DEMONSTRATOR IN ZOOLOGY—The Secretary, Wye College (University of London), near Ashford, Kent (March 1).

READER IN APPLIED MATHEMATICS at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 4).

READER IN NUMERICAL ANALYSIS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (March 4).

CHAIR OF ZOOLOGY—The Registrar, The University, Hull (March 5).

LECTURER/ASSISTANT LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Hull (March 5).

CHAIR OF MATHEMATICS—The Secretary, University of Stirling, P.O. Box 1, Stirling (March 7).

LECTURER IN THEORETICAL PHYSICS—The Registrar, The University, Newcastle upon Tyne, 2 (March 7).

LECTURER IN ZOOLOGY at University College, Dar-es-Salaam (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 8).

READER IN EXPERIMENTAL PHYSICS at Royal Holloway College, Englefield Green, Surrey—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 9).

SENIOR LECTURER or LECTURER IN MATHEMATICS at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Inter-University Council, 33 Bedford Place, London, W.C.1 (March 11).

RESEARCH ASSOCIATE (preferably young graduate with biological, surgical or pathological training, with or without previous research experience) for a two-year project on various experimental aspects of fibrosing mechanisms and wound healing in pigs, etc.—The Secretary, A.R.C. Institute of Animal Physiology, Babraham, Cambridge (March 15).

CHAIR OF HUMAN BIOLOGY—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (March 31).

CHAIR OF METALLURGY—The Registrar, The University, Sheffield (March 31).

THIRD CHAIR OF PHYSICS—The Registrar, The University, Sheffield (March 31).

UNIVERSITY LECTURER and a UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ANIMAL PATHOLOGY in the School of Veterinary Medicine, for duties which will include teaching and research in either morbid anatomy or microbiology—T. D. Kellaway, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge (March 31).

UNIVERSITY LECTURER IN THE DEPARTMENT OF METALLURGY—The Secretary of the Appointments Committee of the Faculty of Physics and Chemistry, Chemical Laboratory, The University, Lensfield Road, Cambridge (March 31).

PROFESSOR (with registrable medical qualifications) IN THE DEPARTMENT OF ANATOMY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (April 12).

LECTURER or ASSISTANT LECTURER (with a good honours degree and experience in modern semiconductor circuit design, and preferably an interest in communications engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Secretary, The University, Edinburgh (April 30).

CHIEF LIBRARIAN (scientist with an aptitude for, and experience in, library work, or trained librarian with an interest in science, particularly chemistry)—The Director, Paint Research Station, Waldegrave Road, Teddington, Middlesex.

HEAD (man or woman, with a good honours degree and teaching experience, and preferably previous service in a College of Education) OF THE GEOGRAPHY DEPARTMENT—The Clerk to the Trustees, Homerton College, Cambridge.

PHYSICAL CHEMIST capable of pursuing and supervising a range of basic and applied researches mainly on electrodeposition and electroforming—The Secretary, The British Non-Ferrous Metals Research Association, Euston Street, London, N.W.1.

PHYSICIST (with a good honours degree) to join a small group working on radiation protection problems associated with neutron radiation—The Director, Radiological Protection Service (Ministry of Health and Medical Research Council), Clifton Avenue, Belmont, Sutton, Surrey.

RESEARCH ASSISTANT IN THE DEPARTMENT OF FORENSIC MEDICINE to assist in a programme of research into the development of rapid methods of toxicological analysis—Dr. Harold V. Street, Department of Forensic Medicine, University of Edinburgh, Teviot Place Edinburgh.

RESEARCH DEMONSTRATORS (with a good degree in botany or horticulture) IN THE DEPARTMENT OF HORTICULTURE to assist with practical classes and carry out research in the physiology of horticultural plants—The Assistant Bursar (Personnel), University of Reading, Reading.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Zoological Record, Vol. 100, Section 2 (1963): Protozoa. Part 1 compiled by Dr. R. A. Neal and Pauline Curds. Part 2 compiled by Pauline Curds and Sarah E. Bunney. Pp. 156. (London: The Zoological Society of London, 1965.) 30*s*.; 4.23 dollars. [812

Johnson Matthey and Co., Ltd. Platinum Metal Catalysts. Pp. 20. (London: Johnson Matthey and Co., Ltd., 1965.) [812

Bulletin of the British Museum (Natural History). Geology. Vol. 10, No. 10: Cretaceous Ammonites and Nautiloids from Angola. By Dr. Michael Kingsley Howarth. Pp. 335–412+13 plates. 55*s*. Vol. 10, No. 11: The Fauna of the Portrane Limestone, III—The Corals. By Dimitri Kaljo and Einar Klaamann. Pp. 413–434+4 plates 18*s*. Vol. 11, No. 3: Middle Jurassic Ostracoda from the Grey Limestone Series, Yorkshire By R. H. Bate. Pp. 73–133+21 plates. 80*s*. Vol. 11, No. 4. Human Skeletal Material from Ceylon, with an Analysis of the Island's Prehistoric and Contemporary Populations. By Dr. Kenneth A. R. Kennedy. Pp. 135–213+15 plates. 45*s*. Zoology. Vol. 13, No. 8: Studies on the British Dermanyssidae (Acari: Mesostigmata). Part 1: External Morphology. By G. Owen Evans and W. M. Till. Pp. 247–294. 21*s*. (London: British Museum (Natural History), 1965.) [812

Philosophical Transactions of the Royal Society of London Series A: Mathematical and Physical Sciences. No 1095, Vol. 259 (9 December 1965): Second Order Vibration of Flexible Shafts. By R. E. D. Bishop and A. G. Parkinson. Pp. 1–31+plate 1. 13*s*. 6*d*.; 2 dollars. Series B: Biological Sciences No. 763, Vol. 250 (9 December 1965): A Study in the Tornoceratidae: The Succession of *Tornoceras* and Related Genera in the North American Devonian. By M. R. House. Pp. 79–130+plates 5–11. 33*s*; 4.95 dollars. (London: The Royal Society, 1965.) [812

Building Research Station Digest (Second Series), No. 65 (December 1965): The Selection of Clay Building Bricks—1. Pp. 4. (London: H.M. Stationery Office, 1965) 4*d*.

Southern Regional Council for Further Education. Triennial Report 1962–1965. Pp. 59. (Reading: Southern Regional Council for Further Education, 1965.) [2212

The Gas Laser and Its Experimental Applications. By R. J. Horton and Dr. P. D. Cook. Pp. 31. (London: Scientifica and Cook Electronics, Ltd., 1965.) 12*s*. 6*d*. [2212

The Overseas Development Institute. Aid in the Commonwealth. By Peter Williams. Pp. 56. (London: The Overseas Development Institute, 1965.) 6*s*. [2212

Chemistry and Industry Buyers' Guide. Chemicals, Chemical Plant, Laboratory Equipment and Apparatus. Pp. 146. (London: The Society of Chemical Industry, 1966) 7*s*. 6*d*. [2212

Other Countries

Annals of the New York Academy of Sciences. Vol. 131, Article 2: Mechanisms of Dental Caries. By Theodore X. Barber and 48 other authors. Pp. 685–930. (New York: New York Academy of Sciences, 1965.) 5 dollars. [812

New Zealand. National Roads Board: Road Research Unit. R.R.U. Bibliography No. 2. Liquids in Soils—an Alphabetical Author Index, 1955–1964. Compiled by E. E. Swinbourn. Pp. 46. (Wellington, N.Z.: National Roads Board, 1965.) [812

Transactions of the American Philosophical Society. New Series, Vol. 55, Part 7: Open-Field Husbandry and the Village Community—a Study of Agrarian By-Laws in Medieval England. By Warren O. Ault. Pp. 102. 2.50 dollars. New Series, Vol. 55, Part 8. Eleven-Digit Regular Sexagesimals and Their Reciprocals. By Owen Gingerich. Pp. 38. 1 dollar. (Philadelphia: American Philosophical Society, 1965.) [812

United States Department of the Interior: Geological Survey Bulletin 1175: Geology of the Cortez Quadrangle, Nevada. By James Gilluly and Harold Masursky Pp. v+117+plates 1–3. Water-Supply Paper 1580–E: A Comparison of Methods Used in Flood-Frequency Studies for Coastal Basins in California. By R. W. Cruff and S. E. Rantz. Pp. v+56+plates 1 and 2. 70 cents. (Washington, D.C.: Government Printing Office, 1965.) [812

United States Department of the Interior: Geological Survey. Professional Paper 433–D: Mineralogy and Cation-Exchange Capacity of Sediments from Selected Streams. By Vance C. Kennedy. Pp. iii+28. 25 cents. Professional Paper 448–H: Low-Flow Characteristics of Streams in the Mississippi Embayment in Tennessee, Kentucky and Illinois. By Paul R. Speer, W. J. Perry, John A. McCabe, O. G. Lawa and others. With a section on Quality of the Water by H. G. Jeffery. Pp. iii+36+plates 1 and 2. Professional Paper 473–C: Geologic and Hydrologic Studies in the Birmingham Red-Iron-Ore District, Alabama. By Thomas A. Simpson. Pp. iv+47+plates 1 and 2. Professional Paper 488: Geomorphology and Quaternary Geology of the Owensboro Quadrangle, Indiana and Kentucky. By Louis L. Ray. Pp iv+72+plate 1. Professional Paper 495–A: Stratigraphy and Geologic History of the Uppermost Cretaceous, Paleocene and Lower Eocene Rocks in the Wind River Basin, Wyoming. By William R. Keefer. Pp. iv+77+plates 1–4. Professional Paper 503–D: Marine Jurassic Gastropods, Central and Southern Utah. By Norman F. Sohl. Pp. iii+29+plates 1–5 45 cents. Professional Paper 503–E: Revision of Some Paleozoic Coral Species from the Western United States. By William J. Sando. Pp. iii+38+plates 1–15. 65 cents. (Washington, D.C.: Government Printing Office, 1965.) [812

Organization for Economic Co-operation and Development. The Research and Development Effort in Western Europe, North America and the Soviet Union: An Experimental International Comparison of Research Expenditures and Manpower in 1962. By C. Freeman and A. Young. Pp. 152. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1965.) 10 francs; 15*s*.; 2.50 dollars. [812

United States Department of the Interior: Geological Survey. Water-Supply Paper 1759–B: Water Resources in the Vicinity of Municipalities on the Western Mesabi Iron Range, Northeastern Minnesota. By R. D. Cotter, H. L. Young, L. R. Petri and C. H. Prior. Pp iv+24+plate 1. Water-Supply Paper 1759–D: Water Resources in the Vicinity of Municipalities on the Central Mesabi Iron Range, Northeastern Minnesota. By R. D. Cotter, H. L. Young, L. R. Petri and C. H. Prior. Pp. iv+20+plate 1. Water-Supply Paper 1759–E: Water Resources in the Vicinity of Municipalities on the East-Central Mesabi Iron Range, Northeastern Minnesota By R. D. Cotter, H. L. Young, L. R. Petri and C. H. Prior. Pp. iv+23+plate 1. Water-Supply Paper 1799: Geology and Occurrence of Ground Water in Otero County and the Southern Part of Crowley County, Colorado. By William G. Weist, Jr. Pp. vi+90+plates 1–5. Water-Supply Paper 1809–K: Hydrology of Melt-Water Channels in Southwestern Minnesota. By Gerald L. Thompson. Pp. iii+11+plates 1–3. Water-Supply Paper 1809–P: Water Supply Potential from an Asphalt-lined Catchment near Holualoa, Kona, Hawaii. By S. S. W. Chinn. Pp. iv+25. Water-Supply Paper 1811: Hydrology of the Little Plover River Basin, Portage County, Wisconsin, and the Effects of Water Resource Development. By E. P. Weeks, D. W. Ericson and C. L. R. Holt, Jr Pp. v+78+plates 1–6 Water-Supply Paper 1953: Quality of Surface Waters of Alaska, 1961–63. Prepared under the direction of S. K. Love. Pp. vi+95. 35 cents. (Washington, D.C.: Government Printing Office, 1965.) [2212

East African Common Services Organization. East African Trypanosomiasis Research Organization Report, July 1963–December 1964 Pp. ii+70. (Tororo, Uganda: East African Trypanosomiasis Research Organization, 1965) 4*s*. [2212

Canada. National Research Council and Medical Research Council Annual Report on Support of University Research, 1964–65. Pp. vii+271. (N.R.C. No. 8627.) (Ottawa: National Research Council and Medical Research Council, 1965.) 50 cents. [2212

United States Department of the Interior: Geological Survey. Bulletin 1187: Quicksilver Deposits of Southwestern Alaska. By C. L. Sainsbury and E. M. MacKevett, Jr. Pp. vi+89+plates 1–8. Bulletin 1194–H: Mesozoic Formations in the Comobabi and Roskruge Mountains, Papago Indian Reservation, Arizona. By L. A. Heindl. Pp. iii+15. 10 cents. Bulletin 1201–E: Bedrock Geology of the Grand Lake Area, Aroostook, Hancock, Penobscot, and Washington Counties, Maine. By David M. Larrabee, Charles W. Spencer and Donald J. P. Swift. Pp. iv+38+plate 1. Bulletin 1224–C: The Lexington Limestone (Middle Ordovician) of Central Kentucky. By Douglas F. B. Black, Earle R. Cressman and William C. Macquown, Jr. Pp. iii+29. 15 cents. (Washington, D.C.: Government Printing Office, 1965.) [2212

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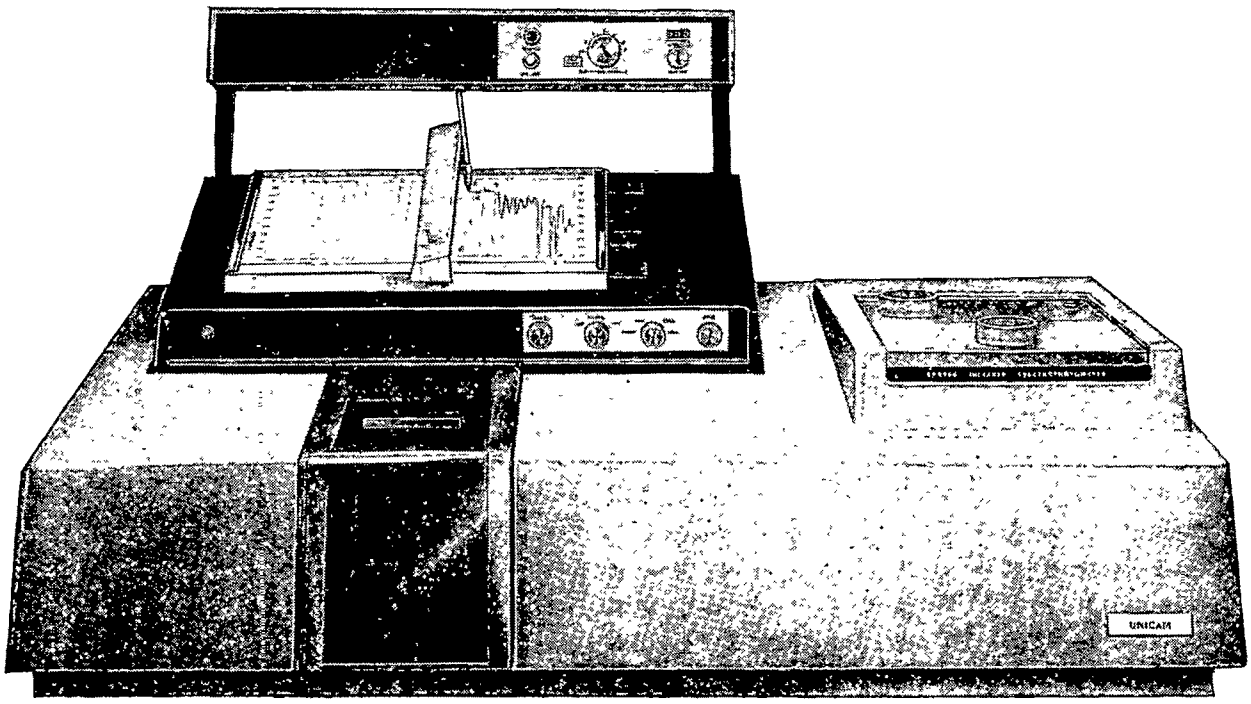
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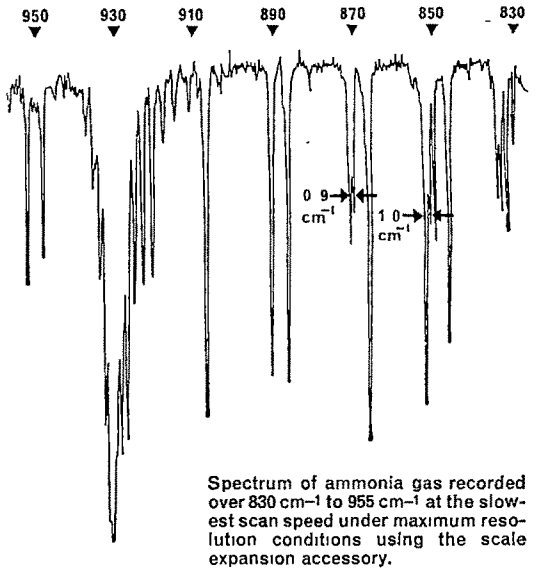
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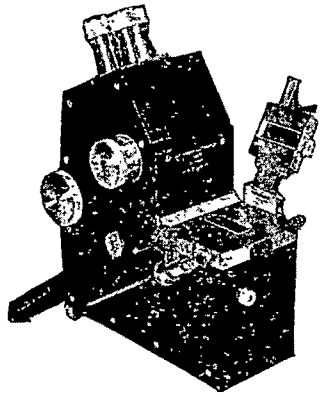
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CERN: AN OPPORTUNITY IN HIGH-ENERGY PHYSICS

AFTER the session of the Council of the European Organization for Nuclear Research (CERN) in December 1965, it was announced that twelve of the member States (that is, all of them with the exception of Greece) had decided to participate in the supplementary programme for the construction of a pair of intersecting storage rings to be operated in conjunction with the 28-GeV CERN proton synchrotron. This decision, the culmination of a long period of discussion, study and planning, has great significance in relation to the future development of the CERN Laboratory and of high-energy physics in Europe as a whole.

When, in the early 'fifties, a handful of enthusiastic and far-sighted senior physicists from a number of European countries came together to plan the CERN laboratory, few of them could have foreseen how successful their efforts would prove. In little more than 10 years a great centre of high-energy physics has been established with a world-wide reputation for carrying out research of high quality and originality. The movement of some of the brightest and most talented young scientists in this field from Europe to the United States has not been halted, but it has been supplemented by a similar, if smaller, movement in the opposite direction, so that it is beginning to take the form of a very desirable two-way stream. CERN has in fact become a centre of research attracting scientists from all over the world. As a centre of international scientific collaboration it has inspired, by its success, similar co-operation in other fields and serves as a model for the development of such centres.

The intersecting storage rings project envisages the construction alongside the CERN proton synchrotron of two roughly circular concentric rings about 300 m in diameter which are distorted so that they intertwine, crossing in eight places with an intersection angle of 15°. Pulses of protons will be injected from the proton synchrotron into the rings. Systems of magnets will maintain these injected protons moving in opposite senses round the two rings so that at the intersection regions the two beams of protons will clash almost head on. Many hundreds of pulses will be injected into each ring until a circulating current of up to 20 amp will be stored in each and it should be possible to maintain these stored beams of protons for a day or more.

The great attraction of clashing beams stems from the fact that in the interaction between two protons, one in either beam, the mass centre of the two particles is almost at rest so that the resultant total momentum of the system is small. This is in contrast to the situation in a conventional accelerator where interactions occur between one energetic and one stationary particle. Since in the final system the total momentum of the products has to be the same after as before the interaction, the greater part of the energy, in the case of particles accelerated in a conventional accelerator, has to go in providing this momentum. In the intersecting storage rings, on the other hand, almost the whole of the energy will be available for producing nuclear interactions.

The total energy of about 56 GeV that will be available in the intersecting storage rings should therefore produce interactions between protons which, for a conventional accelerator, would require an energy of 1,700 GeV. On present indications a conventional machine of such a high energy seems unlikely to be built within the next generation, if at all Although the collision rate obtainable in the intersecting storage rings will be much smaller than that for a conventional accelerator of equivalent energy, the estimated rate of 10^5 interactions per sec is as much as can comfortably be handled with the types of detection techniques suitable for use with this system. Indeed, for many experiments it will be advantageous to operate with a smaller circulating current.

The construction and operation of the intersecting storage rings will present some challenging technical problems. In the kilometre-long tubes that carry the beam a pressure not greater than 10^{-9} mm of mercury will have to be maintained, while in the neighbourhood of the intersections the pressure will need to be kept down to at most one-tenth of this. In the course of the time during which the beams will frequently be stored the protons will traverse a distance hundreds of times as great as that from the Earth to the Sun. While traversing this great distance they will need to be confined within a tube with a maximum cross-section of 16 cm.

The very magnitude and novelty of the technical challenge are only slightly less exciting than the physics which will be made possible when these problems have been successfully overcome. Nobody has any real doubt that they will be overcome. The team that has been working on the design of the intersecting storage rings and will be responsible for the technical supervision of their construction embraces a quite unique range of experience, technical know-how and capacity for daring innovation.

The substantial increase in effective interaction energy which will become available as a result of the construction of this machine should yield information of great significance for the solution of some of the most fundamental problems relating to the nature of matter and of interactions between particles of great energy and at very small separations. The range of new physical phenomena revealed by experiments with existing accelerators has proved to be unexpectedly rich. The lines of the interpretation of these phenomena have been suggested, but decisive tests of these interpretations seem likely to be possible only when much greater effective interaction energies are available. This is the case in the investigation of the so-called strong interactions—which include the class of interactions responsible for the stability of atomic nuclei—where there is evidence to suggest that the striking symmetries that have been recognized in the past few years may perhaps be understood in terms of the existence of three kinds of very massive primeval particles (quarks). The isolation of these quarks, would, however, require interaction energies much greater than those available from any existing accelerator.

It is also the case in the investigation of the weak interactions—which include the class of interaction responsible for β-radioactivity—where the available experimental evidence strongly suggests moderation of the interaction through the so-called W particle (or inter-

mediate boson) of mass too large to be produced with presently available accelerator energies.

The actual experimental programme of the CERN proton synchrotron has proved very much more diverse and interesting than any that was predicted for it when it was being planned in 1954. This is likely to be the case also for the intersecting storage rings. Nature is far richer than the mind of the most imaginative physicist can foresee.

Experimentation with the intersecting storage rings presents problems of great difficulty. It will be necessary to develop detectors with better time resolution than is generally available at present, capable of operating under conditions where many particles are produced in the same interaction, and of discriminating between colliding beam interactions and interactions with the background gas. Considering the pace of technical development, however, these difficulties do not seem more daunting than others that have already been successfully solved in the field of high-energy physics instrumentation.

The cost of the intersecting storage rings is estimated at approximately £28 million spread over 6 years. It will involve an increase of about 35 per cent in the CERN budget, one-quarter of which is covered by the British contribution. This extra expenditure has, however, to be measured against the returns it will bring in the form of an extension of the useful working life of the CERN laboratory throughout the period 1970–80 when it should be in a position to make a unique contribution in this field of study that could be made neither by the 70-GeV proton synchrotron of the Serpukhov Laboratory in the U.S.S.R., due to come into operation at the end of 1967, nor by the 200-GeV United States machine which is expected to be operational by 1972 or 1973. Judged from the point of view of its role in maintaining a centre of excellence in high-energy physics research in Europe, it appears to be an imaginative and worth-while investment.

It is most gratifying that firm commitments to proceed with the construction of the intersecting storage rings came just prior to the retirement of V. F. Weisskopf from the post of director-general of CERN. During his period of office, Weisskopf worked tirelessly to impress on the appropriate authorities in the member States of CERN the need for long-term planning of the future European programme in high-energy physics. His initiative and enthusiasm led to the setting up in 1963 of the European Committee for Future Accelerators under the chairmanship of E. Amaldi. With the agreement on the intersecting storage rings, the first part of the programme recommended by this Committee is in the process of being implemented. The second, even more important and certainly much more costly part of the programme recommended by this Committee, the construction of a 300-GeV proton synchrotron in Europe, is still being studied. The intersecting storage rings are in no way an alternative to such an accelerator, because the latter can also produce beams of energetic mesons and other particles which are a very important additional experimental resource for high-energy physics. For the production of such beams, the motion of the centre of mass in proton–proton collisions is an essential advantage, as it provides much of their energy. In view, therefore, of the complementary nature of the two parts of this programme, European high-energy physicists will hope that a favourable decision on this other part of the programme will not be too long delayed.

HIGHER EDUCATION IN BRITAIN

THE debate on higher education in the House of Lords on December 1, which was opened by Lord Robbins, did not re-examine the question of the scale of university expansion, except in so far as to express doubts about whether the agreed target could now be achieved, in view of the 6 months standstill imposed on new university building. This standstill; as Lord James of Rusholme promptly pointed out, in practice meant a delay of at least a year. The question of organization, which had been the other main theme of the debate in December 1963, was again to the forefront, and Lord Robbins strongly attacked what he termed the 'binary system' set up in the Ministry of Education and Science by the Government. The other two main topics of the debate were the development of the colleges of advanced technology and the position of the teachers' training colleges.

Lord Robbins questioned the wisdom of the decision that no new universities, beyond the six recommended by the Committee on Higher Education, should be created during the next 10 years, and suggested that this decision might create difficulties for forward planning in later years. He was more concerned, however, that the assumption of university status by the colleges of advanced technology might be accompanied by a change in work or function as well as position. He emphasized the vital importance of their continuing to develop on their existing basis and in this he received strong support from Lord Kings Norton, who insisted that the preponderating position of the sandwich course must be maintained, as well as the collaboration between industry, commerce, the professions and the teaching staff.

Lord Robbins's other anxiety concerned the teacher training colleges, and he argued strongly, as did the report of his committee, that the administration of the colleges should be transferred to the universities. He thought that this question of status could be of vital importance in ensuring that an adequate supply of candidates of high ability was forthcoming; he regarded the failure to implement this recommendation as an outstanding weakness of the new binary system. Lord Robbins did not receive general support in this view, though he was strongly supported by Lord Murray of Newhaven. Lord Murray was also concerned about the effect of centralizing administrative arrangements in the Department of Education and Science on higher education and research generally. He was disturbed at the possibility that complete absorption of the universities and the research councils in one Ministry might prejudice the development of higher education as well as research. Lord James of Rusholme, although he supported the idea of a single Ministry, strongly attacked the financial policy which the Government had in practice pursued towards the universities. He insisted that the financial arrangements required overhaul and, while willing to accept closer financial controls, emphasized that such controls must provide security and flexibility. His argument was strengthened by a powerful aside in pleading for adequate finance for

university libraries, which at the lowest were as essential as laboratories or apparatus.

Lord Snow, conceding the crucial importance of academic freedom, was more optimistic as to the possibility of achieving it within the framework of existing arrangements, particularly if information was distributed effectively. He defended the binary system, and in his reference to the teachers' training colleges went a long way towards accepting Lord Robbins's argument. On the question of strengthening the University Grants Committee he was silent, and although Lord Robbins's original plea here was taken up by Lord Hankey and Lord Sherfield, and particularly by Lord Annan, who made several constructive suggestions, it received no comment from the Earl of Longford, who concluded for the Government. Lord Todd also was dubious about the Government's financial arrangements for university building, but the main point in his speech related to the use of television, particularly in the development of what had been described as a 'university of the air'. He thought that there were three educational objectives of major importance which should receive priority at this time. First, there was the provision of school courses in science and mathematics to offset the present shortage of teachers of these subjects; secondly, the provision of courses in science and technology for re-education and training; and thirdly, the development of extra-mural education by the universities.

Lord Todd thought it was in regard to the two last-mentioned objectives that television had a vital contribution to make. If the problems of technical training and adult education were to be tackled, the universities and colleges of technology would have to play their part, and for this they needed a 'university of the air'. Lord Todd was supported by Lord Annan but, while the Earl of Longford confidently affirmed the Government's belief that the target figures of 218,000 university places in 1973–74 would be achieved despite the fact that building starts for 1967–68 and 1968–69 had not yet been agreed, on the 'university of the air' he said no more than that all the implications were being carefully considered. He thought that a 'university of the air' would need a substantial share in a television channel with national coverage. He agreed, however, that the University of Strathclyde could play a useful part in any national scheme by training staff and by preparing and producing programmes that could be transmitted nationally. He declined to commit himself as to licences for low-powered transmission by this or other universities. He did not reply to Lord Wynne-Jones's observation that the estimates in *The National Plan* provided for only 170,000 university students by 1970 and not 199,000, nor was he forthcoming as to alternative sources of university finance, such as higher fees or student loans which had been mentioned in the debate. On the colleges of advanced technology, he merely said that the Government recognized that the National Council for Academic Awards had a major part to play in shaping the future of the technical colleges as centres of higher education.

The impression of 'woolly' thinking on the part of Government spokesmen in this debate is strengthened, rather than otherwise, by some of the papers contributed to the symposium on "The Modern University" at the University of Strathclyde in November. Sir Denis Brogan, dealing with "The Democratic University: American and British", and Dr. Magnus Pyke, discussing "Universities in the Modern Age of Scientific Understanding", dealt with some of the wider issues which had been raised in the debate in the House of Lords. Dr. Pyke made a powerful plea that the universities should endeavour to produce the 'all-rounder' as well as the specialist. Prof. S. G. E. Lythe also gave a firm warning against yielding to professional pressure, and maintained that the first task of a modern university was to ensure that all its graduates had experienced a rigorous intellectual discipline in one field of investigation in which they have penetrated to fundamentals. G. E. Hartley offered some wise words about research and teaching and about the danger of forgetting that only a few students eventually become university teachers; while I. F. Clarke insisted on the importance of a broad curriculum.

One significant characteristic of the local contributions to the symposium was the way in which they pointed to practical means of increasing efficiency or diminishing the costs of university education by attention to points which, possibly trivial in themselves, could in the aggregate be considerable. Mr. E. W. Hughes, of Newcastle upon Tyne, spoke on "New Patterns in University Organization" and was followed by a very practical contribution from Mr. L. McGougan, "A Philosophy for University Administration". The contributions on library services and students' book grants, and notably Mr. W. Scott's paper on "Technological Services in a Technological University", were characterized by a like valuable pragmatism. It would seem from this symposium that the possibilities of increasing efficiency are far more clearly grasped within the universities than by the Government. This was very conspicuous in the papers from K. J. W. Alexander, on "The University and Adult Education", and from A. Ward on "The University of the Air". Such arguments as were put forward were by no means disposed of by the Earl of Longford in the House of Lords debate or by what was added in a written answer in the House of Commons on December 21.

The existence of serious concern over the Government's attitude to the universities was even more apparent in a later article by Lord Robbins in the *Universities Quarterly*. Such concern is also reflected in the report of the Flowers Committee, in the annual survey of the University Grants Committee for the academic year 1964–65*, and in some remarks of Dr. W. H. F. Barnes, vice-chancellor of the University of Liverpool, at the annual meeting of the University Court. Immediately before the Christmas recess, on December 21, the Secretary of State for Education and Science, Mr. A. Crosland, did announce that the Government accepted the recommendations of the Flowers Report, but proposed to spread the expenditure over 6 instead of the 5 years recommended. Little else emerged to remove the misgivings expressed, and the argument for the five essential freedoms of the universities could not well have been better expressed than by Dr. Barnes.

Dr. Barnes agreed that the universities must expect to account to the Government for the expenditure of the large sums voted to them. They must, however, remain free to select their staff and choose their students, to determine their degrees and standards and courses for undergraduates, to decide on their own research programmes and to allocate their financial resources. Dr. Barnes insisted in particular that universities should not accept the extreme thesis that their function was to supply graduates in the proportions required by the latest economic blueprint. He was also concerned that variety in sources of financial support should be encour-

* University Grants Committee—*Annual Survey, Academic Year 1964–1965*. Pp. 15. Cmnd. 2846. (London: H.M.S.O., 1965.) 1s. 6*d*. net.

aged, particularly if the freedom of a university to decide on its own research programme was not to be affected by over-dependence on grants from research councils or from industry.

Apart from the final emphasis on the difficulties and uncertainties which the building deferment policy inevitably involves in the expansion of the universities to meet the Government's objectives and in the arrangements to be made for the next quinquennium, the University Grants Committee in its annual survey only touches on its differences with the Government in muted terms: the frustrations and anxieties are obvious but are allowed to speak for themselves. The Committee, however, is in full agreement with the concept of a dual system of higher education, with each sector—the autonomous and the public—making its own contribution. The Secretary of State for Education and Science developed this idea in a speech at the Woolwich Polytechnic on April 27, 1965, and has discussed its implications with the Committee of Vice-Chancellors and Principals. It regards the Minister's decision not to support the proposed integration of the Lanchester College of Technology at Coventry with the University of Warwick, while encouraging the closest possible academic co-operation, as giving a guide for the practical application of the policy of the dual system.

On university expansion and building programmes, the Committee decided to make an immediate allocation among the universities of the £33 million for building starts in 1966–67, but to postpone the allocation of the provisional £25 million for each of the following two years until it had completed the investigations and discussions necessary to establish final figures for these 2 years. The Committee foresaw considerable difficulties, both for itself and for the universities, if decisions about the priorities of particular building projects had to be taken in relation to allocations which might later be significantly altered to take account of under-capitalization and obsolescence. The Committee is grateful to the universities for their response to the request for a detailed return of the total amount of accommodation available, or expected to be available, by 1967–68, together with an assessment of the suitability of buildings for continued use for university purposes. Owing to such co-operation in this massive and laborious operation, the University Grants Committee was able to submit in May a comprehensive analysis of the whole accommodation available by 1967–68 in relation to the student population at that date and the extent of the deficiencies which it would be necessary to make good. The analysis was discussed with the Department of Education and Science, but the discussions had not been concluded when the Chancellor of the Exchequer announced the Government's economic measures on July 27. Since then, the University Grants Committee has been ascertaining from the universities the precise effect of the deferment policy on their building programmes for 1965–66 and has discussed with the Department of Education and Science the extent to which exemptions from the general rule may be considered in particular cases and the repercussions of the deferment policy on the allocations of building starts for later years.

The Committee and its Medical Sub-Committee were much concerned during the year with planning measures to increase the student intake of the medical schools. There is still a large gap between the actual annual intake and the intake which would be needed to correct the increasingly critical shortage of doctors—the present bottleneck being at the preclinical stage. In the present financial situation there is no prospect of embarking on a comprehensive programme of modernization, but the Committee has been invited to submit evidence to the Royal Commission on Medical Education, under the chairmanship of Lord Todd, appointed on June 29. Arrangements have been made to carry forward the planning for the new Medical School and Teaching Hospital at Nottingham where the Committee has already given financial assistance towards the initial cost of the staff and the development of the library.

The University Grants Committee was also asked to advise on the additional provision of a recurrent grant which it would be appropriate to make during the last 2 years of the present quinquennium to continue the build-up of the Imperial College of Science and Technology, the Manchester College of Science and Technology and the University of Strathclyde, while encouraging and expanding promising developments in the technological departments of other universities. Supplementary recurrent grants totalling £1 million for 1965–66 and 1966–67 were announced on June 29 for allocation to these three institutions to rectify certain serious deficiencies in technology, to consolidate existing activities, and to support selected important projects which were developing out of existing activities. At the same time, the Government indicated that it intended to make available up to £400,000 over the same period for selected approved technological projects in the remaining university fields. The Committee has also advised on the recurrent grant requirements of the colleges of advanced technology for these 2 years and on proposals for the acquisition of additional sites for development submitted by the Birmingham College of Advanced Technology and the Bradford Institute of Technology.

The remaining matters with which the survey is mainly concerned are the comparative analysis of university costs and the control of capital expenditure. On the former, the Committee has become increasingly conscious of the need to obtain more detailed information about university costs and to develop improved techniques of comparative analysis. The Working Party set up in March 1965 under the chairmanship of Sir Harold Sanders "to review the content and layout of the financial and other returns submitted to the University Grants Committee by universities with a view of improving them as instruments of comparative analysis of university past and projected expenditure" has already reported, and the University Grants Committee has accepted its recommendations. The Committee has also discussed the proposed new arrangements with representatives of the Committee of Vice-Chancellors and Principals.

The Working Party agreed that the essential basic requirements for the purpose of comparative analysis were to separate expenditure on research and to calculate an undergraduate unit cost. This would necessitate separating and quantifying, on a consistent basis, the cost of undergraduate teaching, the cost of postgraduate work, and the cost of research carried out by university staffs. The Working Party recommended, accordingly, that revised forms of return should be issued to universities for completion in respect of the academic year 1965–66, and that the operation, once introduced, should be a continuing one, though the arrangements might need to be revised and adjusted in the light of the first year's working. The universities have agreed to put these arrangements into operation for 1965–66, subject to further discussions on the precise techniques to be used. They have reservations, however, about the reliability of

the information in the form in which it is sought, particularly as regards the division of effort between teaching and research, and about the use of the information when it becomes available.

In the light of the study made by the joint Working Party, set up by the Committee and the Department of Education and Science, to review the existing arrangements for controlling the cost of residential accommodation in institutions of higher education, the Committee and the Department concluded that there was no justification for any variation in standards between residential accommodation in universities, colleges of advanced technology and colleges of education. It believed that a single-cost system, flexible enough to deal with the varying requirements, was both possible and desirable. The revised system recommended by the Working Party is in essence a refined version of the previous system, operated on the basis of a 'study–bedroom unit' and designed to retain and increase the flexibility allowed to universities and their architects. The new arrangements were conveyed to the universities in April 1965; essentially they apply to all residential schemes programmed to start in 1965 and subsequent years for which cost lists have not already been fixed.

A joint Working Party with the Department of Education and Science has also been set up to review the cost–control procedures applying to academic buildings where the problems are far more complex. The requirements for teaching and research vary from one university or department to another, and with science buildings a wide range of specialized services has to be covered. The Working Party is accordingly proceeding by stages, starting with the simpler types of teaching and office building. In order to encourage the application of industrialized building techniques to university buildings, the University Grants Committee and the Department of Education and Science are collaborating with the Bristol College of Science and Technology and its architects in developing and applying the 'C.L.A.S.P.' system of construction [see *Nature*, 209, 768; 1966] to increase its suitability for science and technology buildings. The Committee has also been giving much attention to developing a system of monetary allowances which would serve as 'norms' for assessing the value of the equipment needed in new university buildings, both for teaching and for research. The present arrangement is laborious and time-consuming, and it would simplify the Committee's control procedures if monetary allowances could be worked out in relation to the subjects concerned and to the numbers of students and staffs in the new buildings.

The Committee is also conscious of the serious problems which face universities in replacing and modernizing equipment in existing buildings. The problem of financing university purchases of equipment has been comprehensively examined by a joint Working Party of officials of the Committee, the Department of Education and Science, the Treasury and the Science Research Council. The report of this Working Party is now under consideration, and a Working Party of officers of the Committee and of the Ministry of Public Building and Works is examining how the use by universities of the Ministry's facilities for the supply of furniture can be encouraged and extended.

The survey makes no comment on the report of Prof. B. H. Flowers' Working Group on the supply of computers beyond stating that the Committee considered it and passed its views on to the Government at the beginning of August. The two things that stand out from this survey, however, are, first, the extent to which Government policy has itself enhanced the difficulties and uncertainties of the University Grants Committee, and secondly, the persistent failure of the Government to strengthen the Committee's powers to discharge its growing responsibilities. Part of the pressure on the Committee is directly of the Government's making and could be removed by a consistent policy on the part of the Government. Part, however, is the direct outcome of the Committee's steadily increasing responsibilities.

The survey shows how admirably the Committee is itself seeking to meet the new situation and to face new and legitimate demands. It substantiates all that was said in the Robbins Report and has been reiterated in Parliament as to the imperative need to strengthen the staff of the Committee. The Department of Education and Science, however, on which the main responsibility for the failure to strengthen the Committee must fall, does not present such a satisfactory picture. Doubts as to the wisdom of placing the University Grants Committee under the Department of Education and Science cannot but be raised by the frequent references to that Department in this brief survey, especially in the absence of any positive action by the Department to strengthen the Committee or of evidence of a real understanding of the problems of university expansion and development on the part of the Minister himself. Co-operation with the Department of Education and Science there must clearly be, but without including a word of criticism this survey cannot but raise serious misgivings in the universities and elsewhere as to Government policy in higher education. If the debate in the House of Lords on December 1 was largely inconclusive and somewhat disappointing, any subsequent debate that may take place in the House of Commons in the light of this annual survey is likely to be far more incisive and could well be extremely disconcerting for the Department of Education and Science.

POLITICAL AND PHILOSOPHICAL IMPACT OF SCIENCE

Science as a Cultural Force

Edited with an Introduction by Harry Woolf. (The Shell Companies Foundation Lectures.) Pp. viii+110. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1965.) 32*s*. net.

THE four lectures collected in *Science as a Cultural Force* fall neatly into two groups : the administrative and the philosophical. The first two lectures by former science advisers to the President of the United States are concerned with the challenge from without, repeatedly identified as the fact that the U.S.S.R. is producing twice as many scientists as the United States. But their main preoccupation is with the challenge from within: the new pattern of relations that is emerging between the Government and the scientific community. Dr. James R. Killian believes that the executive branch of the Government should make a greater use of scientists in policy-making and in the co-ordination of scientific activities. There is increased governmental pressure resulting from larger grants to universities for research, and the consequent danger of unwittingly imposing constraint on the creative process on the one hand. On the other, society as a whole is becoming more and more research-conscious and research-reliant. This entails the risk that every state, every district, will consider itself thwarted if it does not have its own research centre. These problems must be faced by the scientists themselves. Their traditional fear of overarching bureaucracy has kept them

aloof, but they can no longer elude the fact that the problem of priorities and the political pressure for geographical distribution of Government funds are likely to mean more politics in science. Somehow they will have to learn to work effectively in the political arena.

This leads to the question of the allocation of public funds. How is the State to distribute money for research? Or rather, how is the scientist to enlighten the politician on the research that is important but that yields no immediate results? This is by no means an easy task if we believe Dr. Jerome B. Wiesner. As a Special Assistant to President Kennedy, he was confronted with angry demands to explain how particular pieces of research could possibly be useful. The problem lies in the fact that while the research scientist is primarily motivated by an urge to explore and understand, society supports him primarily because experience has demonstrated how essential research is for the continuing progress in technology. The trick would be to convince down-to-earth Congressmen that acquiring scientific knowledge is a form of capital investment. They might be willing then to consider Dr. Wiesner's proposal that the United States spends a billion dollars on fundamental research, which, after all, would still be less than 20 per cent of the space budget.

The third lecturer, Dr. Michael Polanyi, raises the discussion to the philosophical level when he argues against the ideal of scientific 'detachment'. His two contentions are that all knowledge is based on a measure of personal participation and that we know more than we can tell. The evidence he adduces are cases of 'subception', when an individual becomes aware of an anomaly and subconsciously reacts to adjust himself to its requirements. Whether we can conclude from these examples that knowing is indwelling is open to doubt. So is the claim that the explanation of all living processes by physics and chemistry collapses unless we deny altogether the existence of consciousness. It is, of course, impossible to do justice to such topics in a lecture or a short brief essay. Yet they may whet the reader's appetite and entice him to look up what Dr. Polanyi has written about this elsewhere.

Dr. Gerald Holton tries to bridge the gap between science and the humanities by showing that a simple-minded view of the inductive process (which he seems to identify with the hypothetico-deductive method) is misleading. In order to understand how scientific theories are constructed we must be aware of the presuppositions that underlie them. To empirical and analytical statements, which he likens to x- and y-axes, Dr. Holton adds a third or z-axis. This is the dimension of the fundamental propositions, notions, terms, methodological judgments or decisions—in short, the themata or themes that underpin and penetrate a scientific explanation. This thematic analysis has been fruitful in other fields and it is only recently that it has attracted the attention of the historians and the philosophers of science. Possible examples would be the heuristic concept of 'force' in physics, or the pervasive laws of conservation. This kind of enquiry would help us to a view that goes beyond the usual antithetical juxtaposition between science and the humanities. The lamented separation between science and the other components of our culture depends on the oversimplification which implies that science is done only in the contingent plane, whereas scholarly or artistic work involves basic decisions of a different kind, with predominantly aesthetic, qualitative, mythical elements. In Holton's view, the dichotomy is attenuated, if not eliminated, if we see that science, too, needs more than the contingent plane. But what is there beyond the contingent that is more than a merely provisional absolute? The thematic analysis that is suggested is becoming increasingly common, but we have yet to be told how themes are to be compared, and according to what norms.

W. J. Shea

SOCIAL IMPLICATIONS OF EDUCATION

Education and Values

Essays in the Theory of Education. By G. H. Bantock. Pp. 182. (London: Faber and Faber, Ltd., 1965.) 25*s*. net.

Educational Values in an Age of Technology

By F. B. Pinion. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies. Education Division, Vol. 1.) Pp. xi+184. (London: Pergamon Press; New York: The Macmillan Company, Ltd., 1964.) 17*s*. 6*d*. net.

LIKE G. H. Bantock in the essays collected under the title *Education and Values*, Mr. Pinion is concerned with educational and other values, but what the two books have in common both with Dr. Michael Young's *Innovation and Research in Education* and with Mr. F. C. Palmer's little book on *Student Guidance* is very striking. It is perhaps not surprising to find Mr. Palmer's insistence on the importance of the teaching of English reflected in one or two of Mr. Bantock's essays and in different context by Mr. Pinion. More impressive is not merely the insistence on the importance of more research into education in all four books but also the emphasis on the social implications of education and its place in modern society. Moreover, it is not just an expansion of educational research that is urged: searching questions are asked about its nature and the methods of investigation as well as the choice of subjects.

This consensus of opinion, if not altogether unexpected, is encouraging. Mr. Bantock discusses educational research in only one of his eight essays, but, even so, in that essay his criticism endorses much of Dr. Young's thesis, while elsewhere he enters more fully into some of the characteristics of society to-day from which that thesis is developed. He demonstrates how the facing of questions of values stimulates fresh thinking about the content of curricula as well as about methods of teaching, and that the decisions we take as to values determine the appropriateness of our whole educational system for this age of technology. It involves considering afresh the different roles which the arts and sciences fulfil in developing the complete human being. Nor can our decisions on values be wisely made without regard to the relations between school or university and the community, and the value we place on the individual. Just as firmly as Dr. Young or Mr. Palmer, he challenges accepted principles and preconceived ideas and stimulates fresh thought about the moral issues involved, and how the needs of a technological and changing society are to be reconciled with the development of human personality. Again, like Dr. Young and Mr. Palmer, he maintains that learning remains the most vital prerogative and task of our schools.

Mr. Bantock, nevertheless, realizes that an education concerned simply with a social justice limited to economic purposes would be imperfect, and he makes his point in the context of technological and technical education. Mr. Pinion is more especially concerned with these subjects, though his warning against obsession with class and opportunity interpreted in political terms is given also in a discussion of education, social justice and sociologists. It, too, is reflected by Mr. Pinion, particularly in reviewing the general problems in educational organization, where he comments trenchantly on the influence of class prejudices and emphasizes the need for safeguards. "Education", he observes, "is too precious and costly to be the shuttlecock between opposite political parties".

Educational Values in an Age of Technology is in two parts. The first part is essentially a factual appraisal of the situation in Great Britain. Besides the chapter just noted in which he refers to such diverse matters as administration, the supply of teachers, curricula reform, examinations, finance and research, it includes separate chapters on the growth of technology, the schools, the universities

and colleges of advanced technology, and on other forms of further education, which he reminds us are for many more appropriate than either a university institution or a college of technology. The second part, which occupies less than 50 of his 180-odd pages, is more philosophical and exploratory. Its three chapters, on the cultural crisscross, on the limitation of intellectualism, and on science and religion, seek to outline the dilemma in which the rising generation is placed by the steady erosion by specialization of the areas of agreement and mutual understanding.

Even the factual survey is packed with sense and constructive suggestions and makes a useful contribution to any discussion on the impact of science on society which does not make comfortable reading for those responsible for policy. He has no use for policy which is not determined on educational grounds but by political ideology. Mr. Pinion has an eye for the essentials and is every bit as forthright as Dr. Young in condemning the waste of resources on trivialities and in asserting the need for fresh and unprejudiced thinking. It is, however, above all in what he writes, in his second part, about communication between scientist and non-scientist and on the limitations of intellectualism that he is most challenging. In contrast to Mr. Bantock, who has reservations regarding technology, he has, like Sir Eric Ashby, a deep sense of the value of science and of technology as cultural elements themselves, and his final chapter on science and religion is outstanding in its insight and balance. The greatness and value of science are that, besides teaching us the wonders of creation, it also, seen in the perspective of its conclusions, gives us a sense both of man's power for good and of his fallibility and weakness. Science and religion, in Mr. Pinion's view, can meet, but both must adjust their attitudes. His book is well written as well as stimulating and his wide and apt quotations are supported by an admirable reading list. Fully as effectively as either Mr. Bantock or Dr. Young, he brings us face to face with some of the vital issues with which the advent of compulsory education and the prospect of universal literacy has confronted civilization for the first time within the past hundred years. He points alike to the need for fresh thinking and enquiry if the issues are to be resolved in terms of respect for human personality and on the basis of fact rather than fiction or prejudice.

R. BRIGHTMAN

GEOGRAPHY OF THE ICE AGE

Environment and Archeology

An Introduction to Pleistocene Geography. By K. W. Butzer. Pp. xvii+524. (London: Methuen and Co., Ltd., 1965.) 84*s*.

ENVIRONMENT and Archeology is an attempt to show how the geography of the Pleistocene period may be reconstructed and to illustrate the extent to which such reconstruction is at present possible. Part 1 is introductory and largely concerned with the abnormal nature of the Pleistocene, its extent in geological time and the present state of success in dating it in absolute terms. Part 2 is concerned with the interpretation of vegetation, soils and landforms in terms of climate, Part 3 with the interpretation of sedimentary evidence, Part 4 with the interpretation of biological evidence, mostly pollen and macroscopic plant analyses and studies of mammalian faunas. Part 5 is a series of reconstructions mostly of the Late Pleistocene geography of different regions with a natural bias towards the Mediterranean and African areas with which the author is most familiar. Part 6 is virtually a series of essays on aspects of prehistoric human geography.

It may be appreciated from the foregoing summary that the field attempted is vast, and this is the source of both the strength and weakness of the work. No one Pleistocene specialist will attempt to cover more than one or at most two specialized aspects: he may be a 'beetle man' or a 'snail man' but rarely both; he may be a 'plant and landform man' but not a 'plant, landform and mammal man'. Thus, any synthesis such as this must place a great deal of reliance on secondary sources, on review papers and on the standard teaching manuals on landforms, soils and vegetation. This is inevitable, but it does lead in places to an air of certainty which the specialist would probably disclaim. Practically all questions on such subjects as the origin of landforms and the precise controls of plant and animal species distributions are open ones. But when such evidence is used in palaeogeographic reconstruction one particular interpretation must be chosen for each line of evidence and the interpretation of one piece of evidence may be conditioned by that already put on another. The result may be a sum of truths or a sum of errors, or more commonly a mixture. Perhaps it would have been better not to have attempted to teach the skeletal and hence seemingly dogmatic treatments of soil science, climatology and geomorphology of Part 2, but to refer the reader to reference works for systematic information and to discuss more fully the interpretative doubts.

Yet the apparent dogmatism is conditioned more by the telescoping necessary to cover the field than by any lack of critical appreciation on the part of the author, who deals critically with such vexed questions as the differences between modern and Pleistocene periglacial climates consequent on the different latitudinal positions, with the possibility of the vegetation succession being either climatic or edaphic and with many other awkward problems. Perhaps he relies more on vertebrate evidence, which is subject to human as well as to physiographic controls, than on the invertebrate evidence of such creatures as beetles and snails, which more nearly reflect only physiographic controls—but this may only be a difference of taste between the author and myself. All in all, the book is very valuable and very welcome. In a period when Pleistocene investigations, like many other branches of knowledge, are in danger of disintegrating into mutually semi-comprehensible specialisms, it performs the invaluable constructive and difficult task of synthesis. As such, it should be received with praise and charity by undergraduates, researchers and all interested in the fascinating field of Pleistocene geography.

B. W. SPARKS

EQUILIBRIA IN IRON AND STEEL MAKING

Phase Equilibria Among Oxides in Steelmaking

By Prof. Arnulf Muan and Prof. E. F. Osborn. Pp. xx+236. (Reading, Mass.: Addison-Wesley Publishing Company, Inc.; London: Pergamon Press, Ltd., 1965.) 135*s*.

IN any long-established industry there is a well-defined art which, as knowledge increases, develops into a science. The art exists because it is successful and, although it may have complexities based more on tradition than on logic, the majority of the practice has evolved from sound, factual experience. Following as it does on the art, the science is not always received as enthusiastically as the scientist may desire: this arises, partly, from the difficulties of communicating the use of new ideas to technologists whose training has preceded the advancement of the science. Furthermore, it can happen that the facility to manipulate scientific data is lost in a disastrously short time by the scientist working in industry. Any attempt to overcome these difficulties in converting an art to a science is laudable, but the task is difficult, criticism is freely given, and failure is fairly easy.

The American Iron and Steel Institute has for some years sponsored investigations of phase equilibria in

steel-plant refractories in the College of Mineral Studies at the Pennsylvania State University. The College has made many original contributions to this subject since the project started in 1949 : this work has, in one sense, culminated in the publication of the book, *Phase Equilibria Among Oxides in Steelmaking*. The authors, Profs. Muan and Osborn, have aimed to bring together all available data on relevant oxide systems, and to apply this scientific information to process problems in the iron and steel industry. The presentation of the information has been designed for use by those meeting these problems as they assist in practising the art of operating the production plants.

The first three-quarters of the book gives data relating to equilibria in some sixty oxide systems of importance in iron and steel making, and sets out the basic principles underlying the information. This part of the book is excellent. No attempt is made to over-describe the basic science of the subject, references being given to many of the excellent texts already available: the essential principles are made clear by reference to the equilibria in the different types of system discussed. The special interest of the authors in this part of the work may be clearly discerned; more than three hundred original references are cited, and the subject is treated in an objective and critical manner. Whenever possible, the information is presented in a standard diagrammatic form, and the authors have redrawn many published diagrams in order to give a commendably uniform presentation. The diagrams are, in general, clear, but some of the more complex illustrations would benefit from the use of colour, a device which would, unfortunately, increase the price of an already expensive book.

The three final chapters of the book deal with the application of the equilibrium data to slags, oxide inclusions, and refractories. As in the preceding chapters, the information is handled in a clear and constructive manner ; bearing in mind the audience at which the book is aimed, however, these sections do not maintain the high standard set by the earlier chapters. A few slag systems are discussed in reasonable detail, but more examples would have helped the average reader to extend the treatment to other systems. The validity of this criticism is emphasized by the authors themselves, when they point out that many simplifications and assumptions are necessary in order to discuss the chemistry of steel-making slags. Similarly, the logically presented information on oxide inclusions could have been extended, with benefit, to make more clear the ways in which the different phase relationships can affect the morphology of the precipitates.

Despite these criticisms, however, the book will be of undoubted value to those involved in the practical problems of iron and steel making. It should also find considerable use in providing examples for the amplification of university courses dealing with problems of high-temperature equilibria. D. S Oliver

SKIN AND HAIR—A FORWARD-LOOKING PROSPECT

Biology of the Skin and Hair Growth

Edited by A. G. Lyne and B. F. Short. (Proceedings of a Symposium held at Canberra, Australia, August 1964.) Pp. xi+806. (Sydney: Angus and Robertson, Ltd., 1965.) A.210*s*.; 21 dollars.

Biology of the Skin and Hair Growth is a large and wide-ranging book. Among the subjects covered are the skin structure of snakes and lizards; the development of feathers and hair; studies of hair growth waves and rhythms in cattle, sheep, rodents and seals; radiation effects on hair; the detailed biochemistry of keratin structure and fibre formation; endocrine effects on fibre growth; and the cytology and morphology of hair follicles.

Inevitably, in a book of 46 chapters, many of which incorporate original work, the quality of papers varies. But the average standard is very high and the overall impression is that each subject is treated authoritatively. The emphasis of the book is on dynamic features of the skin–hair complex rather than on the classical aspects of anatomy and morphology. As with most symposia, complete coverage of the field was understandably not achieved. In this particular case, several important but specialized topics are virtually excluded; for example, the work of Bullough and his associates on the control of cell division in the hair follicle and the epidermis, and the subject of skin transplantation immunity. There has been no attempt to make a general synthesis, but in view of the variety of material presented this might well have proved an almost impossible task. The nearest approach to generalization comes in the opening paper by Billingham and Silvers which deals with some of the important remaining problems of skin biology.

Of the numerous sections deserving comment it may be permissible to sample a few. Papers by Straile and by Priestley and Rudall both suggest that the morphology and rate of growth of hair fibres may be controlled by a 'cellular shunt' mechanism determining which of the cells formed in the follicle bulb enter the hair fibre and which enter the follicle inner root sheath which invests it. Such a mechanism involves the spatial relationships between streams of newly divided cells moving distally from the follicle bulb matrix. These relationships may be determined by changes in the shape of the dermal papilla. This work and the studies of Fraser and of Short, Wilson and Schinckel on the proliferation rate of the follicle matrix cells may together hold the key to a complete understanding of the process of hair growth at the cellular level.

Cohen, by means of an audacious technique involving the transplantation of individual follicle papillae, has carried out a successful experimental attack on the fundamental problem of epigenetic specificity as between the dermal papilla and the epidermis. Chapman has constructed a highly ingenious theory which accounts for the formation of wool crimp by postulating periodic contractions of the follicle-attached pilomotor muscles. However, there still seems on this theory a difficulty in accounting for the situation in certain British breeds of hill sheep. Here the primary fibres (which are all associated with arrector pili muscles) may comprise a mixed population of loosely-crimped and uncrimped fibres, whereas adjacent secondary fibres (which lack associated muscles) are all clearly crimped. One hopes that Chapman will be able to direct his attention to this problem in the future. Different types of hair-growth rhythm and the factors controlling them in a variety of species are thoroughly dealt with in chapters by Chase, Lyne, Elizabeth Johnson, Ebling, Ling, Hutchinson and Hayman. Aspects of blood-flow to the skin are discussed in several papers, and here Molyneux revives, from a new point of view, the subject of arterio-venous anastomoses in the skin and their possible role in thermoregulation. Endocrine effects on hair growth are considered by Rougeot, by Houssay, Epper and Pazo, and by Downes and Wallace. A comprehensive paper by Ferguson, Wallace and Lindner indicates the bewildering complexity of present knowledge concerning hormonal influences on wool growth.

It should be evident, even from this selective discussion, that here is an essential reference book for all workers interested in the fields of skin and hair growth. Others on the fringe, such as endocrinologists, physiologists, pure zoologists, histochemists and biochemists, will find many chapters of value.

The book is very well produced, both editing and presentation being of an unusually high standard.

J. Slee

Comparative Atherosclerosis

The Morphology of Spontaneous and Induced Atherosclerotic Lesions in Animals and its Relation to Human Disease. Edited by James C. Roberts and Reuben Straus, with Miriam S. Cooper. Pp. xxi+426+Atlas of Colour Plates. (New York and London: Hoeber Medical Division, Harper and Row, 1965.) 150*s*.

THIS volume arose primarily under the impetus of a conference on comparative atherosclerosis, and its fifty-six authors are leading investigators in this field.

The primary purpose of the volume was "to ferret out and consolidate valuable knowledge buried amid the welter of accumulated information in the literature. A need was recognized for an authoritative reference which would compile and catalogue this knowledge in logical sequence and accessible form, sharply delineate differences and similarities of the disease in all species of animals, and determine which animals are acceptable as models for the most vital phase of all this research effort, which is the testing of mechanisms and materials to understand, prevent, and/or treat atherosclerotic disease in man".

Observations on more than four hundred species of animals are discussed and co-ordinated in seven principal sections dealing, respectively, with atherosclerosis in swine, birds, rats and wild animals, canines, primates, rabbits and man. The eighth section is devoted to summaries of more general problems such as: (*a*) the histochemistry of spontaneous and experimental arterial lesions; (*b*) the relationship between spontaneous atherosclerosis in animals and man; (*c*) comparison of experimentally induced atherosclerosis in animals with spontaneous human atherosclerosis; (*d*) an outlook which probes the probable future of research in this field.

The professional investigator will undoubtedly profit by the exceptionally clear and well-organized presentation of this immense material. It will permit him to select those animal species which suit his immediate purpose best and help him to find his way across the enormous literature which deals with this important field.

Not the least attractive feature of this 426-page volume are the 672 illustrations, including an atlas of 416 excellent coloured photographs and a meticulously prepared index.

H. SELYE

Canine and Feline Nutritional Requirements

Edited by Oliver Graham-Jones. (Proceedings of a Symposium organized by the British Small Animals Veterinary Association, London, May 1964.) Pp. x+160. (London and New York. Pergamon Press, Ltd., 1965.) 63*s*. net.

THIS volume contains the proceedings of a symposium organized in 1964 by the British Small Animals Veterinary Association, with the object of bringing together information gained by scientific workers in the field of nutrition, and the practical experience of veterinary surgeons.

The book is divided into two sections; the first is mainly theoretical, and contains a review of the growth, development, and chemical maturation of pups and kittens during the suckling period, and leads naturally to a discussion of protein and calorie requirements, and of the problems involved in the design and evaluation of diets. Some special aspects of the nutrition of the cat are dealt with in separate chapters. Two chapters in this section emphasize the importance of the interrelationships between nutrients and between the diet and other factors, as exemplified by investigations of the effects of different diets on the course of *Toxocara canis* infestation in dogs. The section closes with a description of some diets designed for special purposes such as the treatment of obesity and diabetes in dogs.

The second section, on practical dietetics, contains a review of the requirements of Antarctic sledge dogs, including recommendations for the calorie intakes needed for weight maintenance at different work loads. Following chapters contain information about the breeding and rearing, for laboratory purposes, of dogs and cats on standardized diets and tinned foods, and the concluding chapter describes diets in use at Basle Zoo for all canine and feline animals.

P. R. PAYNE

Coincidence Tables for Atomic Spectroscopy

By J. Kuba, L. Kučera, F. Plzák, M. Dvořák and J. Mráz. Pp. xxxi+1,136. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 130*s*.

THIS large work gives essentially a series of tables showing, for the strongest lines of each element, the likely lines from other elements which might coincide in wave-length over intervals corresponding to three ranges for instruments with dispersions 0·5–20 Å/mm. The authors have been most painstaking and one admires their enthusiasm; one wonders, however, whether such a task could not have been handled both in selection and reproduction by computer techniques. The wave-lengths are based on the lists of G. R. Harrison dating from before 1939, and the opportunity has not been taken of making corrections and improvements from more recent investigations. Thus, work during the past few years on rare earth lines (R. N. Niseley, V. A. Fassel and C. F. Lentz, *Spectrochem. Acta*, 16, 863; 1960) and spectral-line intensities (W. F. Meggers, C. H. Corliss and B. F. Scribner, U.S. National Bureau of Standards Monograph 32, 1961) has not been included. In some ways recent advances in spectroscopic technique, particularly the increasing use of gratings to give higher dispersions, have helped to decrease errors in analysis arising from the influence of overlapping lines.

British spectroscopists will be grateful that, although the authors are distinguished Czechoslovakian spectrochemists, the book is in English with only minor difficulties of translation, for example, 'self-inversions', and 'ionisation tension'. As a general guide to spectrochemical procedure in wave-length measurement and a convenient summary for identifying lines, the book will form a useful although expensive addition to an analytical laboratory library.

L. BOVEY

Collected Papers of L. D. Landau

Edited and with an Introduction by D. Ter Haar. Pp. xx+836. (New York: Gordon and Breach; London. Pergamon Press, Ltd., 1965.) 200*s*.

PERHAPS the most valuable feature of this collection of papers is concerned with the nationality of the author. The most important (in fact, the majority) of Landau's published works are presented in this rather large book, all in English and in chronological order. A few outdated or incorrect works are listed, with comments, in an appendix. The introduction consists of a very short biography and a brief indication of the contents of the papers. My only criticism of the introduction is its brevity and not its existence, the need of which the editor seems to doubt. There are few physicists to-day who are not specialists, which surely implies that the majority of us need some guidance through the works of a man of Landau's range of interests. Those who agree with the editor's sentiment can, of course, skip the introduction.

The publishers are quite right in stating that this book is an "essential addition to the shelves of every library", but it is unlikely that many individual workers will wish to acquire it, due to its high price. There is, however, some hope for the impecunious, since the same publishers are introducing a new series of books entitled *Men of Physics*, two volumes being devoted to Landau. The combined volumes will contain twenty of the more important papers and will cost 17*s*. 6*d*. each (paperback).

L. L. J. VICK

OBSERVATIONS OF THE RUSSIAN MOON PROBE *LUNA* 9

By Prof. J. G. DAVIES, SIR BERNARD LOVELL, O.B.E., F.R.S., R. S. PRITCHARD and Prof. F. G. SMITH
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ON January 31, 1966, Tass announced the launching of the Moon probe *Luna* 9. Signals from the probe were acquired at Jodrell Bank on February 1 and on February 2 the observations were transferred to the Mark I 250-ft. diameter radio telescope. From previous information available from the flights of *Lunas* 5, 7 and 8 it was anticipated that the probe would arrive at the Moon during the evening of February 3, an expectation supported by a second Tass announcement that *Luna* 9 would reach the Moon at 22h 00m U.T. on February 3. In the event, the landing of *Luna* 9 on the Moon occurred at 18h 45m. The purpose of this article is to give a short account of the observations made at Jodrell Bank of the landing phase and reception of the television photographs from this first successful landing of scientific apparatus on the lunar surface.

Experimental Arrangement

The receiving system consisted essentially of the Mark I 250-ft. radio telescope working on a frequency of 183·535 Mc/s. The gain of the telescope on this frequency is 40 dB and its beamwidth 2 deg. to half power. The full gain was not realized in this experiment because the telescope was simultaneously engaged on other research programmes and the pre-amplifier had to be placed at some distance from the primary feed involving a cable loss of about 2·5 dB. The pre-amplifier was a *DET*29 grounded grid triode with a 2-dB noise factor. After further amplification the output of the pre-amplifier was converted to an intermediate frequency of 10·555 Mc/s using an oscillator locked by means of a Gertsch *FM*-6 frequency meter to a 1-Mc/s frequency standard with a short-term stability of $\pm 2 \times 10^{-10}$. The 10·555 Mc/s signal was then fed into a Racal *RA*17 receiver. The local oscillator of this receiver had a frequency stability of 2 c/s over 1 min with a maximum excursion of about 20 c/s.

The signal from the 100 kc/s intermediate frequency of the Racal with an 8-kc/s bandwidth was fed to a phase-locked oscillator and by synchronous detection the phase modulation was extracted. The detected phase-modulated signal was recorded together with appropriate time and frequency standards on an Ampex *FR*100*A* tape recorder. The frequency of the phase-locked oscillator, which was proportional to the signal frequency, was measured using a digital counter and printed out automatically every 2 sec. The printed figure was the integral frequency count of the previous 1-sec interval.

The Landing Phase

The variation in received frequency from 17h 50m to 18h 45m U.T. is shown in Fig. 1. From 17h 50m to 18h 44m U.T. the intermediate frequency decreased from 100,932 to 100,638 c/s, representing an increase in the line of slight component of the velocity of 494 m/sec,

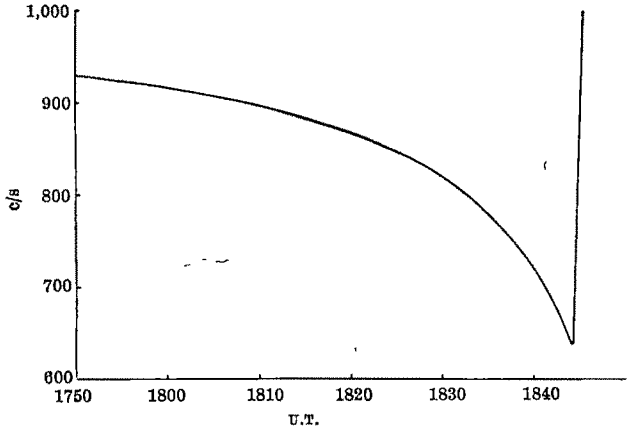


Fig. 1 The variation of received signal frequency from *Luna* 9 between 17h 50m U.T. and 18h 45m U.T. on 1966 Febr. 3 The ordinate is the intermediate frequency minus 100 kc/s Abscissa is time in U.T. on February 3, 1966

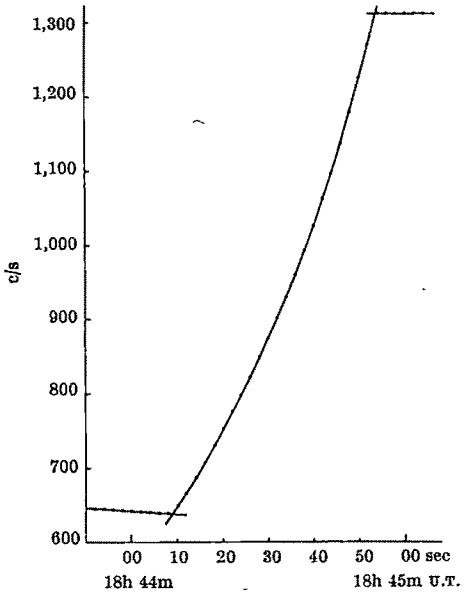


Fig. 2. The variation of received signal frequency from *Luna* 9 between 18h 44m U.T. and 18h 45m U.T. on 1966 Febr. 3. The ordinate is the intermediate frequency minus 100 kc/s. Abscissa is time in U.T. on February 3, 1966

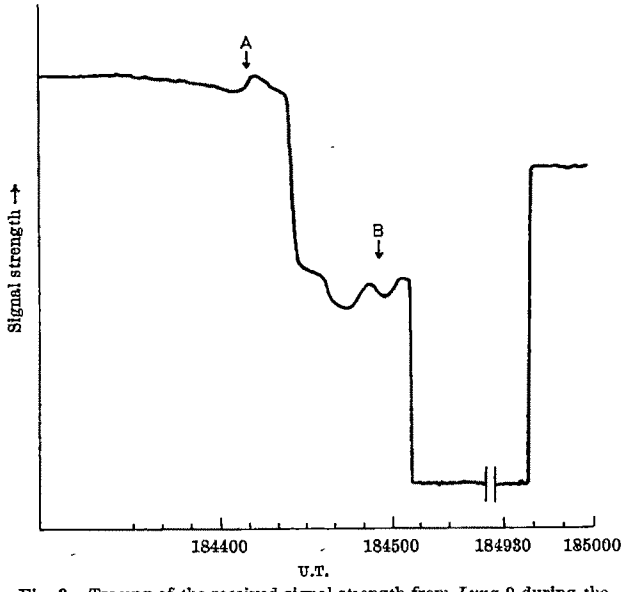


Fig. 3. Tracing of the received signal strength from *Luna* 9 during the landing phase The ordinate is the signal amplitude in arbitrary units (the level at *A* is about 12 dB signal · noise ratio). The abscissa is the time in U.T. on February 3, 1966

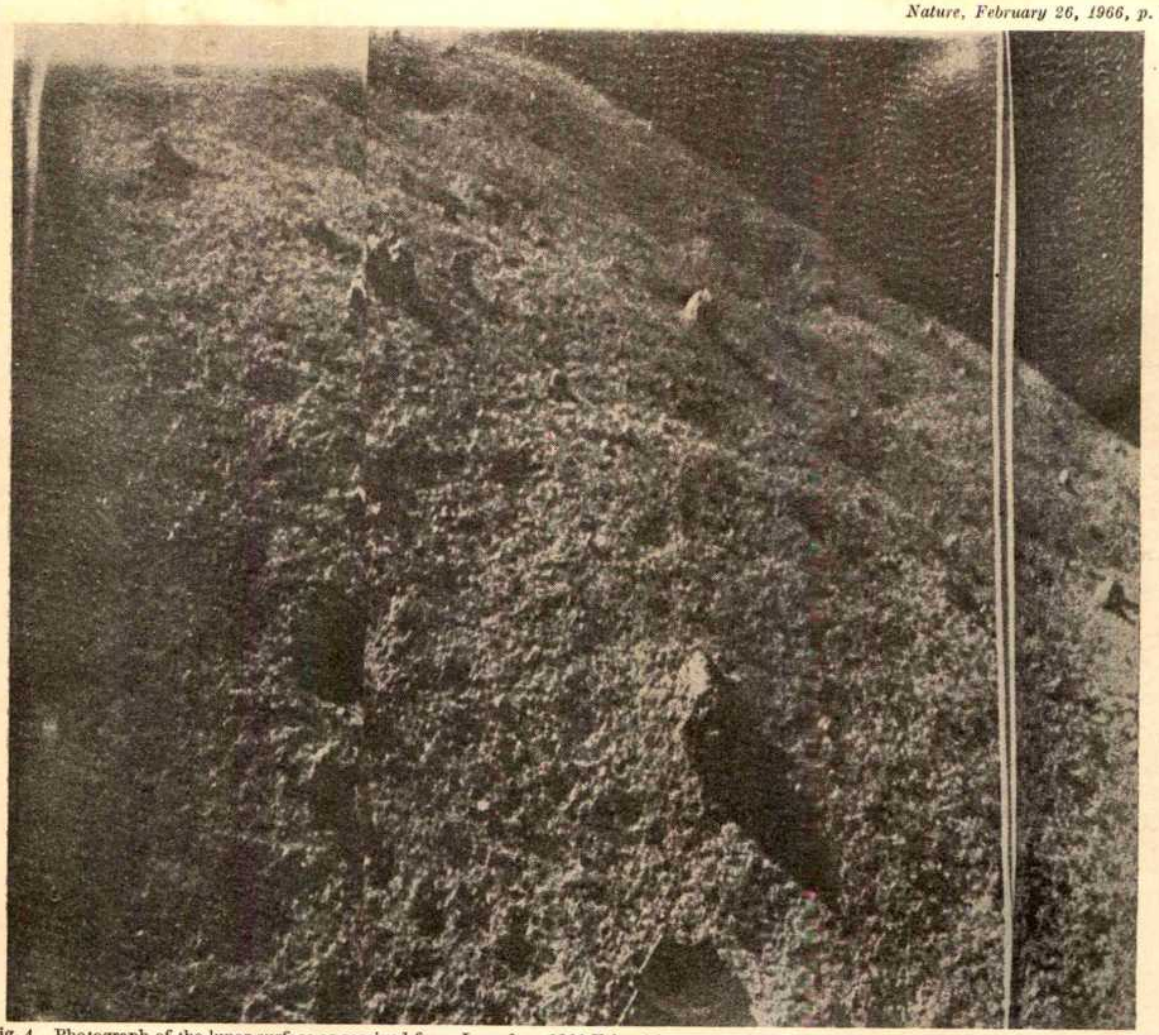


Fig. 4. Photograph of the lunar surface as received from *Luna* 9 on 1966 Febr. 4 between 15h 30m and 16h 55m U.T. A part of the protective fairing of the probe is visible in the foreground. According to information received from the U.S.S.R. the horizontal scale is compressed by 2·5 times

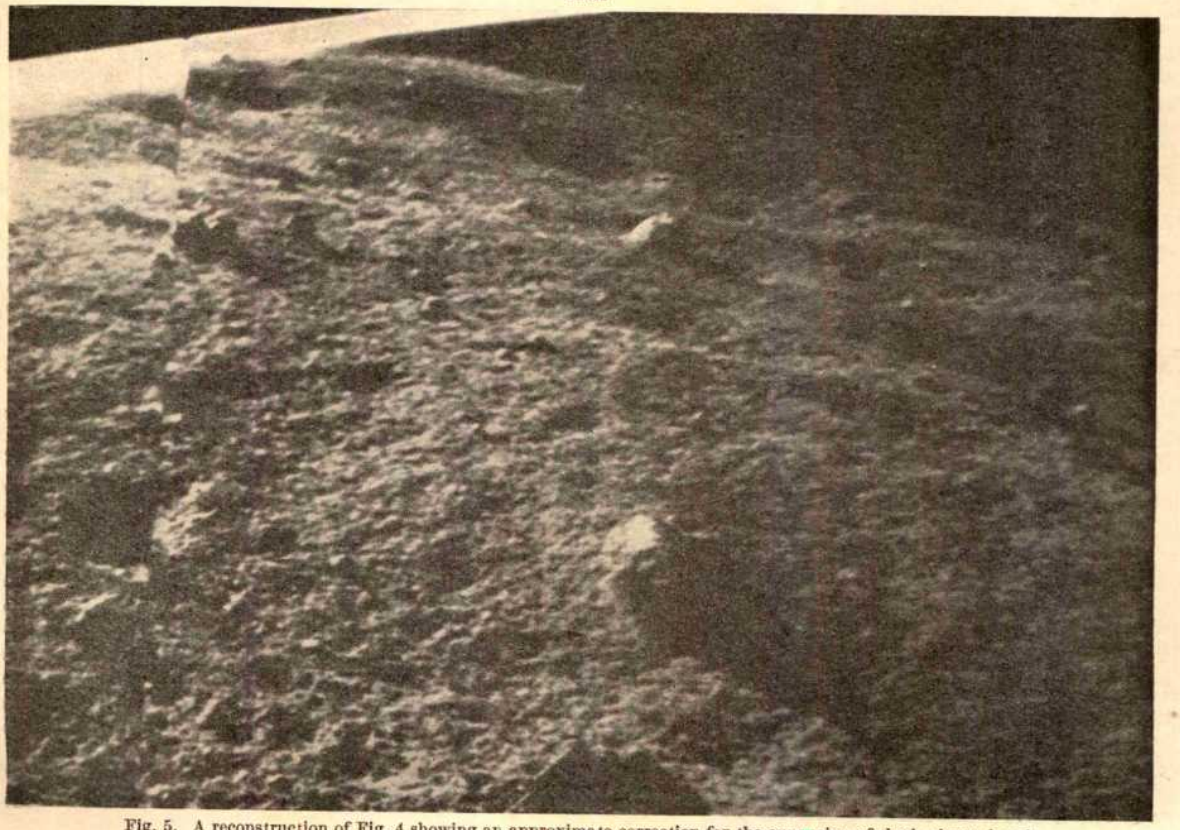


Fig. 5. A reconstruction of Fig. 4 showing an approximate correction for the expansion of the horizontal scale

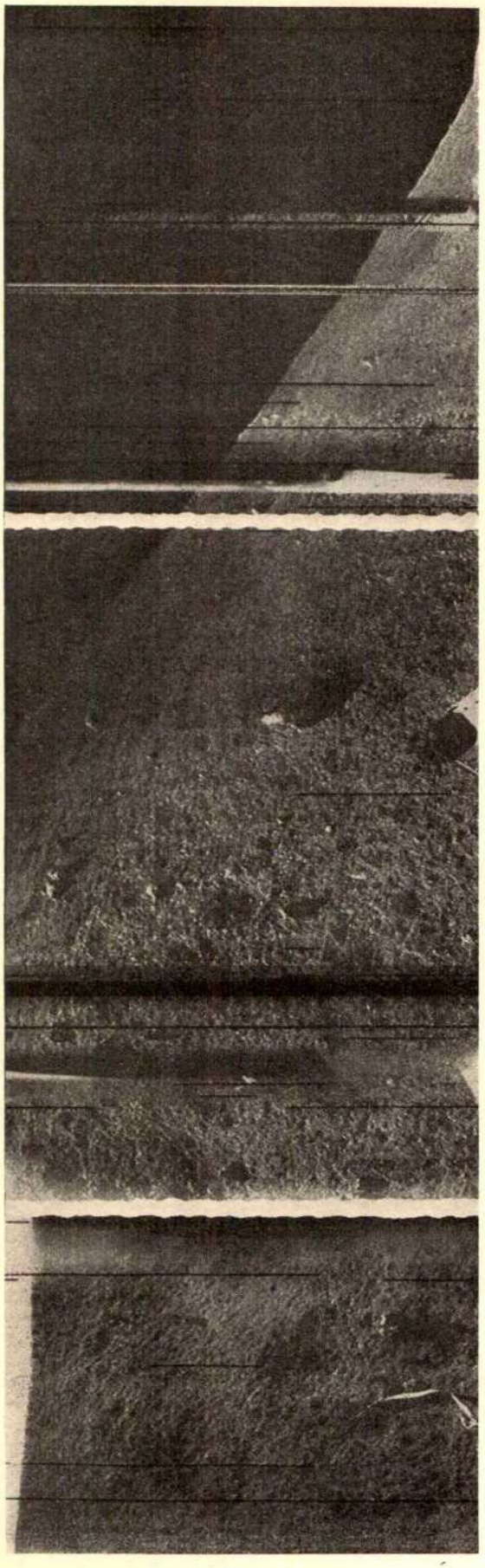


Fig. 6. A reconstruction of a panoramic sequence of the lunar surface as received from *Luna* 9 on 1966 Febr. 5 between 16h 40m U.T. and 17h 40m U.T. According to information received from the U.S.S.R. the horizontal scale is compressed by 2·5 times

and the rate of change of frequency increased from 2 c/s/min to 24 c/s/min. Extrapolating this curve to 18h 44m 30s, the time at which impact would have taken place had the retro-rockets not been fired, the rate of change of frequency at impact would be 25·8 c/s/min, corresponding to an acceleration of 0·705 m/sec². Comparing this with the Moon's surface gravity of 1·62 m/sec², it appears that the landing occurred approximately 64° from the centre of the Moon's visible disk. This compares well with the value of 61° computed from the published landing area and allowing for libration.

The variation of frequency from 18h 44m to 18h 45m is shown in more detail in Fig. 2. Each measured point lies on one of three smooth curves. Up to 18h 44m 08s, the points lie on the curve corresponding to free fall; from 18h 44m 10s to 18h 44m 52s a rapid deceleration occurs, increasing from 32·4 to 76·0 m/sec² over the interval. After 18h 44m 54s, the frequency remained constant within 2 c/s, corresponding to the transmitter being at rest on the Moon's surface. The total frequency change from 18h 44m 08s to 18h 44m 54s is 675 c/s, corresponding to a line of sight velocity of 1,108 m/sec, or total velocity of 2,540 m/sec, or 1·07 times the escape velocity from the lunar surface. Integration of the deceleration curve indicates that the retro-rockets were fired at a height of 68·5 km. Insufficient detail of the moment of landing is available, but it is clear from the shape of the curve that the impact velocity was very low.

The variation of strength of the received signal over the landing phase is shown in Fig. 3. The deceleration commenced at the point marked *A*, and produced no significant change in strength. Approximately 15 sec later the strength decreased over about 1 sec to about a third of the original power. At the point marked *B*, the deceleration ceased, once again without much change in signal strength. Twelve seconds later, at 18h 45m 07s, the signals ceased. During all this time the carrier was modulated with telemetry information.

The Reception of the Photographs

Approximately 4 min after landing at 18h 49m 38s the signal reappeared at precisely the same frequency, with an amplitude somewhat less than the original and with telemetry of a type different from the previous transmissions. At 18h 58m 45s the type of telemetry again changed and the detected phase modulated signal consisted of a variable tone in the range 1·4–2·6 kc/s. This was quickly recognized to be a facsimile transmission with an approximate one-second line scan. This variable tone was fed through a Muirhead Type *D* 665 FM/AM convertor into a Muirhead picture receiver Type *D*-700-*A*. Fig. 4 is an example of one of the photographs received on February 4 during the facsimile transmissions which began at 15h 30m and ceased at 16h 55m U.T. The Russians stated that the horizontal scale of the pictures issued from Jodrell Bank was compressed in the horizontal direction by 2·5 times. An approximate correction of Fig. 4 to allow for this extension is shown in Fig. 5. Fig. 6 is the panoramic view received on February 5 between 16h 40m and 17h 40m U.T. The signal strength during the reception of the facsimile transmissions decreased from 12 dB in an 8 kc/s bandwidth on February 4 to 3 dB on February 6.

The variation in the length of the shadows between February 4 and 5 is clearly visible by comparison of Figs. 4 and 6, and an idea of the scale can be obtained from the appearance of the protective fairing in the foreground of both pictures. For example, the conspicuous stone near the fairing is about 6 in. across and, on the assumption that the camera was 2–3 ft. above the surface, the horizon is 0·5–1 mile distant. Other parts of the probe are visible in Fig. 6.

We thank the editor of the *Daily Express* for his co-operation in lending us the Muirhead convertor and picture receiver.

THE MOON FROM *LUNA* 9

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THE Russian probe *Luna* 9 was reported to have landed at the selenographic position $\lambda = 64°\ 22'$ W., $\beta = 7°\ 8'$ N. (astronautical convention). This places it 70 km north-east of the rim of the well-known 64-km diameter structure, Cavalerius, on the border of Oceanus Procellarum. The touchdown appears to have been on a bright, slightly elevated area within a few kilometres of a group of hills known, from Earth-based studies, to mark out the arc of a partial ring some 100 km in diameter. When *Luna* 9 landed, this whole region was in darkness; morning broke on the scene about an Earth-day later and picture transmission commenced soon afterwards. As a consequence of the Sun being only a few degrees above the horizon, even small obstacles cast long shadows; contrast was high and detail sized appreciably less than 1 cm was recorded in the near field of each photograph.

Blocks of rock 1 ft. or so in diameter and depressions are not uncommon and, at smaller sizes, the terrain is extremely rough. In the highly successful *Ranger* experiments carried out by the United States, 1 ft. was about the best resolution attained by the last photographs of each series; yet, even at this resolution, positive topography, such as blocks of rocks, was rare and virtually all the easily identifiable topography in the *Ranger* photography was negative. *Rangers* 7, 8 and 9 impacted in mare-type country, and the greater proximity of the touchdown point of *Luna* 9 to lunarite hills may be thought to explain the increased numbers of rock blocks per unit of surface. However, one clear—perhaps the most important—explanation of the difference between the American and Russian pictures is that ground resolution attained by *Luna* 9 is better by two orders of magnitude than that of any of the *Ranger* cameras.

The difference is not simply a function of the respective camera-to-ground distances and the lens characteristics; on the Moon the amount of detail recorded on photographs is critically dependent on the altitude (A) of the Sun and, while the first pictures from *Luna* 9 were taken with $A = 7°$, the later pictures of the three *Ranger* series were taken with $A = \sim 20°$, $\sim 15°$ and $\sim 10°$, respectively.

Thus the terrain would necessarily appear smoother on the *Ranger* photographs. Although some astronomers argued that the *Ranger* programme supported the 'deep-dust' theory, it was known that, in order to satisfy the Moon's peculiar light-scattering properties, the surface must in fact be highly porous. Elementary visual[1], photographic[2] and millimetric[3] observations combined had argued convincingly against an appreciable dust cover. Furthermore, evidence from the *Ranger* photographs themselves has been used[4] to demonstrate that apparent smoothness on the photographs may not be real. We believe that the greater part of the Moon will show a roughness comparable with that shown by *Luna* 9. For the first time there would seem to be complete proof that this part of the Moon does not have an appreciable cover of unsintered dust.

The first pictures from *Luna* 9 reveal a remarkable array of detail down to half a centimetre in size. *A* (Fig. 1) is apparently one of the petal-shaped covers that dropped away from the camera housing after touchdown. *B* is the sunrise shadow of the rock *C*, which itself is very pitted and may be as porous—possibly with a bulk density as low as 0·5 g/c.c.—as the surrounding terrain. It is a rounded rock about 15 cm in diameter, some 2 m from the camera. *D* is a second, rounded rock-block much larger than *C*. We have used two methods to estimate its size. First, the apparent roughness of the terrain between *C* and *D* changes by a factor of 10, so, assuming the average size of irregularities is constant over this distance, unit apparent transverse length at *D* is actually ten times the same unit at *C* and the distance from the camera to *D* is ten times the distance from the camera to *C*, quoted as 2 m. Thus the distance to *D* is about 20 m. This, and a second method based on the fact that the apparent (foreshortened) width of the shadow of *D* is a monotonic function of distance from the camera and the height of the camera above the ground, yield results compatible to within a factor of 2 on the assumption that the terrain is flat: the estimated diameter of block *D* is ~ 1 m. Many other blocks of rock or boulders may be seen in Fig. 1. The closest appear to be rounded and to be buried by only a small fraction of their diameters.

This finding and the observation that the vehicle did not penetrate to any great extent indicate that the surface has an appreciable bearing strength. From calculations based on possible ejecta from a supposed impact crater photographed by *Ranger* 9, G. P. Kuiper[5] estimated a crushing strength of > 1 kg cm^{-2} and this appears to be consistent with what is known of the *Luna* 9 landing vehicle.

It is unlikely that a spherical rock mass is an extrusive or the eroded top of an underlying rock mass. Rather, it appears that the blocks have been deposited on the surface. A cluster of blocks may be seen at *E*. They cannot be meteorites; even at the minimum velocity of 2·4 km sec^{-1} they would have penetrated some distance and scooped out impact craters explosively, breaking up in the process. Furthermore, it is unlikely that the blocks are ejecta from a primary impact crater, since the velocities of the secondary particles would still be of the order of 1 km sec^{-1} and, at these velocities, the blocks would probably break up on impact. Now towards the skyline in Fig. 1 there is a crater-like depression *DA* estimated by us to be of the order of 10 or 20 m in diameter. Could it be that this is a secondary impact crater—either of meteoritic or volcanic origin—and that the blocks were jettisoned as tertiary objects at low velocity? On another picture a similar-sized depression occurs a short distance away. Undoubtedly, these two objects would have been interpreted on *Ranger* photography as depressions without raised rims. Such depressions are generally regarded to be collapse craters. The *Luna* 9 pictures do not show clearly whether the rounded rims of these craters are raised above the surrounding terrain—which is hummocky—or not. If the depressions are due to collapse, then it would seem that the blocks strewn around outside could be volcanic bombs tossed out during mild eruptive phases. A similar distribution of particles can be seen around certain terrestrial volcanic centres[6–8].

There appear to be some sub-parallel features *FF* running across Fig. 1. The fact that there is more than

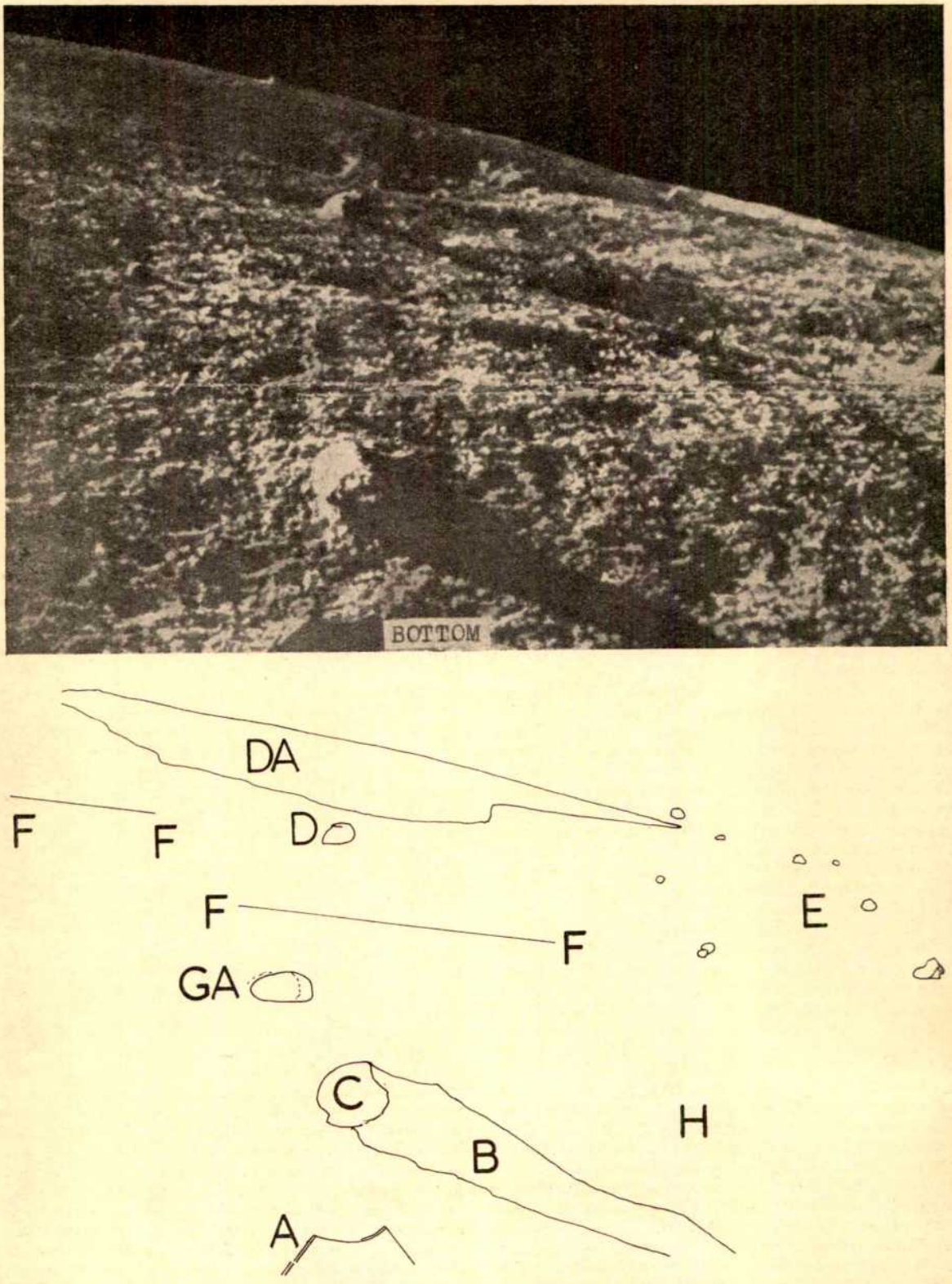


Fig. 1. *Luna* 9 photograph received from the U.S.S.R., February 4, 1966. This shows the closest view yet taken of the lunar surface. (Associated Press picture issued by the Official Soviet News Agency, Tass.) Bottom, key (see text)

one apparent lineament and that the terrain appears to change character across the lineaments suggests that they are real. These markings could be flow lines on, or fronts of, a lava flow; or they could be part of a concentric fracture system related to impact. Because of the similarity of the terrain to a lava terrain we prefer the former interpretation.

The skyline is reported to be only 1·5 km away. It undulates, like all the terrain, gently; slopes on a metre scale being of the order of 4°, excepting those of crater walls such as *GA* which have slopes steeper than 13°. Such craters may be primary or secondary impact craters. Secondary craters may be produced by the throw-out from a primary impact site or they may be produced

when blocks hurled from an explosive volcanic eruption dig into the surface materials.

A whole plethora of steep-sided irregularities are, however, found on the centimetre scale. At this scale (*H* in Fig. 1) the lunar surface bears a remarkable similarity to the scoriaceous surface of an aa (Hawaii) type of lava flow, but we recognize that the irregularities might equally well have been sculptured by meteoritic churning. In any event, solar sputtering must have played a part in shaping the sub-centimetric irregularities and the short-wave and corpuscular radiation from the Sun must, in addition, have darkened the rocks from their original lighter tone.

It is well known that the lunar surface has reflexion properties unlike those of any terrestrial materials. These unusual properties are well illustrated by the graph in Fig. 2. The figure shows the brightness of marial material as a function of angle of observation for a given constant angle of incidence (in this case 30°), and is constructed by reducing the lunation brightness curves of many points to the curve of mean albedo; data have been derived from measurements by Fedoretz[9] and Bennett[10]. It seems that the observation that the Moon's surface (in both maria and highland areas) scatters a large fraction of the incident light back in the direction of incidence can only be explained satisfactorily by assuming that the surface is very rough and, in particular, that individual holes or pores are interconnected.

We have attempted to explain these features on the assumption that at least some parts of the lunar surface consist of lava flows. If this is so it will be appreciated that the lava reaching the surface encounters not an atmosphere as in the case of the Earth, but a high vacuum, and as a result any release of entrapped gas is likely to cause a higher degree of vesicularity than in the corresponding terrestrial case. Following Dobar *et al.*[11], we have, therefore, rapidly evacuated the atmosphere from vessels containing molten igneous rocks and examined the resulting solids after cooling to room temperature. In all cases a highly vesicular material is found consisting of interconnected vesicles from 0·1 to 2 or 3 cm in diameter. Such material, when darkened by solar radiation, is consistent in appearance with the terrain so far resolved in the Russian photographs, and our material also reproduces the observed lunar photometric properties. It is

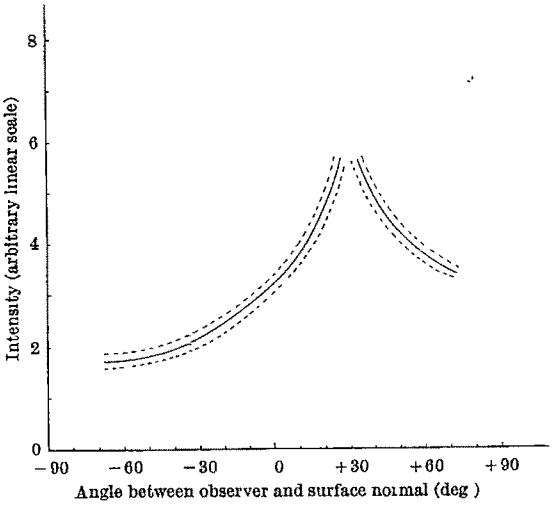


Fig 2 Scattering diagram for maria with angle of incidence of sunlight =30 degrees The dashed lines indicate the limits of probable error

not yet possible to decide to what extent the surface has been broken up by meteoritic impacts and re-sintered by the solar bombardment; photographs taken with a resolution of less than 1 mm will be needed to resolve such questions. There is, however, strong evidence based on polarimetric observations[12] that the irregularities photographed by *Luna* 9 will be found to be peppered with darkened but individual grains that are certainly produced by micrometeorites.

Part of this work was supported by a research grant from the Science Research Council.

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OBITUARIES

Sir Gordon Morgan Holmes, C.M.G., C.B.E., F.R.S.

Sir Gordon Morgan Holmes died at his home in Farnham, Surrey, on December 29, 1965. He was in his ninetieth year.

Holmes was born in Castlebellingham, Co. Louth, Ireland, in 1876. After gaining his M.D. with the gold medal of the year from Trinity College, Dublin, he was for a short period a resident medical officer at the Richmond Hospital in Dublin, but then turned to the study of the comparative anatomy of the nervous system and proceeded to the Anatomical Institute at Frankfurt, then directed by the famous neuro-anatomist, Ludwig Edinger, where Holmes also encountered another pioneer in the field, Carl Weigert.

During the two years that he remained at Frankfurt he produced work on the nervus acusticus, on the forebrain of the bird, and was given by Edinger the task of working out the residual anatomy of one of Goltz's 'dogs without forebrain'. So impressed was Edinger by his young student that he offered Holmes a position as his assistant, and this might well have determined Holmes's future place and order of study. However, he came to England and turned to neurological medicine, filling successively the roles of resident medical officer, pathologist, director of research and finally physician to the National Hospital, Queen Square, London, until his retirement during the Second World War.

From his first arrival at this well-known neurological hospital, which enjoyed an international reputation for research many years before it finally achieved university recognition after Holmes's retirement, his output of original work was continuous. It covered the anatomical and morbid anatomical investigation of the human nervous system and the manifestations of diseases of the nervous system over a wide range.

The study and analysis of cerebellar defect, due to disease or injury, began in 1904 when, with Grainger Stewart, he published the first adequate study of cerebellar tumours. Later, during the First World War, which he spent in France as neurological consultant to the British Forces, he amplified his analysis of cerebellar function by investigations on acute injuries from gunshot wounds. The final results of all his investigations on this subject were published in 1917 and 1922.

During the opening decade of the century he worked in collaboration with Henry Head on disturbances of sensation associated with cerebral lesions, drawing from them inferences as to the role of the cerebral cortex in sensory function.

This was a remarkable collaboration, for Holmes, his training soundly anchored in anatomy, not only devised quantitative methods of sensory assessment, but also was a brake on Head's eager fancy. It will be remembered that Head's early studies with Rivers on the afferent sensory system, though brilliant, had a fatal defect, in that Nature had not provided the structural facilities for the theories which they had put forward. Holmes, therefore, was an essential complement to his partner in this work, and between them they produced a more delicate and precise concept of the role of the cortex in sensation than any earlier one.

Also during the period spent in France, Holmes made a number of valuable studies on disturbances of visual function from brain lesions. These included a somatopic cortical representation of the afferent visual pathway, and the bases of visual orientation and attention. These considerable cerebellar and visual studies were collected and published as a supplement to *Brain* (of which journal Holmes was editor during 1922–37) in 1956 on his eightieth birthday. Appended to that supplement is a bibliography of his published work which reveals his immense productivity and the wide range of his thought over the fields of neurological anatomy, physiology, morbid anatomy and nosography.

During the years between 1919 and 1939 Holmes also gained an international reputation as an incomparable teacher of postgraduate students in neurological medicine. These young men flocked to the National Hospital from the British Commonwealth and from the United States to join Holmes's staff as clinical clerks. These clerks and his own house physicians formed a generation of neurologists still to be found in all parts of the English-speaking world, holding senior positions in neurological departments. Holmes was an exacting taskmaster to these men, but he had the gift of lucid and logical exposition so that the 'how' and 'why' of his conclusions and of his diagnoses were apparent. He was not a showman anxious to impress, but a born teacher.

Personally, Holmes was a man of great strength and inexhaustible energy and of a forceful temperament, sometimes frightening but never malicious, always gaining the respect and later the affection of those he taught. To be trained by him was a salutary discipline. Committees he loathed, and was an impatient and sometimes intolerant member of them. But for younger men in whom he saw a keenness and a capacity for original work he had infinite patience and sympathy.

Finally, it is worthy of note that Holmes was one of the last representatives of those physicians of an earlier generation who depended entirely on the private practice of consulting medicine, whose research was carried out in their hard-won spare time, and who wholly lacked academic or other support. With the death of the voluntary hospitals this way of life ceased to be.

Holmes was appointed C.M.G. and C.B.E. during the First World War and was knighted in 1951. He was elected a Fellow of the Royal Society in 1935; he was an honorary member of numerous foreign medical societies, and received honorary degrees from the Universities of Dublin, Durham and Edinburgh and from the National University of Ireland. He married, in 1918, Rosalie, daughter of Brigade-Surgeon Jobson, and had three daughters.

F. M. R. Walshe

Prof. William Wilson, F.R.S.

William Wilson died on October 14, 1965, at the age of 90. He retired in 1944 from the Hildred Carlile chair of physics at Bedford College, University of London, which he held from the time of its creation in 1921. He came from a farming family and spent his boyhood at Goody Hills in Cumberland. From the village school at Holme St. Cuthbert he obtained scholarships to the Aspatria Agricultural College and to the Royal College of Science. At this stage, he found difficulty in changing his course of study from one in biological subjects and temporarily abandoned his scientific career for school teaching. He taught English in the Berlitz School of Languages in Dortmund for a time, but was able to return to scientific work by studying physics and mathematics in German universities. Later in life he often praised the freedom of choice which was given to students in Germany and held that the British system of examinations imposed an undesirable and discouraging rigidity.

His first published work, in the *Annalen der Physik* for 1907, is an extract from his Dr. Phil., Leipzig, inaugural dissertation. It is an account of experiments on photoelectric emission and on the photoconductivity of such materials as silver iodide. He long retained an interest in the photoelectric effect and published a quantal theory of thermionic emission based on the idea that the electrons were ejected photoelectrically by quanta of radiation in thermal equilibrium. He showed that the temperature dependence would be similar to that deduced by O. W. Richardson, who used non-quantal arguments. It later became clear that these photoelectrons could account for only a small fraction of the thermionic current.

After becoming an assistant lecturer at King's College, London, in 1906, his interest was aroused by the atomic speculations on nebulium of Prof. J. W. Nicholson, professor of mathematics at the College, which antedated Bohr's theory of the hydrogen spectrum. In 1915 Wilson's famous paper "The Quantum Theory of Radiation and Line Spectra" appeared in the *Philosophical Magazine*. In this he first proposed the Wilson–Sommerfeld quantization rules in the form:

$$\int p_1 \, dq_1 = \rho h$$
$$\int p_2 \, dq_2 = \sigma h$$
$$\int p_3 \, dq_3 = \tau h$$

where $\rho, \sigma, \tau, \ldots$ are positive integers (including zero) and the integrations are extended over the values p_s and q_s corresponding to the period $1/\nu_s$. The q are Hamiltonian generalized co-ordinates of position and the p are their conjugate components of generalized momentum. The paper applied the rules to the simple harmonic oscillator, to find $E_\rho = \rho h\nu$. An application of statistical mechanics to an assembly of oscillators gave Planck's radiation formula but failed to give the Bohr frequency relation:

$$h\nu = E_1 - E_2$$

In 1916 appeared "The Quantum of Action", which preceded Sommerfeld's first paper on the subject. In this Wilson deduced that if elliptic orbits existed in the atom they could only have a few discrete values of the eccentricity ε given by

$$\sqrt{1-\varepsilon^2} = \sigma/(\rho+\sigma)$$

where σ and ρ are positive integers as before.

Wilson did not follow this up, but in the quite independent work of Sommerfeld on the other side of the firing lines in Germany it led to the idea of space quantization and to a theory of the fine structure of the lines of hydrogen. In this theory the energies of the levels agreed with the values given later by the relativistic wave mechanics of Dirac.

The sharply defined elliptic orbits used in the theory are incompatible with the indeterminacy relations of Heisenberg, so that their success in explaining the fine structure must be regarded as an accidental coincidence. In this, the theory resembles many other steps forward in

the history of quantum mechanics in which nearly every advance has been shown later to have contained some erroneous components.

Wilson's later work was mainly concerned with basic problems such as a search for a relation between general relativity, electromagnetism and quantum mechanics. In the final volume of his text-book *Theoretical Physics*, which appeared in 1940, he expressed the view that a unified theory may follow the ideas of Kaluza who, in 1925, suggested that the paths of particles were geodesics in a 5-dimensional continuum. The fifth dimension would be related to the conservation of charge.

In all his researches he chose difficult subjects which were insoluble with the state of knowledge at the time. In his last years he was engaged in speculations concerning the shapes of the spiral nebulae.

Wilson was a fluent and remarkable lecturer and teacher. He had an unusual gift for personal contact and discussion with each student, often during the course of a laboratory class. His *Theoretical Physics*, in three volumes, contains many short, clear and original derivations of formulae. After his retirement he published the *Microphysical World* and *A Hundred Years of Physics*.

The latter contains valuable details of the history of recent ideas of the structure of the material world. It is at the same time an excellent, concise text-book of modern physics in which the human interest of the story helps the reader to follow the subtle arguments and experiments by which the facts have been established. The book is one of the "Hundred Years" series, but the title has perhaps deterred undergraduate students from appreciating its virtues as a source of information.

Wilson's wife died in 1957. He is survived by his son and his grandchildren. H. O. W. RICHARDSON

Dr. Basil Roland Record

DR. B. R. RECORD, senior principal scientific officer in the Ministry of Defence, head of the Biophysics Section of the Microbiological Research Establishment, Porton, died at his home in the New Forest on November 17, 1965. He was fifty-four years of age.

He was educated at King Edward School, Birmingham, and as a schoolboy, passionately interested in chemistry, carried out quite advanced chemical preparations in his garden-shed laboratory. With this interest he went on, naturally, to study chemistry at the University of Birmingham under the late Sir Norman Haworth and graduated with first-class honours in 1933. His first research study, also carried out at Birmingham, under the direction of Dr. S. R. Carter, was in the field of physical chemistry—an investigation of the molecular weights of polysaccharide derivatives using osmotic pressure methods. The osmometer designed by Carter and Record for use with organic solvents formed, then, a notable advance in technique and is still quoted approvingly in modern texts.

In 1937, with the aid of an extended scholarship from the Department of Scientific and Industrial Research and with Sir Norman's encouragement, he was one of the earliest of those who went to work in Prof. Svedberg's laboratory in Uppsala to use the ultra-centrifugal and diffusion techniques then recently developed there. He found the 18 months he spent in Sweden in the quiet university town of Uppsala among the happiest of his working days, and it was there that he produced his very successful results on the physico-chemical characteristics of a series of methylated glycogens and of the specific polysaccharides from Types I, II and III pneumococcus. Although the publication of this work was much delayed, the observations must have been the earliest demonstration of the strong dependence of sedimentation and diffusion coefficients on concentration found with some materials.

Although he thought seriously of remaining longer in Sweden, where he found the way of life much to his liking, the prospect of approaching war sent him back to England in 1938 to join Drs. A. S. McFarlane and R. A. Kekwick, who had by then brought both a Svedberg ultra-centrifuge and Tiselius electrophoresis apparatus into operation at the Lister Institute. It is in striking contrast with present-day science that in his first year at the Lister Institute no financial support could be found for him from either the Lister or public funds. In 1939, however, he was awarded a coveted Beit Memorial Fellowship, which he held until he resigned it in 1940 to undertake more active war work. It was during this period at the Lister that he worked with R. A. Kekwick on a physico-chemical investigation of the development of diphtheria anti-toxin in the immunized horse and the characteristics of anti-toxin proteins, the work which he, in retrospect, thought of as his most successful.

From 1941 until 1944 he worked in the Army Operational Research Group under Brig. Schonland, and principally on radar predictors, field fire-control procedures (anti-aircraft) and trials of new equipment. It was typical of him that, conceiving that he could not work freely and scientifically under senior army officers, he refused throughout to accept a commission though at much cost to himself in terms of income and the advantages of commissioned rank in war-time.

In 1944 he was released from the A.O.R.G. at the request of Sir Alan Drury to join the 'Medical Research Council–Lister Blood Products Unit' which had been set up at the Lister Institute to undertake human plasma fractionation and, initially, the preparation of fibrinogen and thrombin which, together with fibrin foam, were urgently required for war-time clinical application: at a later stage the work was extended to cover the production of γ-globulin and albumin. He had a marked aptitude, and took much pleasure in his engineering skills and contributed to the design of pilot plant equipment for plasma fractionation under aseptic conditions.

The final move in his career was made in 1947 when, at the urgent request of the director, Dr. D. W. Henderson, he took up an appointment at the Microbiological Research Establishment. Here he was made responsible for the formation of a biophysics unit together with an extensive freeze-drying laboratory, for which he designed a range of freeze-drying machinery. Under his guidance, physico-chemical investigations of several biologically important macromolecular systems were made, including, particularly, botulinus toxin and polyglutamic acid, the latter of interest both biologically and as a member of the then emerging field of polyelectrolyte systems. He was, however, most particularly concerned with the fundamental aspects of the survival of micro-organisms during the freeze-drying process.

His work was characterized by the most scrupulous attention to the conception, design, performance and analysis of experiment, by his insistence that deductions and conclusions were adequately based on experiment and by the preparation of careful, clearly written and unambiguous reports: nothing aroused his anger more than the inclusion of unwarranted speculation in a scientific paper. Although somewhat retiring, he had a strong personality, and he was devoted to the idea, perhaps no longer really possible, that a scientist should be personally engaged in experimental observation and, in consequence, showed no desire to form a large group of workers, but gathered and retained only a few bound to him by his integrity and friendship.

Conflicting with his laboratory-bound science was his wish to be actively engaged outdoors, and he was fortunate in having spent his last years in a house with a large woodland garden in the New Forest, which gave him the greatest pleasure. R. G. WALLIS

NEWS and VIEWS

The Royal Society: Vice-Presidents

The president of the Royal Society, Prof. P. M. S. Blackett, has appointed the following vice-presidents for the year ending November 30, 1966 (in addition to those already announced in *Nature*, 209, 134; 1966): Dr. S. G. Hooker, technical director (aero), Bristol Siddeley Engines, Ltd.; Dr. R. D. Keynes, director of the Institute of Animal Physiology, Babraham, Cambridge; Sir Hans Krebs, Whitley professor of biochemistry at the University of Oxford; Dr. N. Kurti, reader in physics, University of Oxford, and Senior Research Fellow, Brasenose College.

Director of the Radio and Space Research Station: Mr. J. A. Ratcliffe, C.B., C.B.E., F.R.S.

Mr. J. A. Ratcliffe retires on February 28 from the directorship of the Radio and Space Research Station of the Science Research Council. He went to this post from the Cavendish Laboratory, Cambridge, in 1960. Educated at Giggleswick School, Mr. Ratcliffe proceeded to Sidney Sussex College, Cambridge, where he graduated in physics in 1924. He began research under E. V. Appleton at the time when he was making the important experiments which established the existence of the ionosphere, and it was Mr. Ratcliffe's collaboration in this work which first stimulated his enthusiasm for the subject which has since been his main scientific interest. He was appointed a demonstrator at the Cavendish Laboratory in 1927, and it was also during this pre-war period that he acquired his high reputation as a teacher of physics. After service in radar research and development during the Second World War, for which he was appointed an O.B.E. in 1947, he returned to Cambridge and resumed work at the Cavendish Laboratory, where he became a reader in physics in 1947. The reputation he had acquired as a teacher and research worker attracted many young graduates to his radio research group and it quickly acquired a high international reputation. A firm believer in the importance of international co-operation in science, Mr. Ratcliffe has played a prominent part in the work of the International Union for Scientific Radio, of which he is, at present, chairman of Commission III (Ionospheric Research). He served as an overseas vice-president of the Institute of Radio Engineers of America in 1960. He was president of the Physical Society during 1958–60 and is at present senior vice-president of the Institution of Electrical Engineers. The Institution has just announced the award to him of the Faraday Medal for 1966, in recognition of his "extensive researches on the ionosphere, and for his studies on the propagation of low frequency radio waves". Mr. Ratcliffe was appointed C.B.E. in 1959 and C.B. in 1965. He became a Fellow of the Royal Society in 1951. His College, Sidney Sussex, made him a Fellow in 1927 and an honorary Fellow in 1965.

Dr. J. Saxton

Dr. John Saxton has been appointed director of the Radio and Space Research Station, Ditton Park, Slough, Bucks, in succession to Mr. J. A. Ratcliffe. Dr. Saxton is director of the United Kingdom Scientific Mission in Washington, D.C., and Counsellor (Scientific) at the British Embassy there. Before taking up this appointment he was deputy director of the Radio Research Station. Educated at Loughborough Grammar School and the Imperial College of Science and Technology, London, Dr. Saxton joined the staff of the College after graduation and carried out research on artificial radioactivity before joining the Radio Division of the National Physical Laboratory in 1938. (This developed into the Radio Research Station in the early 1950's.) Dr. Saxton's work on the dielectric properties of the atmosphere and on studies of the propagation of radio waves at very high frequencies have made him well known in Europe and North America. In 1961, at the invitation of the University of Texas, he spent a year there as visiting professor of electrical engineering.

Master of Birkbeck College: Prof. F. K. Hare

Prof. F. Kenneth Hare, professor of geography at King's College, London, has been appointed master of Birkbeck College, in succession to the late Sir John Lockwood. Prof. Hare was born on February 9, 1919, and graduated B.Sc. (special) in geography at King's College in 1939 at the early age of twenty. He had already started research on land use in Galloway and on geochronology in the Thames Valley, combined with graduate work in meteorology. During the Second World War he was one of the small band of scientists behind the plans for the Normandy landings, where such difficult but vital problems as the relation between weather, soil, mud and tank-performance had to be considered. Moving to Canada as assistant professor at McGill University, Hare soon succeeded to the headship of the Department of Geography, and became involved in such diverse activities as establishing research stations in Labrador and Barbados and the air survey of the Northlands. A doctorate from the French-speaking Université de Montréal, a deanship at McGill, an Hon. LL.D. from Queen's University and election as honorary president (being a non-American) of the Association of American Geographers mark stages in North American appreciation of his work. Tempted back to England in 1963 to succeed his old chief, the late Prof. S. W. Wooldridge, Hare has been playing an active part in both the Natural Resources Advisory Committee and the Natural Environment Research Council.

United Kingdom Atomic Energy Authority: Prototype Fast Reactor

In a statement in the House of Commons on February 9, the Minister of Technology, Mr. F. Cousins, said that the Atomic Energy Authority had advised him that the design of a prototype fast reactor was sufficiently advanced to justify starting construction and this had been sanctioned by the Government. The prototype would generate 250 MW and, with the associated fuel production plant, would cost about £30 million. It was expected that the reactor would come into operation in 1971. The Atomic Energy Authority had advised him that only the reactor development establishments at Dounreay and Winfrith would be suitable sites from the technical point of view, and after considering all the relevant factors the Government had decided that it should be built at Dounreay. The fuel production plant would be sited at Windscale. A similar statement was made in the House of Lords, also on February 9, by Lord Snow, who in reply to questions added that while Chapel Cross and Windscale had also been considered, only Dounreay and Winfrith merited serious consideration from the point of view of research and development facilities, although social factors had also been taken into consideration.

Channel Tunnel

In a debate in the House of Commons on February 9, Mr. S. Swingler, Joint Parliamentary Secretary to the Ministry of Transport, stated that the preliminary geo-

logical and geophysical studies for the Channel Tunnel had proved very difficult and complicated. The surveys included the sinking of 73 boreholes, land borings, measurements of current, and weather studies. They were put in hand in agreement with the French Government following the Minister's statement on February 6, 1964, in an endeavour to find the best route for either a bored tunnel or a submerged tube. Besides the marine borings, site work completed last October, costing £2·1 million to be shared between the two Governments, had included several hundred miles of geophysical survey, ten land borings in England, nine land borings in France, measurements of currents and tides in the Channel, many varieties of new tests in the boreholes, and laboratory tests on samples of rocks. For the marine borings, two special platforms and four drilling vessels were used as well as many support ships, and besides five firms of consulting engineers, specialists in geophysics and palaeontology were engaged under the general direction of the Channel Study Group. A supplementary estimate of £845,000 represented the British share of the additional costs due to particularly difficult weather conditions, etc. A report on the survey was expected in March, and the report was likely to show the feasibility of the project. Discussions had also been proceeding between officials of the two Governments on the organization and finance of future stages of a Channel Tunnel project and how the juridical problems involved might be solved.

Concord Project

A FURTHER short debate on the aircraft industry in the House of Commons on February 9, in which Mr. R. Carr again emphasized the critical importance of deciding the stage at which decisions on a highly technical project should be firm, was concerned essentially with the *Concord* project. In replying on the debate, the Parliamentary Secretary to the Ministry of Aviation, Mr. J. Stoneham, said that there had been a slight increase in cost since the estimates were last presented to the House and the specification had been modified from 118 to about 130 seats. The estimates had also been revised by a new system providing a more accurate appreciation of the costs involved, and the assessment, which was being made the part-certificate of airworthiness, would also include considerable costs. There would be a ground test programme of unprecedented scope, including airframe testing with the research facilities at Farnborough. There had been no technical delay, nor any increase in cost resulting from the review of the project, October 1964–January 1965. Much work had been done on the noise factor and the first prototype should be flying early in 1968.

Royal Society Expedition to Tristan da Cunha

THE volcanic eruption that began on Tristan da Cunha in October 1961 and led to the temporary evacuation of the whole population, though not of the domestic animals and birds, was soon followed up by the Royal Society's Expedition in the early months of 1962. Geological and biological reports on this reconnaissance have been published separately. The latter includes the terrestrial botany of the whole island group (Tristan, Nightingale, Inaccessible, also Gough Island) by N. M. Wace and J. H. Dickson; the fauna by M. W. Holdgate; the effects of the eruption on the vegetation of Tristan by J. H. Dickson; and on the fauna by D. E. Baird (*Phil. Trans. R. Soc.*, Series B: Biological Sciences. No. 759, 249 (October 7, 1965). Pp. 257–434+plates 32–47. London: The Royal Society, 1965. 85*s.*; 12.75 dollars). The monograph brings together most of the previous information from other expeditions, with a well-documented analysis of the status of the plant and animal species, both native and introduced by man. Tristan has already more than twice as many invading species of flowering plants as native ones, though they are still largely segregated. The plant communities are described. The faunal analysis is much less complete, but it also shows that there are more introduced species of invertebrates, such as worms, myriapods and beetles, than native ones. Direct damage to vegetation by lava flow and ash was much less than that from toxic fumes which affected a broad zone on the north and north-east sides of the island. The abandoned domestic stock were severely attacked by feral dogs, but the unusual conditions apparently have not severely affected native birds.

The Alaskan Earthquake of March 1964

ON the afternoon of Good Friday, March 27, 1964, Southern Alaska was struck by perhaps the strongest earthquake to have hit North America within historic times, the epicentre being about 80 miles east-south-east of Anchorage, the largest city of Alaska, which suffered damage estimated at about 300 million dollars. Anchorage normally houses a rather large contingent of geologists; and within two days of the earthquake a committee of these local earth scientists, the membership of which rose to forty, was empowered to outline "the necessary and immediate courses of action" to be taken by the city. The group contracted for aerial photography, a drilling programme, soil testing, and geological mapping, producing a preliminary appraisal within two weeks and a final report a month later. Probably no major earthquake has been so thoroughly investigated so soon after its incidence. Contemporaneously, the United States Geological Survey initiated a programme of longer-term investigations, and from the first of its reports to be published it seems likely that this shock will ultimately be more fully documented than any comparable catastrophe (United States Department of the Interior: Geological Survey. Professional Paper 542-A: *Effects of the Earthquake of March 27, 1964, at Anchorage, Alaska.* By Wallace R. Hansen. Pp. iv+68+plates 1 and 2. Washington, D.C.: Government Printing Office, 1965). Damage at Anchorage was caused by direct seismic vibration, by ground cracks, and by landslides, the latter moving on nearly horizontal slip surfaces related to zones of low shear strength and high water content in a glacial clay underlying much of the area. Old landslides predating the settlement of Anchorage and presumably triggered off by earthquakes have now been widely recognized in the vicinity. This first monograph in a continuing series is extensively illustrated and it forms an important addition to the basic literature of engineering geology.

Electrolytic Hydrodimerization

A SCIENTIFIC and technological breakthrough into a new field of electro-organic chemistry has recently been proclaimed by the Central Research Department of the Monsanto Company, St. Louis, Missouri, of which a brief account is given in *Monsanto Magazine* (Monsanto Company, St. Louis, Missouri. 45, No. 5, December, 1965). It is a fascinating story of tantalizing and frustrating research which appears to have been initiated some years ago by Soviet scientists, but their process was not economically feasible. The problem, put into simplest terms, was to convert a plentiful and relatively inexpensive compound, acrylonitrile, into a vital intermediate in the manufacture of nylon; in fact, the compound adiponitrile. The basic argument was that theoretically two molecules of acrylonitrile, if placed end to end and supplied with one more hydrogen atom apiece, would structurally form one molecule of adiponitrile, but hitherto research experiments had failed to discover a *modus operandi* for economically 'splicing' the two acrylonitrile molecules and at the same time adding the necessary hydrogen to complete the adiponitrile structure. This process, as now realized successfully, is an example of hydrodimerization. In the present case the chain of events was started by Dr.

C. A. Hochwalt, Monsanto research and technology vice-president (since retired), who studied the Russian reports. He formed the opinion that some phases of the Russian experiments were of technical interest and were worth following up by Monsanto scientists. It was Dr. M. M. Baizer of the Central Research Department who was critically concerned with one aspect of the Russian approach to the problem, that involving electrolytic reactions; Dr. Baizer disagreed with widely accepted interpretations of the effect of an electric current on organic chemicals, in particular the alleged existence and functions of intermediate fragments, the 'free radicles'; he was convinced that these had been overrated, and were probably not involved at all in the main problem. Dr. Baizer accordingly set to work, on comparatively modest laboratory equipment centring on an electrolytic cell, to see whether his ideas were correct or otherwise. "In a few months, he was ready to report that it did appear, indeed, that free radicles did not necessarily figure in the results. In addition, he was able to report a breakthrough into a new field of electro-organic chemistry. It was the direct electrolytic hydrodimerization of a broad variety of petrochemicals into others twice their molecular size." Later, his process was the basis for many patents, in one case ten being issued on the same day. The mechanism of the process involved is shown diagrammatically in colour in the article and may, in summary, be conveyed as follows: the sequence begins as two acrylonitrile molecules in an electrolyte-water solution are drawn together when current from a cathode breaks the carbon double bond in the first, 'charging' it, causing the carbon double bond in the second to break as the two molecules join ('carbanion'); at this stage two hydrogen atoms from the water are attracted, thus the adiponitrile molecule is formed. Within two years of this discovery, a multi-million-dollar Monsanto plant, with batteries of electrolytic cells many times larger than the original cell used by Dr. Baizer, is busily and efficiently converting acrylonitrile into adiponitrile at Decatur, Alabama; this was described as a technological feat for which, in 1965, Monsanto won one of the most prized honours in this field in America, the Kirkpatrick Award for Chemical Engineering Achievement.

Morphological Analysis by Digital Computer

A COMPUTER-ORIENTED approach to the analysis of microscopic images has been developed by M. L. Mendelsohn, W. A. Kolman, B. Pery and J. M. S. Prewitt, of the Department of Radiology, University of Pennsylvania, around *CYDAC*, a data conversion system which scans through the microscope and records the optical information in digital form on magnetic tape (*Methodik der Information in der Medizin*, 4, No. 4; December 1965). The quality of the optical information thus made available to the computer is surprisingly good, as shown by the reconstruction of the original image using the high-speed printer. Preparations for the computer interpretation of scanned images are under way, using blood cells as a model system. Parallel attempts to classify human chromosomes by means of *CYDAC* scans have given encouraging preliminary results.

Developments in Pure Chemicals

AT present the research and development programme of the B.D.H. Laboratory Chemicals Division is aimed mainly at the production of highly purified inorganic compounds used for optical purposes—principally the hot-pressing of infra-red windows and the growing of crystals for a variety of purposes. The emphasis is always on greater and greater purity since optical properties are profoundly influenced by small traces of some impurities. Associated with this programme is the preparation of highly pure acids and solvents; these are used primarily by the electronics industry for the etching and cleaning of semiconductor surfaces, but they also have an obvious appeal to analysts and those concerned with the preparation of very pure materials. In the past 5 years the company has been engaged in a major effort to make the United Kingdom less dependent on imported biochemicals. Apart from producing many materials formerly available only from the United States, the Biochemical Department has made its own contribution to the range of biochemicals available commercially. Examples include (*a*) a B.D.H. preparation for the world market of the reagent dansyl chloride and a series of dansyl derivatives of amino-acids which are important in protein research; (*b*) the commercial production of high quality ε-(2 : 4-dinitrophenyl)-L-lysine hydrochloride which is important in determining lysine in foodstuffs, especially in milk. It has been important to lipid chemists in Britain that the company has provided reasonably priced methyl esters of very pure higher fatty acids. B.D.H. is playing a large part in the supply of reagents to hospital laboratories. The bacteriologist has benefited from a mucous digestant, *N*-acetyl-L-cysteine; some tetrazolium salts of exceptional quality are speeding the work of the histologist and cytologist (also of those concerned with seed viability testing). Special attention is being paid to the demands of automatic analysis in clinical laboratories by providing ready-made solutions and convenient forms of other reagents.

The Cape Asbestos Company, Ltd.

THE Cape Asbestos Company, Ltd., is the parent company of the Cape Asbestos Group, an international organization with widespread industrial ramifications. The Mines Division includes Cape Asbestos South Africa (Pty.), Ltd., the South African parent company, and affiliated firms, operating amosite mines at Penge, Weltevreden, Kromellenboog and Malipsdrift, blue asbestos mines at Koegas, Westerberg, Pomfret and Warrendale; also the London-based Cape Asbestos Fibres, Ltd., concerned with the promotion, sale and distribution of raw and processed asbestos fibres. The Insulation Division is concerned with various proprietary insulating materials, for example, rockwool, asbestos yarn and cloth, asbestos millboard, rope lagging, packings and jointings, plastic finishing and insulating compositions and asbestos reinforced plastic sheeting, insulation; this Division operates in London, Liverpool, Dublin, and at Benoni, Transvaal. The Board Products Division incorporates Cape Building Products, Ltd., Uxbridge, makers of 'Asbestolux' board and the well-known Uxbridge Flint Bricks, Harefield Lime Company, producing lime for brick manufacture, and Marinite, Ltd., London and Glasgow, who make the popular 'Marinite' asbestos sheet.

Companies operating in the Engineering Division include Small and Parkes, Ltd., Manchester, concerned with brake and clutch linings, disk brake friction pads for automotive and industrial applications, fanbelts, engineering plastics; others trading elsewhere in Britain, in Brussels, and at Benoni South, Transvaal, are concerned with general garage equipment. That at Fareham, Hampshire, is concerned with water jet propulsion units for pleasure and commercial craft. There is a separate Overseas Division, based in Chicago, Toronto and Turin, which handles the sale of various asbestos products. Associated companies, concerned mostly with packings, jointing and insulating products, are located in Argentina, Canada, France, and elsewhere. This information about the activities of the Group is chronicled in the house journal, *Cape Magazine* (14, No. 3; Autumn 1965. 114 Park Street, London, W.1). Although most of this periodical is concerned with internal staff matters, from United Kingdom and overseas centres, a few short articles are of wider interest, one dealing particularly with the new rules (1965) governing structural fire pro-

tection in passenger and cargo ships, another with the safety precautions against accidents operating in the several factories belonging to the Group.

Forest Diseases and Insects

A GREAT deal of effort and investment is being directed to increasing forest production, but there are certain factors which are reducing the desired results. Many of these factors were discussed at a symposium held under the aegis of the Food and Agriculture Organization and the International Union of Forest Research Organizations at Oxford during July 1964 on "Internationally Dangerous Forest Diseases and Insects" (*Unasylva*, 19 (3), No. 78; 1965. Food and Agriculture Organization. 3*s*. 3*d*.). Very little factual information is available on losses due to these causes, but in the United States it was estimated in 1952 that they accounted for 35 per cent of the gross annual growth. Although old natural forest stands suffer most from decay and insect-caused mortality, the main concern of the future may be in the man-made forests, many of which are characterized by exotic species in pure, even-aged stands. Thus new problems are possibly being created. Of the various control measures likely to be practical on a forest scale, it seems that the so-called 'natural' ones hold certain advantages. Appropriate silviculture and forest management can manipulate the species, stand composition and rotation and so help prevent disease and insect damage. This really involves no extra cost and the same may be said of biological control measures which can be self-perpetuating. On the other hand, although chemical control has the advantages of being rapid, easily applied and available for use against many insects and fungi, its economics are complex and its possible side-effects tend to strike a warning note. Very sound recommendations were passed at the symposium and these included the need for more pathologists and entomologists, for more research and the quantitative evaluation of losses, for the certification of seed, the quarantine of plants and their disinfection and disinfestation and for annual reports from all countries on disease and insect conditions. This volume of *Unasylva* is a very fine synopsis of the main subjects discussed at the symposium. It is to be followed by the publication in two volumes of the individual papers and by a third volume on *Diseases of Widely Planted Forest Trees*. These will make available a great deal of useful information on subjects that are becoming more and more important in forestry practice.

Vegetation Types

MAPS of vegetation types can be of great value to the silviculturist and forest manager because they indicate site conditions, and site is a very important factor influencing production. The need for more detailed maps of vegetation types for parts of the Swiss Jura lying to the north-west of Lake Neuchâtel came about as a result of old silver fir stands suffering from the drought years of 1945–50. These stands, with southerly aspects and situated on shallow, calcareous soils, began to perish when, in their weakened state, they suffered from bark beetle attack (*Ips curvidens*). Thus, the Forest Service was faced with reforestation work, and so it was decided to make a detailed investigation of the natural vegetation of the region. This was done during the 10-year period ending in 1961 and the results are now available (*Extraits de la Carte Phytosociologique des Forêts du Canton de Neuchâtel*. Par Jean-Louis Richard. Commission Phytosociographique de la Société Helvétique des Sciences Naturelles. Matériaux pour le levé Géobotanique de la Suisse, Fascicule 47. Pp. 48+1 carte. Berne: Editions Hans Huber, 1965. 25 Sw. francs). It is claimed that in most of the forests of the Swiss Jura, man has nowhere degraded the natural vegetation to such an extent that it is irreversible. Indeed, even some changes in the woody vegetation have scarcely affected the herbaceous cover and the soil, and so there is little difference between the actual and the potential vegetation. Thus, where potential and not actual vegetation has been mapped, this has been done without fear of any risk of serious error. Four blocks of forest totalling more than 26,000 hectares were examined and their physical features, geology, soils and vegetation are described. Twenty-eight natural forest associations are listed, together with their characteristic species and notes on the soils. The vegetation type map with three blocks on a scale of 1 : 25,000 and one at 1:10,000 shows a wealth of detail and the reproduction is excellent. It is true to say that the entire work and its usefulness are typical of the efficiency that is associated with Swiss forestry.

Physiodormancy

DR. JOHN WIEBE, of the Horticultural Experiment Stations, Vineland Station, Ontario, has written to the Editor as follows: "All deciduous, temperate zone, perennial plants cease growth and enter dormancy at some time in summer or autumn. They remain dormant until growth begins again in spring under favourable moisture and temperature conditions. Frequently, temperature and moisture conditions in late summer and autumn are favourable for the continuation of growth, yet the plant remains dormant. If a dormant plant is brought into a greenhouse before its rest period, or cold period requirement, has been satisfied, it will remain dormant. Once the rest period has been satisfied by exposure to a suitably low temperature for a sufficient time, the plant is capable of resuming growth as soon as it is in a favourable environment. Because of continuing unfavourable temperature, it may however, and usually does, remain dormant for some time after its rest period has been satisfied. Because 'rest period' or 'cold period requirement' are ambiguous terms, I propose the term 'physiodormancy' for that component of the dormancy of a plant which is controlled by physiological or biochemical agents. Thus, a plant is said to be physiodormant when it will not start into growth at normally favourable conditions of moisture and temperature. The plant may continue to remain dormant, when it is no longer physiodormant, if temperature or lack of moisture prevents the resumption of growth".

Scientific Research

Scientific Research is a new addition to the list of monthly magazines which may be received gratis by those actively engaged in research in physics, chemistry or the life sciences. J. D. Luntz, editor and publisher of the magazine for McGraw-Hill, Inc., states in the first issue (January 1966) that the contents provide interpretative commentary on technical developments, selected technical articles, and new product information of specific use to experimenters. The feature article in the first issue is by C. Laverick on superconducting magnets, and the new products section consists of comprehensive surveys of commercially available (English, American and Japanese) storage oscilloscopes and American signal-averaging computers, together with a preview of some of the instruments which were exhibited at the Physics Show in New York during the annual meeting of the American Physical Society and American Association of Physics Teachers during January 26–29, 1966. A literature survey and reader service card are included. Three new improved designs of electron-microscopes, computer time-sharing, and a report on the disastrous fire that wrecked the Cambridge (Massachusetts) electron accelerator bubble chamber hall in July 1965, form the main features of "Science Roundup", the section dealing with recent developments.

Acta Physica

Acta Physica, devoted to the publication of original research work in the field of theoretical and experimental physics, is published by the Hungarian Academy of Sciences. Contributions deal with theoretical problems of the principles and applications of quantum mechanics, quantum chemistry, solid-state physics, thermodynamics and statistical mechanics, elementary particles, and experimental investigations in spectroscopy, crystal physics and nuclear and elementary particle physics. Papers are printed in English, German, French or Russian, with abstracts in various languages. Beginning in 1966, *Acta Physica* will appear in two volumes annually, each containing approximately four hundred pages, with four issues per volume. The subscription rate per volume is £3. The editor is P. Combás and the editorial board consists of Z. Gyulai, L. Jánossy, L. Kovács and K. Novobátzky.

New Members of the Advisory Council on Technology

Mr. D. K. Fraser, of G. A. Harvey and Co. (London), Ltd., and Mr. A. Siddall, of Joseph Lucas, Ltd., have been appointed members of the Advisory Council on Technology. They replace Lord Brown, who resigned on appointment as Minister of State at the Board of Trade, and Prof. M. J. Lighthill, who resigned on appointment as Honorary Physical Secretary of the Royal Society.

Scholarship at the National Vegetable Research Station

Applications are invited by the National Vegetable Research Station for a postgraduate scholarship tenable at the Station from October 1. The purpose of the scholarship is to enable the recipient to pursue further studies and to obtain training in one of the following lines of research in progress at the Station: biochemistry; biometry; ecology and control of weeds; entomology; plant pathology. The scholarship, which is of the value of £600 per annum, will be awarded for two years, with possible extension for a third year. Further information can be obtained from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick. The closing date for receipt of applications is March 31.

Smithson Bicentennial Addresses

The Smithsonian Institution has announced that the eleven addresses delivered at the scholarly sessions of the James Smithson bicentennial celebration, held in Washington, D.C., during September 16–18, 1965 (*Nature*, 208, 320; 1965), are to be published in a volume now in the press. The collection will include papers by James S. Bruner, Herbert Butterfield, Sir Kenneth Clark, Ian McTaggart Cowan, G. Evelyn Hutchinson, Arthur Koestler, Claude Lévi-Strauss, Lewis Mumford, J. Robert Oppenheimer, Stephen E. Toulmin, and Fred L. Whipple, together with an introductory essay by S. Dillon Ripley, secretary of the Smithsonian. Readers of *Nature* have already had the benefit of a preview of a few of the papers (208, 923, 1033, 1036, 1145; 1965; and 209, 10; 1966). The book is scheduled for publication in the spring of 1966 by Simon and Schuster, New York, in association with the Smithsonian Institution. Further information can be obtained from the Editorial and Publications Division, Smithsonian Institution, Washington, D.C.

University News:

Birmingham

Mr. M. H. Loretto has been appointed lecturer in the Department of Physical Metallurgy. Dr. G. Sweeney has been appointed senior Research Fellow in the Department of Mechanical Engineering. The following Research Fellows have also been appointed: Mr. D. K. Davies (geology); Mr. J. A. F. Wickramasinghe (physiological chemistry).

Liverpool

Prof. D. Hull, professor of physical metallurgy, has been appointed to the Henry Bell Wortley chair of metallurgy, on the resignation of Prof. W. S. Owen. The following lecturers have also been appointed: Mr. K. G. Evans, Dr. G. R. Jones and Mr. D. P. Sen Gupta (electrical engineering and electronics).

Manchester

Dr. J. G. Davies, reader in radio astronomy, has been appointed to an additional chair of radio astronomy.

Sheffield

The following lecturers have been appointed: Dr. J. D. Harry (physiology); Dr. G. M. Birtwistle (electronic computation in the Department of Applied Mathematics).

Sussex

The following appointments have been made: *Professorships:* Mr. J. F. C. Kingman (mathematics and statistics); Dr. F. J. Bayley (mechanical engineering); *Lectureship:* Dr. S. Shall (biological sciences); *Senior Research Fellowships:* Dr. M T. Gillies and Dr. K. A. Stacey (biological sciences).

Announcements

Mr. J. F. Gaitens has been appointed technical assistant to the scientific director of the Universities Federation for Animal Welfare.

Prof. T. F. Dixon has resigned from his position as head of the Department of Biochemistry in the University of Singapore in order to join the Eastern Mediterranean Region of the World Health Organization.

A seminar on "Atmospheric Physics" will be held by the American Institute of Physics in Washington on March 14. Further information can be obtained from Eugene H. Kone, American Institute of Physics, 335 East 45 Street, New York 17.

A conference on "Historical Aspects of Microscopy" will be held by the Royal Microscopical Society at Oxford on March 18. Further information can be obtained from the General Secretary, Royal Microscopical Society, Tavistock House South, Tavistock Square, London, W.C.1.

The 1965 Guthrie Lecture of the Institute of Physics and the Physical Society will be delivered by Dr. J. B. Adams (director of the Culham Laboratory) on the subject "Nuclear Fusion Research" on March 28. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A meeting of the Biochemical Society will be held at the University of Hull during March 24–25. The programme will include a colloquium on "The Metabolism of C_1 Compounds", a discussion forum on "Mucopolysaccharides", and a visit to the Biological Laboratories of Reckitt and Sons, Ltd. Further information can be obtained from the Administration Officer, the Biochemical Society, 20 Park Crescent, London, W.1.

The anniversary meetings of the Chemical Society will be held in Oxford during March 28–31. The programme will include symposia on complexes of the heavy donor atoms, contemporary topics in organic chemistry and methods of studying molecular interactions in gases, liquids and solids, a discussion on the uses of programmed learning in the teaching of chemistry to degree standard, the Robert Robinson Lecture on "Some New Developments in the Chemistry of Natural Colouring Matters", to be delivered by Lord Todd, and various tours and visits. Further information can be obtained from the General Secretary, the Chemical Society, Burlington House, London, W.1.

THE BRITISH COUNCIL

THE feature article accompanying the annual report for 1964–65 of the British Council is this year concerned with libraries and library development (*Annual Report of the British Council, 1964–1965*. Pp. vii+99+12 plates. London: The British Council, 1965. 2*s*. 6*d*.). It contrasts the present position of libraries in Britain where normally upwards of 250,000 titles are in print at the publishers, 7,000 periodicals are available, and library co-operation offers access to more than 100 million books, and the public library service with a stock of about 80 million volumes issued more than 500 million books on loan from 40,000 points of service with the position of British Council libraries abroad and the work of the Council's libraries. The Council now maintains 141 British libraries overseas and sends regular supplies to a further 55. These 196 libraries, including 62 in Asia and 42 each in Africa and in Europe, cover 83 countries; 252,372 volumes were supplied to them in 1964 and they lent about 4·5 million books. Their three major functions are to provide a ready and comprehensive service of information on any subject falling properly within the Council's field; to keep the serious student using them in touch with recent British developments in his or her field; and to support the Council's work in teaching English with technical works and dictionaries and with appropriate text-books at all levels. They are built up over the years on the detailed advice of Council staff working in the field. In India and Pakistan, and on a smaller scale in Ceylon, Nigeria and Trinidad, the Council has set up special collections of multiple copies of English text-books which are prescribed reading for local university and other courses, concentrating on aspects of science, technology, economics and social sciences, with some medicine and dentistry. In India alone, some 60,000 such text-books have thus been made available to the libraries of 150 universities and colleges.

Use is made of some 2,200 periodicals published in Britain, and subscriptions to these and annual reference books total nearly 50,000. More than 150 professional librarians are now employed by the Council, and the trained librarian occupies a key position in developing the Council's library services overseas. The Council is also giving special attention to the development of public libraries in the new Commonwealth countries, and in the past 6 years 22 schemes in 9 countries have been assisted. Some £370,000 has been spent on capital grants and other forms of aid under this scheme, and the projects have been uniformly welcomed by the Governments of the countries concerned. The grave shortage of qualified and experienced librarians, however, has been a constant brake on library development in all fields and is likely to constitute a limiting factor for some years to come which only assistance from overseas can remove. Even so, the Council is concerned as to whether sufficient is being done to expand library services in the developing countries of the Commonwealth.

The Council's budget of £10,736,000 for 1964–65 included £676,000 from earnings and donations, grants of £5,388,000 from the Foreign Office, £3,977,000 from the Commonwealth Relations Office, £304,000 from the Colonial Office, £371,000 from the Ministry of Overseas Development, and £20,000 from the Board of Trade making up the remaining part. The Council also administered the expenditure on technical assistance schemes of £2·5 million from the Ministry of Overseas Development. There are now 60 posts for English language teaching experts in the Council's career service at a cost of £280,000 a year and these activities tend to concentrate increasingly on training teachers. Television officers have been appointed in Ghana, India, Iran, Kuwait, Nigeria and the Sudan and, in 1965, in Latin America and Ethiopia, while places for nearly 600 teachers of English from overseas are provided in summer schools in Britain. The Council helped to finance a project to develop programmed materials for teaching English, and a large-scale enquiry conducted by the National Foundation for Educational Research into the language difficulties of overseas students and collaboration with the British Broadcasting Corporation in the production of films for teaching English continued.

Further requests were received during the year, particularly from Commonwealth countries, for assistance in training science teachers and in the reform of school curricula in scientific subjects, and the Council has created six posts for scientific officers in East and West Africa. Of some 713 specialist tours, advisory visits and delegations arranged during the year, 113 were concerned with science and technology, 91 with medicine and 240 with education. A scheme of exchanges between British universities and the Centres of Advanced Study in India was started. Of 63 awards toward the cost of travel between Commonwealth countries, 43 were to university teachers on leave, 15 to distinguished scholars invited by universities for short visits and 10 for postgraduate university workers with research grants. Under the foreign scheme, 130 short visits were paid by senior members of university staffs, 3 longer visits and 103 visits under the younger research workers interchange scheme. Of 11,130 overseas visitors to Britain assisted by the Council, 2,664 were concerned with science and technology, 1,284 with medicine, 2,440 with social studies, and 2,612 with education.

Further progress is reported with the Overseas Students Welfare Expansion Programme, and by August 31, 1965, provision for more than 3,750 places had been approved since the scheme was launched in 1961, and grants for hostel development totalling £2,525,000 had been made. An encouraging feature is the extent to which the welfare needs of overseas students are being met by the institutions in which they are studying. More than 340 young Russians visited Britain in 1964–65 under the student exchange programme; and nearly 500 final-year students from Latin America, many of whom were concerned with developments in industry and technology, came to Britain under a programme initiated in 1962 in association with the Institute of Directors. Of the 9,923 overseas students met on arrival, 6,221 were from the Commonwealth and 1,713 from Colonial territories. Permanent accommodation in Council residences was arranged for 297, in lodgings for 6,125, and in university and other hostels and halls of residence for 2,026, apart from transit or temporary accommodation. Hospitality was accepted for 14,567 students; 16,200 were members of Council centres, 2,877 attended holiday courses of 7–10 days, and 22,200 attended weekend courses, study units and surveys. In October 1964, two additional Council scholarships were created to provide undergraduates from the Federal Republic of Germany with an opportunity of taking a first degree at a British university, and the present estimates (1965–66) provide for an increased programme of youth exchange between Germany and Britain.

THE BRITISH RADIOLOGICAL PROTECTION ASSOCIATION AND THE JOINT HEALTH PHYSICS COMMITTEE

SCIENTISTS of many specialities are concerned with some aspects of what, for want of a better name, has usually been referred to as 'health physics'. This may be taken to be the investigation of problems of radiological protection including, for example, radiation dosimetry and operational techniques connected with the use of ionizing radiations in industry, medicine and scientific research. Some co-operation of the various appropriate learned societies and other bodies concerned was therefore felt to be desirable, and in December 1961 the Joint Health Physics Committee was set up to co-ordinate the arrangement of symposia in this field. This Committee had representation from the British Institute of Radiology, the British Occupational Hygiene Society, the Faculty of Radiologists, the Hospital Physicists' Association, the Institute of Physics and the Physical Society, the Radiological Protection Service, and the Health and Safety Branches of the Central Electricity Generating Board and the United Kingdom Atomic Energy Authority. After its formation in 1963, the Society for Radiological Protection was also represented.

This co-operation was fruitful, each organization in turn arranging a symposium open also to interested members of the other organizations. Some twelve symposia have been held during the period 1962–65 with attendance between 150 and 300, the distribution between the various specialities varying with the nature of the subject.

The Health Physics Society, which was formed in the United States in 1955, was intended to be an international organization. In fact, by 1964 it did have some 20 per cent of its membership in some fifty countries outside the United States, and a number of national sections (including the Society for Radiological Protection in the United Kingdom) had been formed. However, it was apparent that other national or regional health physics organizations were also being formed and, with the initiative of Dr. K. Z. Morgan, of the Health Physics Society, discussions were started with representatives of many countries for the formation of an International Radiation Protection Association to promote international scientific co-operation in health physics and, at intervals, to arrange international meetings in this field. A provisional constitution was agreed at a meeting in Paris in December 1964, under which the International Radiation Protection Association was to consist of an affiliation of national societies (one from each country or region) with individual members of these societies having, as appropriate to their qualifications, either full or associate membership of the Association.

To meet this new situation, and after detailed discussions between the societies concerned with the Joint Health Physics Committee, it was agreed to form instead the British Radiological Protection Association. This is an affiliation of learned societies concerned with radiological protection, formed to affiliate itself to the International Radiation Protection Association as the associate society for the United Kingdom. For a small annual subscription (at present 10*s*. per annum) interested members of any of the constituent societies may then join the British and also automatically the International Radiation Protection Association as individual full or associate members, according to their qualifications as laid down in the constitution of the International Radiation Protection Association. The Joint Committee of the British Radiological Protection Association has representation from each of the constituent societies (together with one or two non-voting co-opted members) and will function in place of the Joint Health Physics Committee to co-ordinate the arrangement of symposia on health physics subjects in Britain. It will also arrange for discussion of relevant topics with the British representatives of the British Radiological Protection Association on the executive council of the International Association, who, under the constitution, will have been elected by a ballot of the individual full members of the Association.

The founder constituent societies of the British Radiological Protection Association are the British Institute of Radiology, the British Occupational Hygiene Society, the Faculty of Radiologists, the Hospital Physicists' Association and the Society for Radiological Protection (now no longer a section of the Health Physics Society). The Society of Radiographers has also been accepted as a constituent society. That there is general support for the Association is evident from the fact that already more than three hundred individual full members have been registered and accepted for membership of the British and International Associations. This number will entitle the British Radiological Protection Association to five votes on the executive council of the international body.

Affiliations to the International Radiation Protection Association have now been accepted from a total of eleven societies, including organizations in France, the United States, Luxembourg, Scandinavia, Japan, United Kingdom, Belgium, Israel, Italy, Netherlands and Central Europe, and other applications are pending. Thus, the International Association is well established and its first International Congress is to be held in Rome in September 1966.

Meanwhile, the Joint Committee of the British Radiological Protection Association is planning two symposia in the United Kingdom in November 1966, one to be organized by the Faculty of Radiologists on "Radiation Genetics", and the other to be organized by the British Occupational Hygiene Society on the "Hazards Associated with the Use of Lasers". It is to be hoped, therefore, that such co-operation, both nationally and internationally, will be of general and scientific value to those concerned with the various aspects of radiological protection.

Further details of the British Radiological Protection Association and its functions can be obtained from any of the constituent societies, or from the honorary secretary of the Joint Committee, Mr. R. Oliver, the University Radiation Protection Office, 15 Parks Road, Oxford.

R. Oliver

NUCLEAR AND ENGINEERING CERAMICS

THE position of ceramics in nuclear engineering is now well established. The same cannot be said for their structural applications in engineering if the relative number of papers on these two aspects presented at the meeting of the Basic Science Section of the British Ceramic Society held at the Atomic Energy Research Establishment, Harwell, during October 25–27, 1965, is truly representative. Of the thirty papers presented, only five dealt with potential non-nuclear applications. Mr. N. Parr (Admiralty Materials Laboratory, Poole, Dorset) discussed the difficulties encountered in attempts to establish the technology of reaction-bonded silicon nitride and its

application as a structural engineering material. Of particular interest was the philosophy lying behind his engineering designs to take into account the brittle nature of his material. The complexities attending the pyrolytic preparation of silicon carbide formed the main theme of the paper presented by P. Popper (British Coke Research Association, Stoke-on-Trent). This process, which superficially seems quite simple, was obviously sensitive to a number of process variables which had a considerable effect on the structure and hence the properties of the deposited material. There were three papers dealing with mechanical properties as such, two concerning themselves with beryllia and one with silicon carbide. Quite respectable strengths are now available in carefully fabricated polycrystalline ceramics. The paper by N. A. Hill, J. S. O'Neill and D. T. Livey (Atomic Energy Research Establishment, Harwell) showed that by control of grain size, moduli of rupture of approximately 50,000 lb./in.^{2} were obtained in beryllia. W. P. Rotsey, K. Veevers and N. McDonald (Australian Atomic Energy Commission, Lucas Heights) showed that in addition to grain size, surface preparation and the presence of water vapour were critical features in determining the room temperature strength of beryllia. Results of investigations of the delayed fracture in air of a self-bonded silicon carbide reported by J. C. V. Rumsey and A. L. Roberts (University of Leeds) showed that delayed fracture behaviour could be adequately correlated phenomenologically and that at elevated temperatures well-defined primary and secondary creep stages were exhibited.

With respect to the nuclear aspects of the meeting, it is interesting to compare the nature of the papers presented at this meeting with those presented at a very similar meeting at the same venue 4 years previously. The general headings are approximately the same, but the emphasis has, as one would have expected from the rapid progress in the nuclear field, changed considerably. Whereas 4 years ago interest was mainly centred on the fabrication and properties of uranium and plutonium oxide ceramics with a little attention being paid to the carbides, on this occasion practically no attention was given to the pre-irradiation, preparation and characterization of oxide ceramics, the main interest in these areas now lying with uranium and plutonium carbides with a little attention being given to other uranium compounds. The papers by D. George and N. R. Williams (Atomic Energy Research Establishment, Harwell) and by J. M. Horspool and N. F. Rose (Dounreay Experimental Reactor Establishment, Caithness) showed clearly that the preparation and fabrication by sintering of uranium monocarbide and uranium plutonium monocarbide were close to becoming well-controlled fabrication routes, most of the problems associated with the preparation of fine powders, their handling and the selection of a suitable binder, having been overcome. In both these papers the use of nickel as an additive to promote sintering to greater than 95 per cent theoretical density at temperatures as low as 1,550° C was advocated. It became quite clear, however, that the true role of nickel as a sintering promoter was far from understood, and this was emphasized by the paper of B. L. Eyre, A. F. Bartlett and D. Barry (Atomic Energy Research Establishment, Harwell) which described a transmission electron microscope investigation of sintered uranium monocarbide with and without the nickel additions. Differences in structure were observed and possible effects on sintering behaviour were discussed again without any very definite final conclusions being reached.

The complexity of phase relationships in the uranium–nickel–carbon, uranium carbide–iron–nickel, and uranium carbide–iron–chromium systems was illustrated in a paper by S. K. Dutta and J. White (University of Sheffield). Probably the most interesting point which arose from this work was that solid uranium carbide was likely to be compatible at elevated temperatures with only a very limited range of iron–chromium alloys despite the fact that it was completely compatible with both iron and chromium. This situation arose as a result of the formation of the compounds $Cr_{23}C_6$ and UFe_2. The sensitivity to composition of the mechanical properties of uranium monocarbide and closely associated alloys was the basic theme of the papers by J. S. Pottinger, M. C. Regan and H. J. Hedger (Atomic Energy Research Establishment, Harwell) and A. Accary, P. Magnier and M. Marchal (Commissariat à l'Énergie Atomique, Saclay). The former authors used hot hardness measurements to show that hypostoichiometric compositions softened rapidly above 600° C, while hyperstoichiometric compositions showed a slower decrease of hardness up to about 1,000° C. Partial substitution of tungsten, niobium and vanadium for uranium in the carbides was particularly effective in increasing the resistance to deformation by indentation. At higher temperatures the French investigators showed that mechanical behaviour was strongly dependent on the total carbon content and that at approximately 2,300° C the plastic properties were such that extrusion and die-forming were possible. Measurements of self-diffusion in uranium carbide were reported by H. M. Lee and L. R. Barrett (Imperial College, London), who concluded that the activation energy of bulk diffusion in the slower diffusing species, namely, uranium, was in good agreement with sintering kinetic data, but that the mechanism of creep was likely to be more complicated than that usually described as the Herring–Nebarro mechanism.

The growing importance of fuel in the form of microspheres (approximately 500μ diameter) was represented by papers dealing with the preparation, coating, evaluation and irradiation testing of sol gel oxide micro-spheres by R. G. Wymer and J. H. Coobs (Oak Ridge National Laboratory, United States), and the consolidation of carbon-coated fuel particles in a graphite matrix by hot pressing by G. H. Williams, J. B. Morris and D. W. Savage (Atomic Energy Research Establishment, Harwell). The use of sol-gel techniques for the preparation of very high density oxide spheres at relatively low temperatures was pioneered at Oak Ridge, and they have now extended their work both in scale and in scope to include plutonium dioxide, thorium dioxide–uranium dioxide and plutonium dioxide–uranium dioxide, rare earth oxides and curium oxides. These particles have to be coated usually with pyrolytic carbon to retain fission products and their incorporation into a matrix without cracking the coating is a delicate operation which has apparently been solved by the British authors.

Work reported on the irradiation behaviour of fissile ceramics was confined largely to uranium dioxide, as would be anticipated from its present pre-eminent position as a nuclear fuel. As was re-stated by R. S. Barnes and R. S. Nelson (Atomic Energy Research Establishment, Harwell) in their general paper on the behaviour of inert gases in ceramic fuels, structural changes have an overwhelmingly important effect on gas release. The paper by J. R. McEwan and J. Hayashi (Atomic Energy of Canada, Ltd.) detailed the effect of time, temperature and five other potential variables on isothermal grain growth in uranium dioxide. Of general interest was the observation that porosity elimination was not rate controlling so far as grain growth was concerned. The use of an arc image furnace to study columnar grain growth in a temperature gradient of uranium dioxide, both with and without additions of other oxides, was reported by T. J. Potter and C. A. Elyard. Matters relevant to the dimensional stability of uranium dioxide taken to high burn-ups were the subject of papers by B. T. Bradbury, J. T. Demant and Mrs. P. M. Martin (Atomic Energy Research Establishment, Harwell) and by J. D. B. Lambert (Atomic Energy Research Establishment, Harwell). The former authors were concerned with the nature and distribution of solid fission products in irradiated uranium dioxide. Above approximately 3 cent burn-up, white inclusions were found at grain

boundaries which contained a number of the solid fission product elements. Mr. Lambert reported on the irradiation behaviour of uranium dioxide particles in cermets at burn-ups in the range 5–12 per cent and was mainly concerned with the swelling which appeared to proceed eventually at an inexorable rate of about 1·7 volume per cent per 1 per cent of burn-up.

Detailed investigations of the behaviour of krypton gas bubbles introduced by ion bombardment in uranium dioxide using the electron microscope were described by G. H. Bannister, R. M. Cornell and B. M. Jeffrey (Central Electricity Generating Board, Berkeley).

There were more papers dealing with the irradiation behaviour of non-fissile ceramics than with any other single topic. Both gas release and dimensional stability were, as were fissile oxides, major topics for consideration. In-pile release of helium from beryllia had been investigated by Y. Carteret, J. Bareau and J. Elston (Commissariat à l'Énergie Atomique, Saclay), who showed that after a few hundred hours the release of helium attained a constant level. Measurements of the diffusional release of tritium from beryllia by fast neutron annealing had been made by K. T. Scott and L. L. Wassell (Atomic Energy Research Establishment, Harwell). The behaviour exhibited, namely, that of initial bursts followed by steady-state release, was very similar to that of inert gas in fissile oxides. Activation energies varied from 48 to 59 kcal/mole. Investigations of the nature of fast neutron-induced defects in single crystal alumina and in polycrystalline alumina and magnesia, using transmission electron microscopy, changes in lattice parameter, density and length were reported by R. S. Wilks *et al.* (Atomic Energy, Harwell), by M. Stevanovic and J. Elston (Commissariat à l'Énergie Atomique, Saclay) and by R. P. Thorn and V. C. Howard (Reactor Materials Laboratory, Culcheth). A still more sophisticated technique for studying irradiation-induced defects by the scattering of long wave-length neutrons was reported by D. G. Martin (Atomic Energy Research Establishment, Harwell), who showed that the smaller clusters often visible under the electron microscope as irresolvable dots probably contained less than thirty defects per cluster. The overall situation on irradiation effects in ceramic oxides was well summarized in a paper by B. S. Hickman and D. G. Walker, who discussed at some length correlations between the irradiation behaviour of beryllia, alumina and magnesia as well as zinc oxide. The irradiation behaviour of only one non-fissile carbide, namely, polycrystalline, self-bonded, porous, cubic, silicon carbide, was discussed by R. P. Thorn, V. C. Howard and B. Hope (Reactor Materials Laboratory, Culcheth) in terms of dimensional change and X-ray parameter measurements. The saturation growth obtained was, as is normal, a function of temperature and was paralleled by saturation effects in thermal conductivity. There was, however, an appreciably slower recovery on annealing of thermal conductivity compared with dimensions.

The meeting was organized by Dr. D. T. Livey of the Metallurgy Division, Atomic Energy Research Establishment, Harwell, and, as was apparent, received a considerable amount of international support. It was attended by 152 people and, as has now become the well-established form with the Basic Science Section meetings, approximately half were members of the Society and half were guests. The papers presented at this meeting will be published as a separate volume of the proceedings of the British Ceramic Society.

J. Williams

REVIEW OF THE FOSSIL RECORD

A SYMPOSIUM entitled "A Review of the Fossil Record", organized jointly by the Geological Society of London and the Palaeontological Association, was held at the University College of Swansea during December 20–21, by invitation of Prof. F. H. T. Rhodes, head of the Department of Geology. It was attended by some 160 members.

The meeting had been arranged in order to discuss a projected publication volume by the Geological Society, and in this respect it followed the pattern of the symposium on the Phanerozoic time-scale held at Glasgow in February 1964. The volume in preparation is to consist mainly of contributions by more than a hundred specialists, covering the whole fossil record, both plants and animals, organized into thirty chapters on a systematic basis. Each contributor was asked to recommend a classification of his allotted taxa, and to document the first and last reported fossil occurrence of each taxon. To do this thoroughly was thought to be a sufficient task, and it carried the implication that there was continuity of life between these first and last occurrences, even if no intermediate record had yet come to light. Thus, a survey of the ranges of taxa covering all fossils through geological time could be included in a single volume. It was left to the contributor in each case to decide on the degree of taxonomic sub-division; so in some cases families and genera, and in others, super-families or orders, have been recorded. In this way some idea of the diversification can be given, without departing from the minimum requirement for first and last documented records.

An editorial committee shared the systematic editing and maintained contact with specialist contributors. The committee also worked out a standard stratigraphical scale, so that data could be presented in a uniform way. To this end, questionnaires were first sent out, and after some discussion a scale was agreed on, admittedly arbitrary and temporary, for the use of the symposium only. However, some longer-term benefit may result from this, for although the scale was divided into units of about stage rank and did not attempt any tidy or final codifying of these units, the committee did nevertheless define the base of each selected unit reasonably precisely and unambiguously. This project demonstrated the need for a standard global stratigraphical scale, and illustrated the principle of defining units by single reference horizons at their bases. The degree of division of the stratigraphical scale was a compromise, being on the one hand too fine for much international correlation, and on the other too coarse to plot some precisely known records. The stratigraphical scale was used for a series of charts on which the ranges of taxa were plotted as lines joining first and last records. Some precision was also lost because presence only, and not position, within each division was plotted. These working charts were on display at Swansea, and will be re-drawn for publication in the forthcoming volume.

The object of the symposium meeting was to discuss issues relevant to this compilation. Eight main papers and several short prepared contributions were presented and will appear, with some of the discussion, in the symposium volume.

The symposium was introduced with a review of the origin and development of Pre-Cambrian life and the base of the Cambrian system by J. W. Cowie. Speculation on the origin of life on this planet, as related to hypotheses of the composition and state of the hydrosphere and atmosphere, was briefly surveyed, and was followed by a review of the earliest organized structures in rocks. Mention was made of the recent discovery by Barghoorn of organized carbon residues about 3,000 m.y. old, possibly

older than the better-known stromatolitic dolomite of McGregor from Bulawayo which has been dated only by correlation with distant rocks via 2,700-m.y.-old granite. In rocks less than 2,000 m.y. old, stromatolitic, algal and acritarchal structures are common. The first faunas so far established may not be much older than established Cambrian; how much older they are raises the question of the biostratigraphic definition of the base of the Cambrian. The distinct and now well-known *Ediacara* fauna is only marginally older than archaeocyathid or trilobite-bearing Cambrian. Thus, shelled brachiopods could be the oldest animal fossils yet known, the newly discovered obolids from arctic Canada having been uncertainly argued, from radiometric evidence, to be about 700 m.y. old. The degree of (? explosive) evolution of the known Lower Cambrian fauna was then examined in relation to various hypotheses.

In discussion, it was pointed out by P. C. Sylvester-Bradley that a distinction should be made between complex organic chemicals preserved in ancient rocks, which might be abiogenic, and relics of organized life. It was argued by R. Goldring that trace fossils (except, for example, burrows) were commonly formed at the sediment–water interface, which itself might not be generally preserved. This would account for the discrepancy between ranges of fossils and traces of presumed similar animals. Even so, there was a marked increase in traces of biological activity with the appearance of trilobites.

A computer analysis of the fossil record was presented by B. M. Funnell and J. L. Cutbill. The committee had decided that the large amount of data to be assembled according to a common pattern justified some attempt at computer analysis. It was only possible to report at the meeting results based on the unedited charts already submitted; however, 1,566 taxa from these were used, and it seemed unlikely that later submissions would alter the results in any substantial way. Taxonomic rank depends perhaps as much on the history of palaeontological investigations as on any biological hierarchy, and therefore no particular attempt was made to correct according to their systematic rank the taxa which had been selected by contributors, except that in three cases where large numbers of families had been documented in a limited part of the stratigraphical column these were reduced to super-families. Errors affecting the ranges of taxa were inevitable, not only because of the many limitations of the fossil record, but also as the result of the systematic attribution of fossils and the history and geography of palaeontological research. A significant bias was inevitably introduced by the fact that living representatives of taxa, rather than fossils, had been documented for the latest stratigraphical unit, thus heavily over-weighting this latest interval by comparison with all other intervals, which depended solely on the fossil record. For analyses of ranges standard units were taken as equal, although many were known geochronologically to be unequal, and others were not sufficiently known. The known units averaged about 5–6 m.y. back to the Triassic, and 5–10 m.y. back to the Devonian, increasing to 15 or even 30 m.y. for the early Palaeozoic ages. The authors were under no illusions about the limitations of what could be obtained by numerical abstracting and presentation of the data (for example, in histograms, with successive smoothing), but nevertheless it was possible to draw some general conclusions from the tabulations presented. Frequencies of all taxa, initial or final records per stratal unit with major taxa taken separately or totalled, were not random; some trends were noticeable, reflecting rapid diversification in early Palaeozoic time and a distinct Palaeozoic mode, with a marked 'low' in the curves at about the Permian/Triassic boundary. The Mesozoic/Cenozoic boundary was not so distinctly marked.

Lower Palaeozoic appearances were discussed by M. R. House, who first commented on what the computer analyses had shown independently, namely, the genius of John Phillips 125 years ago in distinguishing the broad Palaeozoic, Mesozoic and Cenozoic divisions of life. House surveyed the progress and diversification of most major taxa through Palaeozoic time. In assessing claims for an evolutionary explosion it would be important to distinguish between taxonomic diversification and the numerical abundance of individuals within each taxon. However, an initial multiplication of genera to a high early maximum was followed by a slower decline. Moreover, early diversification was more rapid in higher ranks of taxa. Although rocks of Cambrian age have yielded records of most phyla, the Ordovician might well prove to be the time of most marked explosion and proliferation. The detailed pattern was probably controlled ecologically, and might have been markedly affected by the feeding habits of different groups.

By reference to the *Dalmanellacea*, V. G. Walmsley showed how relatively minor and continuous taxonomic revision could produce major changes in range charts. But, as pointed out by B. M. Funnell, when many records are combined small changes will be self-cancelling and so will not seriously affect the kind of statistical study conducted.

Permo–Triassic changes were discussed in a paper by F. H. T. Rhodes (read by M. J. S. Rudwick), who argued first that the Permian was a time of rich and diversified faunas, even those that were shortly to become extinct; thus there was little prior indication of impending changes. Although substantial extinctions took place towards the end of Permian time, the noticeable effect was due to many gaps not being immediately filled, so that early Triassic faunas lacked variety and contrast. Certainly, although none of the many hypotheses for these changes has yet been substantiated, and few continuous marine successions across the boundary are known, the marked change in the fossil record does not appear to be an artefact and demands explanation.

Triassic/Jurassic vertebrate changes were discussed by C. B. Cox, who showed that the most fundamental changes on land took place in Triassic time, though correlation of some of the continental strata is uncertain. Extensive adaptation to marine conditions was a distinctive feature of Triassic time. This pattern gave some coherence to the Mesozoic era so far as vertebrates were concerned.

Cretaceous–Tertiary marine faunal changes were discussed by J. M. Hancock, who limited himself to a careful study of the commonly accepted dramatic extinction of ammonites and possible continuation of belemnites. Decline was already evident in Campanian time and, indeed, gradual extinction was also taking place in Maestrichtian time, although world-wide regression at the close of Maestrichtian time obscured the record. Many important occurrences have been recorded recently from Danian and Upper Palaeocene rocks so that changes, while gradual in detail, were yet distinctive when viewed on a sufficiently broad scale.

G. M. Phillip commented on the record of the Echinoids, which evolved slowly through Ordovician to Triassic time, with about one new family per period, and then dramatically diversified in Lower Lias time, with new families continuing to appear at about 15 times that rate to the present day.

Plant–insect relationships were next discussed. The apparent dramatic changes in the insect record are largely attributed to the relatively few rich fossil insect horizons, but some established stages of insect evolution were nevertheless outlined by J. M. Smart. A number of suggestions were made by N. F. Hughes about plant evolution and its possible relationship to insect development. Most insects depend directly on plants for food, and the suggested later Cretaceous development of angiosperms may not have had much impact on insect evolution because the pre-angiosperms (pteridophytes and gymno-

sperms) provided a similar habitat when equatorial. A critical stage may have been reached with the emergence of tall land plants in Devonian time; insects would have crawled up these plants and developed flight. The important diversification of reproductive structures in plants in early Carboniferous time was probably a protective response to attack by insects, which themselves became adapted in diverse manners. Insect pollination could have begun in Permian time.

The last principal contribution, biochemical evolution and the fossil record, by L. B. Tarlo, reviewed a rapidly expanding field of investigation, largely consequent on recently developed techniques using amino-acid analysis. Comparative biochemistry has long provided some independent taxonomic criteria, but only recently has the systematic investigation of the biochemical record of the rocks been stimulated by the possibilities of protein taxonomy. With what reliability particular combinations of amino-acids may be used to infer the presence of, for example, collagen is still uncertain, but this line of investigation promises well. It is complicated by the discovery that not only do many fossils yield rich biochemical results, but so do their enclosing rocks; hence the source of any particular compound is often difficult to determine.

A. Hallam, from studies of strontium/calcium ratios in cephalopod shells, suggested that later Palaeozoic nautiloids may be related to more 'primitive' forms than goniatites.

Throughout the discussion the basic questions of evolutionary cause and effect recurred, often inconclusively. Linguistic care was necessary to avoid 'loaded' descriptions such as one form 'ousting' another from its 'ecological niche'. But not all was negative. Some correlation between changes due to physical environment and those due to selective pressure for evolutionary adaptation seemed reasonable. If this were pressed in terms of what is becoming known about the inevitable interplay of tectonic, eustatic, gradational and climatic changes, for example, then the evident rate of biological change may be partly explicable.

It is hoped that the whole symposium and volume will stimulate palaeontologists and others to extend this study to progressively lower levels of taxa as a contribution to the record of evolution of the biosphere as a whole.

W. B. Harland

CALEDONIAN MAGMATISM IN NORTH WALES

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In a survey of the British Caledonides, Read[1] emphasized the need for more information on the many aspects of Caledonian magmatism in Britain. He briefly discussed the few available isotopic ages for the Caledonian granites of the 'metamorphic' and 'non-metamorphic' belts, but pointed out that further determinations were clearly required. More recently, Fitch *et al.*[2] have published geochronological data relating to magmatic activity within the 'non-metamorphic Caledonides' of North Wales, and have reviewed aspects of Caledonian orogeny and metamorphism in Britain[3,4]. This article reports the results of isotopic age determinations on biotites from hornfelses associated with the Tan-y-grisiau 'microgranite', Ffestiniog, North Wales, and briefly discusses their significance in investigations of Caledonian magmatism.

Several authors[5–10] have made reference to the Tan-y-grisiau intrusion, but little detailed work has been published concerning its petrology, form and age. Recently, one of us (J. E. T.) has made a petrological and petrochemical examination of the intrusion and its aureole. During this work biotite-bearing metamorphic rocks were collected from the south-east margin of the intrusion (350 yd. west of Brynmelyn cottage, *SH*687430) which have since proved suitable for age determinations by the potassium–argon and the rubidium–strontium methods. The samples are representative of two rock types: a biotite-rich contact hornfels and a biotite-bearing xenolithic rock included in granite veins penetrating the country rock. Biotites in the granite itself were examined in thin-section but were found to be extensively chloritized and unsuitable for age determinations. The dating of an intrusion solely by means of measurements on associated hornfelses does not appear to have been reported previously, although Erickson and Kulp[11] and Hurley *et al.*[12] have given data for fine-grained hornfelses, which agreed well with results from associated igneous mica. Whole-rock determinations on the granite itself are in hand.

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Potassium–argon determinations were made at Oxford, where a Franz isodynamic separator was used for mineral separation. Argon was determined on a Reynolds mass spectrometer using an isotope dilution technique with argon-38 tracer. Potassium was determined by flame photometry. The analytical precision for both elements is estimated as ±2 per cent from replicate determinations. The uncertainties in the ages given in Table 1 are based on this figure, giving a precision of ±3 per cent in the Ar/K ratio. The rubidium–strontium determinations were done at Pisa. The biotite sample used for the determination on the contact hornfels was taken from a separation done at Oxford, part of which had already been used for duplicate potassium–argon determinations. The xenolithic biotite was separated at Pisa using a Franz separator. A selected sample was further purified by grinding under alcohol and resieving. The rubidium and strontium contents of the biotite were determined by an isotope dilution method using an Atlas *CH*4 mass spectrometer with normal collector. The analytical results are given in Table 2.

The 'uncorrected ages' in Table 2 are based on an assumed normal $^{87}Sr/^{86}Sr$ ratio of 0·712 at the time of metamorphism. Because of the difference between these and potassium–argon ages, it was decided to make some 'whole-

Table 1. Potassium–Argon Data

Sample No. and description	K (per cent)	^{40}Ar (p.p.m.)	Atm Ar / Total Ar (per cent)	Age (m.y.)
JET/1 Contact-hornfels (biotite)	5·54	0·117	4·6	398 ± 11
	5·54	0·180	3·3	409 ± 11
			mean	404 ± 8
JET/2 Xenolith (biotite)	5·58	0·183(5)	4·7	413 ± 11
JET/3 Dolerite (whole-rock)	1·75	0·363	4·4	314 ± 9

Decay constants: $\lambda_e = 0.584 \times 10^{-10}$ yr.$^{-1}$; $\lambda_\beta = 4.72 \times 10^{-10}$ yr.$^{-1}$.
Isotopic abundance of ^{40}K = 0·0119 atomic per cent.

Table 2. RUBIDIUM–STRONTIUM DATA

Sample No. and description	Rb (p.p.m.)	Com.Sr (p.p.m.)	^{87}Sr rad (p.p.m.)	^{87}Sr rad / ^{87}Sr tot	Age uncorrected (m.y.)	Age corrected (m.y.)
JET/1 Contact-hornfels (biotite)	502 ± 8	24·7 ± 0·4	0·889 ± 0·035	0·34	424 ± 21	412
JET/2 Xenolith (biotite)	327 ± 6	48·9 ± 0·8	0·590 ± 0·041	0·15	433 ± 30	429

$t_{\frac{1}{2}} = 4{\cdot}7 \times 10^{10}$ yr.

rock' rubidium–strontium measurements on the hornfelses. The method by which such measurements can be used to give a calculated age which takes account of variation in isotopic composition of strontium at the date of metamorphism has been demonstrated for the Moine Series by Long and Lambert[13].

For *JET*/1 the whole-rock measurements gave the ratios: total Rb/Sr = 0·8 by weight, $^{87}Sr/^{86}Sr = 0{\cdot}737$ (atomic ratio) and a corrected biotite age of 412 m.y. For *JET*/2 the corresponding figures were 1·04, 0·732 and 429 m.y. This latter apparently discrepant age may be attributable to lack of uniformity of composition in sample *JET*/2. It was not possible to make the whole-rock determinations on exactly the same sample from which the biotites had been separated.

The data support 408 ± 7 m.y. as the date of metamorphism of the hornfelses, which on the simplest interpretation would be the date of emplacement of the granite. However, a long period of cooling would mean that these ages were younger than the date of emplacement. Alternatively, they could be the result of a post-hornfelsing thermal event in the history of the rocks. In the absence of evidence in support of the latter two possibilities we shall, for the present, consider the simplest interpretation to be the correct one.

Isotopic dating of a magmatic episode is of greatest value when the age of the magmatism is known relative to other events comprising the geological history of an area. In discussing the relative age of the acid magmatism represented by the Tan-y-grisiau intrusion, reference should be made to the following events in the structural evolution of North Wales: emplacement of basic magma (as represented by ophitic augite-dolerite sills and dykes); the impression of the regional cleavage(s); folding; faulting and crushing; and mineralization. A brief summary is given here of the available information which is relevant to such a discussion.

The Cambrian and Ordovician strata of Caernarvonshire and Merionethshire have been intruded by an immense bulk of basic magma in the form of dykes and sills[8], the majority of which are ophitic augite-dolerites. Although many basic intrusions of Ordovician age are known to occur, for example the intrusive sheets of Cader Idris (pre-Upper Ordovician)[14], most of the dykes and sills are thought to be late Caledonian[8,9]. Recent field-work has shown that the Tan-y-grisiau intrusion is cut by several of such dykes, and, therefore, pre-dates the episode of basic magmatism represented by them. Some of these dykes have been affected by subsequent shear movements while others are only slightly sheared. (A whole-rock potassium–argon determination has been made on such a dyke, see Table 1.) The previously held view[8] that the Tan-y-grisiau granite "must be appreciably younger than the dolerites" has to be reconsidered. If there were but one episode of late Caledonian injection of basic magma (namely, that which produced the dykes cutting the granite), then this view is incorrect. If more than one phase is envisaged, then the Tan-y-grisiau intrusion pre-dates at least one of them. It is interesting to note that Caledonian microgranophyres cut by late Caledonian ophitic dolerites have been reported from the Moel Hebog area[9]. The recognition of post-granite dolerite injection means that Read's[1] catalogue of events for the "non-metamorphic Caledonides" must be modified. Further modifications seem likely in the light of recent discussion[4].

There is no general agreement regarding the age of the late Caledonian dolerites relative to the impression of the regional cleavage. Both pre-cleavage[6,9,14,15] and post-cleavage[7,16,17] ophitic dolerites have been described from Caernarvonshire and Merionethshire. Consequently, it is not feasible to establish the date of emplacement of the Tan-y-grisiau intrusion relative to the impression of the regional cleavage by reference to dolerite/cleavage age relations. Furthermore, the recognition of polyphase folding in the Caledonides of North Wales[18], each phase accompanied by the development of cleavage, underlines the need for restraint in interpreting records of cleavage/dolerite age relations until the chronology of the regional structure has been fully determined. Preliminary work by C. T. Harper at Oxford (personal communication) suggests that the cleavage associated with F_1 folding was impressed 415 m.y. ago. This compares with an average age of 410 ± 10 m.y. quoted for a cleavage event which affected rocks in Anglesey and Arvon[4].

More direct evidence concerning the date of emplacement of the intrusion has been noted by several authors. It has been tentatively suggested[5] that emplacement post-dated the cleaving of the surrounding Tremadoc rocks, as "angular cleaved fragments" of the latter were said to lie in offshoots from the intrusion. However, an examination (by J. E. T.) of localities where such fragments show bedding and cleavage suggests that the cleavage may well be related to pre-intrusion faulting and not to the regional cleavage. In the spotted Tremadoc slates of the south-west part of the Tan-y-grisiau aureole the spots are said to have grown in slate which was already cleaved[7], implying that the intrusion is younger than the regional cleavage. The occurrence of distorted fossils in massive hornstones close to the margin of the intrusion has been cited in support of this view[17]. However, observations on spotted rocks near the margin of the granite suggest that the intrusion pre-dates the regional cleavage[20]. More work is obviously needed to resolve these conflicting views.

The date of emplacement of the granite relative to the regional thrusting, which produced the zone of shear disturbances running from the Tremadoc area into Deudraeth, has been demonstrated in the area a mile and a half south-west of the main outcrop of the granite[7]. Here the spotted aureole rocks have been disrupted in a zone of minor thrusting which is later than the formation of the spots. Hence the thrusting post-dates the emplacement of the intrusion. This has been confirmed by observations made in an area a quarter of a mile south of the granite margin[20]. It has been stated that the augite-dolerite sills of Deudraeth post-date the regional thrusting[7]. If so, they must post-date the Tan-y-grisiau intrusion and confirm the observation that there was injection of basic magma later than the acid magmatism which produced the Tan-y-grisiau mass.

Research in the Aberglaslyn area[10] has shown that an inferred concealed intrusion "produced local thermal metamorphism and tourmalinization of the early Caradoc sediments in post-Llarnagian and pre-Longvillian times". If, as Fearnsides[17] and Shackleton[9] have suggested, this thermal metamorphism is attributable to a hidden extension of the Tan-y-grisiau granite, then the intrusion is of Caradocian age, that is of the same general age as the intrusive rhyolites of Moel Hebog[9]. Indeed, Beavon[10] has suggested that the Tan-y-grisiau intrusion "is a sub-volcanic granophyre related to the Snowdon Volcanic Series, rather than a Caledonian 'granite'". However, there is no direct evidence for relating the Aberglaslyn thermal metamorphism to the Tan-y-grisiau intrusion. Dating of the Bwich-y-Cywion intrusion would be of interest for comparison.

Until recently the mineralization associated with the Cambrian and Ordovician rocks of the Harlech Dome was thought to be of either Ordovician[21] or post-Carboniferous[22]

age, although part of it has been regarded as Caledonian[23]. Moorbath[24], however, on the basis of investigations of lead isotope abundance, suggested a probable Silurian age for the mineralization (mean model age of 430 ± 40 m.y.). If the lead dates are accepted the Tan-y-grisiau intrusion post-dates the mineralization.

The data given in Tables 1 and 2 suggest that the age of the Tan-y-grisiau intrusion is slightly, but not significantly, greater than the ages of the "Caledonian Newer Granites" given by Lambert and Mills[25]. The statement of Fitch *et al.*[2] that the principal Caledonian magmatism in North Wales occurred some 400–420 million years ago is supported by the data presented here. Although Beavon's contention that the age of the intrusion is Caradocian[10] cannot be conclusively refuted, the possibility of a late Silurian age must still be borne in mind, especially as a minimum age of 445 m.y. has recently been recorded for alleged Lower Caradocian volcanic rocks in Scotland[26].

The single apparent age of 314 ± 9 m.y. for the dolerite is anomalous and, since the rock is slightly sheared, is possibly the result of argon loss. However, it does confirm the view, based on the absence of olivine in the rock, that the dykes are unrelated to the early Tertiary dykes of Snowdonia.

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STEREOCHEMICAL RELATIONSHIP BETWEEN CODING TRIPLETS AND AMINO-ACIDS

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BASED on the genetic code as published by Nirenberg *et al.*[1], the following correlations between codons and amino-acids were deduced[2]: the amino-acids with hydrocarbon residues have U (uridine) or C (cytosine) as the second base, those with branched methyl groups have U as the second base; the basic and acidic amino-acids have A (adenine) or G (guanine) as the second base; the aromatic amino-acids and amino-acids derived from a common organic acid are grouped together; a simple structural change in an amino-acid is accompanied by a change of one base only, such that 16 out of 20 amino-acids are interconnected (Fig. 1); if the third base in a triplet influences the coding, it does so only by being either a purine or a pyrimidine. It seems unlikely that such a logical arrangement could have arisen by chance, and it was suggested that a structural relationship between coding triplets and amino-acids exists. Similar suggestions were made by Nirenberg and his collaborators[1], Weinstein[3] and Woese[4].

The hypothesis of a stereochemical relationship between nucleotide triplets and amino-acids was tested by constructing models: this article reports some results of this investigation.

Models were built using Courtaulds space-filling components in the usual way. Triplets were made up as if they were part of an RNA-chain, comprising bases, ribose sugar and phosphate bonds in the 3′-5′ sequence. Full flexibility of the RNA within the limits given by the models was assumed. Amino-acid molecules were assembled separately, and then fitted into the triplets by means of hydrogen bonds, stacking bonds and covalent bonds. Hydrogen bonds were only accepted when they were within the distances and angles defined by the components. Stacking of the heterocyclic structures was used when they were in contact or nearly so, and when they overlapped to within approximately 2 Å. Amino-acids with hydrocarbon residues possess no sites for the formation of hydrogen bonds on the hydrophobic residue. It was assumed that by removing hydrogen from a CH_3 group on the residue and from a base, a covalent bond between residue and base could be formed. For example, after the bonding of a terminal methyl group on leucine to uracil in this way, uracil resembles thymine. Methyl bonding is referred to in more detail in the discussion.

Possible bonds to the ribose sugar or phosphate link were not used. The —OH group of the carboxyl group

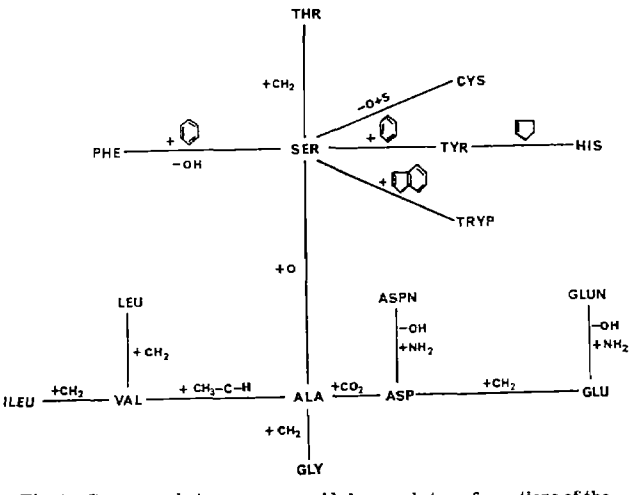


Fig. 1 Connexion between amino-acids by simple transformations of the genetic code. Each line marks a change in the structure of an amino-acid accompanied by a change of one letter of the code. For example, the structural difference between Gly (GGU) and Ala (GCU) is due to one CH_2 group. Note that biochemical relationships are different

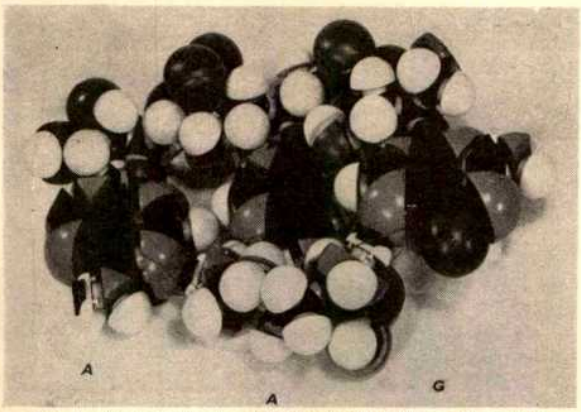


Fig. 2. Photograph of model of lysine fitted to model of AAG

on the head of the amino-acid was left free, since it is known that this provides the link with the *t*RNA during transfer as well as participating in the peptide linkage.

We started with the assumption that the specificity of the third base might be low because it codes for a part common to all amino-acid molecules, namely, the $H_2N.CH.COOH$ group; the residue was then fitted to the first and second bases. An assembly was considered satisfactory when at least four possible bonds, of which at least two must be with the residue, could be effected without strain and without undue distortion of the triplets. The head of an amino-acid can always be fitted with hydrogen bonds to one or two bases. To provide specificity additional bonds to the residue are required.

In the following examples of amino-acids fitted into triplets, the diagrammatic representation of the arrangement is distorted due to the transfer of a three-dimensional pattern to two dimensions; in the models all bonds are at permissible angles and distances.

Amino-acids with acidic or basic residues could be fitted by hydrogen bonds alone to their codons in a satisfactory manner. The fitting of other amino-acids was more complicated.

Lysine. Forms four hydrogen bonds with its codons AAG (Fig. 2) and AAA. The first two bases AA— are planar, with the third base stacked above the second.

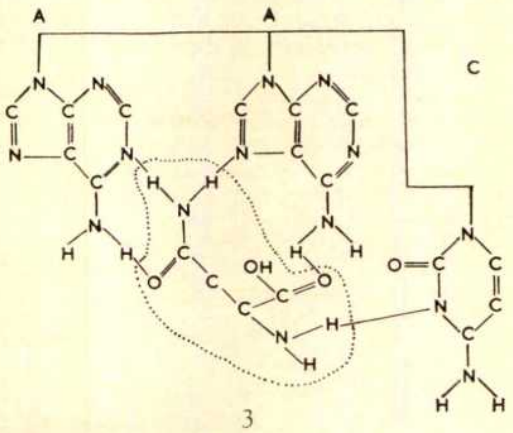
Asparagine. Can form five hydrogen bonds with its codons, AAU (Fig. 3), and AAC.

Tyrosine. Can be fitted into UAU (Fig. 4) and UAC using three hydrogen bonds and one stacking bond. The amino-acid lies above the central base (A), the three bases lying approximately in the same plane.

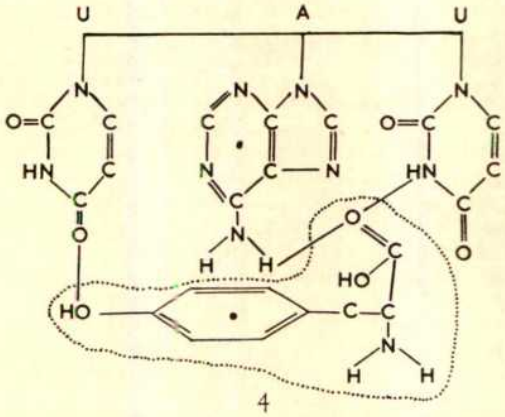
Leucine. Since no sites for hydrogen bonding are available on the residue, this can be fitted with hydrogen bonds to the head only, and by shielding the hydrophobic residue of the amino-acid in a fairly satisfactory way to CUA and CUC and less satisfactorily to UUG. Leucine can also be fitted in ways which are strained or otherwise unsatisfactory to UUC and UUU. This shielding of the residue using Courtaulds models is a very subjective criterion of a fit.

If it is assumed that terminal methyl groups can bond to bases (Fig. 5), leucine can be fitted in a planar arrangement to UUG, CUG, CUU, CUC, CUA and UUA. Thus leucine fits the triplets of the genetic code in a manner which can be clearly defined.

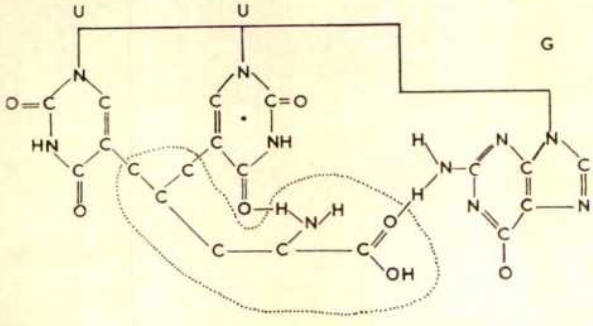
Methionine. Will form a planar arrangement with four hydrogen bonds, two of which are to the sulphur atom with AUG and AUA. If it is assumed that terminal methyl groups can bond to a base (see later) methionine



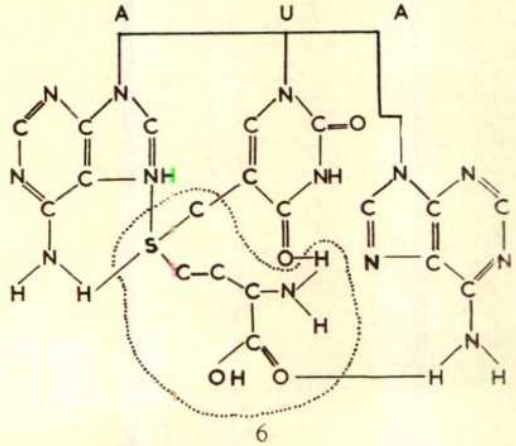
3



4



5



6

Figs. 3–6. Diagrammatic representations of models. The distortion of some bonds is due to the transfer of a three-dimensional to a two-dimensional pattern. Note that in all cases the =O of the head of the amino-acid is hydrogen-bonded to the third base, the hydroxyl group is free. Fig. 3. Asparagine-AAC. Fig. 4. Tyrosine-UAU. Note the stacking bond (0). Fig. 5. Leucine-UUG. Note the bonding of the two terminal methyl groups. Fig. 6. Methionine-AUA. Note the two hydrogen bonds to S

Table 1. Survey of amino-acids fitted to codons

AA	Codons				Bonds H	Bonds π	Bonds M	Remarks
ala	GCU	GCC	GCA	GCG	2	0	1	
arg	CGU+	CGC	CGA	CGG	5	0	0	
	AGA	(s)	AGG	(s)				
asp	GAU	GAC			4	0	0	
asp N	AAU	AAC			5	0	0	
cys	UGU	UGC			4	0	0	
glu	GAA	GAG			5	0	0	
gly	GGU	GGC	GGA	GGG	3	0	0	+2 possible to chain
his	CAU	CAC			4	1	0	
leu	UUA	UUG	CUU	CUC	2	0	2	
	CUA	CUG						
lys	AAA	AAG			4	0	0	
ileu	AUU	AUC			2	0	2	
met	AUA	AUG			4	0	1	
phe	UUU	UUC			2	1	0	
pro	CCU	CCC	CCA	CCG	2	1	0	Uncertain
ser	UCU	UCC	UCG	UCA+	3	0	1	
	AGU+	AGC+						
thr	ACU	ACC	ACA	ACG	3	0	1	
tryp	UGA	UGG			3	1	1	
tyr	UAU	UAC			3	1	0	
val	GUU	GUC	GUA	GUG (s)	2	0	2	

Codons as assigned by Nirenberg *et al.* (ref. 1), marked +, could not be fitted; (s) strained fit.
Bonds; number of H, hydrogen bonds; π, stacking bonds; M, methyl bonds (for ser a similar bond was used).

will form four hydrogen bonds and one such methyl bond, the methyl bond being to the second base, with AUA (Fig. 6) and in a strained model with AUG.

Other amino-acids. The results of attempts to build models of the possible combinations of other amino-acids to triplets assigned to them in the genetic code are shown in Table 1. A more extensive investigation into other possible fits and possible specificity of the system will be reported separately.

If utilizing the methyl bond is accepted, only four combinations of codon and amino-acid cannot be effected, three of which involve uncertain placings in the code, namely, ser-UCA, AGU, AGC and arg-CGU (ref. 5). Combinations regarded as strained are doubtful since the parameters of the components used cannot be assumed to represent the dimensions of molecules with perfect accuracy. There is thus good, though not perfect, agreement with the genetic code, so far as the criterion for a fit is concerned.

Except for the smallest amino-acids all satisfactory models were built by fitting first the $H_2N.CH.COOH$ group, the head of the amino-acid, to the third base of the triplet; attempts to do otherwise ended in failure. Since, as is shown in Table 1, most amino-acids can be successfully fitted to the respective codons, it can be concluded that this is the correct way to approach the problem. So far as these models are concerned the angle sub-tended between the base and the rest of the RNA forms the difference between purines and pyrimidines in aligning the residue for bonding to the other bases. It can be concluded that the limited specificity of the third base can be explained by these findings.

The residue can be fitted to the other bases by hydrogen bonds, stacking or by means of a terminal methyl group. The restraints within a system are therefore given (*a*) by the number of possible bonds which is a function of suitable configurations on the amino-acid residue and on the bases, and (*b*) by the spatial relationship of the atoms suitable to form the bonds. The substitution of pyrimidine for purine in the third base does not significantly change the number of possible bonds between the amino-acid and the bases, but the spatial relationship can be altered in such a way that the amino-acid will not successfully fit some combinations.

No sites for possible hydrogen bonds are available on the residues of some amino-acids. In a few instances good fits were obtained when the bases formed a cavity enveloping the residue and protecting it from contact with water, but the arrangement cannot be considered satisfactory until forces to effect and to stabilize such a system are defined. It was assumed that terminal methyl groups on an amino-acid residue could be bonded to a base. The formation of such bonds, which change bases in such a way that they resemble methylated bases, would require an enzyme. Methylated bases in *t*RNA and the required enzymes (*t*RNA methylases) have been found[6,7]; it seems possible that the function of these enzymes is connected with the assembly of complexes of amino-acids and RNA. Utilizing this principle, more satisfactory assemblies were constructed.

The small amino-acids, glycine and alanine, follow a genuine double code because only two bases are bound to the amino-acid; other amino-acids, such as threonine, follow a code which is only double in appearance, since all three bases are bound to the amino-acid, but the third base is non-specific because the difference in angle between purine and pyrimidine is insufficient to make attachment impossible.

It can be concluded that it is possible to fit amino-acids stereochemically to their codons and that the low specificity of the third base can be explained by our findings.

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SPECIFICITY OF THE STEREOCHEMICAL RELATIONSHIP BETWEEN RIBONUCLEIC ACID-TRIPLETS AND AMINO-ACIDS

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In the preceding article the stereochemical fitting of amino-acids to the codons ascribed to them by Nirenberg *et al.*[1] was reported. The possible existence of structural relationships between amino-acids and their coding triplets had already been suggested by Nirenberg *et al.*[1], Weinstein[2] and Woese[3], and the correlations between amino-acids and their coding bases have been established by Pelc[4].

We used Courtaulds space-filling components to construct amino-acid molecules and sections of RNA chain complete with bases. The amino-acids were then stereochemically fitted to the base triplets. Except for glycine and alanine, the two smallest amino-acids, the head of the amino-acid, $NH_2.CH.COOH$, is always bonded to the third base, and the similarity of the hydrogen bonds used explains the low specificity of the third base. In fitting the amino-acids to their codons we realized that changing a purine for a pyrimidine, or vice versa, as third base, did not alter the possible bonds, but did alter the alignment of the amino-acid with the other two bases. In fact the bonds made with the third base orientate the amino-acid relative to the RNA chain. The amino-acid is then fitted to the other two bases using hydrogen bonds, stacking bonds, and methyl bonds (see preceding article for further details).

Models of 90 per cent of the codon-amino-acid complexes required by the genetic code could be achieved without introducing undue strain. The present article reports

Table 1. SURVEY OF AMINO-ACIDS FITTED TO TRIPLETS

AA	Codons	Other good fits	Strained	Imposs.	Not accounted
arg	CGU*, CGC, CGA, CGG, AGA, AGG	3	1 (+2?)	48	6
asp	GAC, GAU	0	0	32	30
aspN	AAU, AAC	2	7	36	17
gluN	CAA, CAG	4	0	20	38
his	CAU, CAC	(0)	(0)	(62)	(0)
leu 1	UUA*, UUG*, CUU*, CUC, CUA, CUG*	(0)	(4)	(49)	(9)
leu 2	UUA, UUG, CUU, CUC, CUA, CUG	2	0	56	0
lys	AAA, AAG	1	3	37	21
met 1	AUA, AUG	(12)	(2)	(40)	(8)
met 2	AUA, AUG	0	1	58	3
ser	UCU, UCC, UCA*, UCG, AGU*, AGC*	3	3	52	0
thr	ACC, ACU, ACA, ACG	0	8	40	12
tyr	UAC, UAU	(0)	(0)	(52)	(10)
val	GUC, GUU, GUA, GUG	4	40	16	0
10	36 (4*)	19	67	395	123
% of 640	5·0 fitted, 0·6 not fitted	3·0	10·0	62·0	19·3
% of 517	6·2 fitted, 0·8 not fitted	3·7	14·0	76·0	

Total No. of triplets for 10 amino-acids is 640, of which 517 were tried.
leu 2 and met 2: models were constructed utilizing methyl bonds. leu 1 and met 1: constructed without methyl bonds. Codons: as assigned by Nirenberg *et al.* (ref. 1). Codons marked with an asterisk (*) could not be fitted; all others were good fits with no strain. Strained and impossible: some uncertainty is attached to these placings since, if standards of accuracy are relaxed, some combinations which we deemed impossible might be regarded as strained or even good fits; furthermore, the limitations imposed by the standardized sizes and angles of the components have to be borne in mind.
Not accounted: the number of combinations that were not tried at all or not sufficiently tried to justify assignment. Per cent of 640: percentages relating to all possible combinations; since there are 64 triplets and 10 amino-acids were examined in sufficient detail, there exist 640 possibilities. Per cent of 517: percentages relating to all combinations which were examined, that is (640—not accounted). Figures with question mark (?) are uncertain placements.
All figures in parentheses were disregarded for statistical evaluation—leu 1 and met 1 because the use of methyl bonds gives better agreement with experiments, the aromatic amino-acids his and tyr because of the uncertain specificity of the stacking bonds.

results of further investigations on the specificity of these stereochemical fits. For this purpose a triplet was built up and the head of the amino-acid was bonded to the third base. Fitting of the amino-acid to the first and second bases was then attempted. The head of an amino-acid can always be fitted to one or two bases and will make at least two hydrogen bonds with them; this bonding is therefore non-specific. An assembly was considered satisfactory when the residue formed at least two bonds with the triplet so that, including the bonds to the head, a minimum of four bonds between amino-acid and triplet is achieved without undue strain or distortion of the molecules. The larger amino-acids have more possible binding sites.

Triplets marked with an asterisk (*) are those found in the genetic code[1].

Asparagine. Can form five hydrogen bonds with a suitable triplet. It was found that neither a pyrimidine nor G can be the second letter; the last letter is always a pyrimidine. U, and probably G, cannot be the first letter. Satisfactory arrangements with five hydrogen bonds can be formed with AAU*, AAC*, CAC and CAU.

If the hydroxyl group is utilized for hydrogen bonding, asparagine can also be fitted to the following triplets: AGC, AGU, CGC, CGU and AGA. We consider such an arrangement unsuitable because then the —OH group would not be available for peptide forming or enzyme–ATP binding.

Methionine. Will form a planar arrangement with four hydrogen bonds, two of which are to the sulphur atom with AUG* and AUA*. If it is assumed that terminal methyl groups can bond to a base (see preceding article and later in this article) methionine will form four hydrogen bonds and one such methyl bond in a satisfactory arrangement with AUA* and in a strained configuration with AUG* and AUC.

Leucine. Since no sites for hydrogen bonding are available on the residue, this can be fitted with hydrogen bonds to the head only, and by shielding of the hydrocarbon residue of the amino-acid by the bases, to CUA* and CUC* which are satisfactory, and to UUG* which is less so. Leucine can also be fitted in ways which are strained or otherwise unsatisfactory to GUG, UUC and UUU. It can be fitted neither to CUU*, CUG*, UUA*, nor to any triplets with CC— as the first two bases, or triplets with G or A as the second base. However, these fits involving shielding of the residue tend to be very subjective.

If it is assumed that terminal methyl groups can bond to bases, leucine can be fitted in a planar arrangement to UUG*, CUG*, CUA*, CUU*, CUC*, UUA*, UUU and UUC. It will be seen that in this case the fitting of leucine agrees with the genetic code and, since leucine is bound to ribosomes with UUU and UUC as 'messenger' in biochemical experiments, with the experimental evidence as well.

Other amino-acids. The results of attempts to build models of the possible combination of other amino-acids to triplets are shown in Table 1.

Out of ten amino-acids (Table 1), if methyl bonding to the base can be assumed, methionine is specific and leucine agrees with the incorporation experiments, although not entirely with the genetic code. Of the nineteen combinations shown in the column 'Other good fits', six are to be regarded as possible if incorporation in biochemical experiments, rather than inclusion in the code, is regarded as a criterion. The combinations mentioned in the column 'strained fits' have to be regarded as doubtful since the components used cannot be assumed to represent the parameters of the molecules with perfect accuracy.

The ten amino-acids examined in detail could *a priori* have been expected to fit into $10 \times 64 = 640$ possible triplets. Of these 517 were tested and it was found that 395 plus 67 strained ones, or 76·0 per cent plus 14 per cent, respectively, could not be fitted. Of the 8 per cent which could be fitted, approximately 5 per cent agree with the genetic code of Nirenberg *et al.*[1]. In Table 2 are listed seventeen triplets which stimulated incorporation of amino-acids but were not accepted for the genetic code by Nirenberg and his collaborators[1,5]. Of thirteen that were tried, eleven, that is, 85 per cent, could be fitted, which cannot be due to chance since on the large sample 90 per cent could not be fitted.

Our results indicate a high degree of specificity in bonding amino-acids to codons, very good agreement with the experimental findings of Nirenberg and his collaborators, and good though not perfect agreement with the genetic code. The placing of arginine and serine in the genetic code is not certain[6]. There is thus the possibility of even better agreement between the models and the genetic code.

The way in which our model system can explain the limited specificity of the third base has already been discussed in the preceding article. For example, lysine will fit equally well into AAA* or AAG*, but not AAU or AAC, the pyrimidine molecules being too small to effect the necessary bonds in this case as third base.

It can be concluded from the results in the preceding article that amino-acids can be fitted to codons and from the results summarized in Table 1 that the system provides some specificity. In view of the almost full specificity of threonine and some impossible fits, a correlation between amino-acid and anti-codon can be excluded. No reliable criteria for stacking bonds are available, and therefore the high degree of specificity of the fits for histidine and tyrosine, based on an overlap of the heterocyclic rings within 2 Å, can only be provisionally accepted.

The specificity of our system is determined by three factors, the success of fitting of amino-acids into their codons, the possibility of fitting amino-acids to triplets other than codons and the impossible fits (Table 1). There is good correlation between the triplets listed under the heading 'Other good fits' and the experiments on the stimulation of amino-acid incorporation by synthetic

Table 2. Incorporation of more than 1·4 Times Background observed* but not accepted for the Genetic Code

Triplet	Amino-acid	Fitted
UGC	ala	+
CGC	ala†	+
AGC	ala	+
UAG	aspN	+
GAU	aspN‡	+
GAC	aspN‡	+
UGC	aspN	0
UGA	aspN	0
UGA	cys	−
AGU	cys	−
AGC	cys	−
AGA	glu	+
UGA	glu	+
UUU	leu	+
UUC	leu	+
GAA	lys	+
GCG	thr	−
17		11 +
		2 0
		4 −

Models: +, Can be fitted; 0, cannot be fitted; −, not tried.
* Nirenberg *et al.* (ref. 1), Brimacombe *et al.* (ref. 5).
† 1·3 times background.
‡ *t*RNA may have contained some ^{14}C-asp-*t*RNA (Nirenberg *et al.* (ref. 1)).

*m*RNA (Table 2). The biological significance of these combinations ultimately depends on the possibility and number of 'mistakes' in an amino-acid sequence in a given protein synthesized *in vivo*. Little evidence on this point is available and it would seem prudent to defer discussion. The combinations listed as impossible in Table 1 form the majority of all possibilities. A few of them might ultimately be found to be possible, especially if different rules for building models are accepted. However, we feel sure that in most cases no such possibility exists, since the relevant parameters are too different. A minor shift from impossible to strained or even good fits would not materially affect the conclusion that the system is of limited specificity and that the degree of specificity is very similar to that of the relevant biochemical experiments.

In view of the good correlation with experimental findings and the fact that Ingram and Sullivan[7] observed that some amino-acids were attached to *s*RNA by bonds other than amino-acyl ribose bonds, it seems unlikely that such complexes are not used in biological systems. This point is also supported by Weinstein's[2] argument that mutations of the genetic code would be expected in the absence of structural correlations between amino-acids and triplets; since the code is universal, that is, non-mutated, there must be a relationship which is still of importance.

In 1958 Crick[8], under the assumption that RNA could not form 'cavities' to accept amino-acids, suggested the existence of an adaptor system for the recognition of codon and amino-acid. In the combination of adaptive enzyme and *t*RNA, this adaptor was found. Two possibilities of utilizing the findings reported here for the system of protein synthesis can be visualized. (*a*) With the help of an adaptive enzyme an amino-acid molecule is fitted to, or recognized by, the codon on the *t*RNA and the anti-codon on the same later recognizes the codon on the *m*RNA. (*b*) The adaptor system is used to insert an amino-acid molecule into the respective codon on the *m*RNA and protein synthesis then proceeds along the *m*RNA attached to a ribosome. The former suggestion does not lead to any major changes in the adaptor system as visualized at present (for reviews see refs. 9 and 10). In its favour also is the fact that both a codon (GCG) as well as an anti-codon (CGC) for alanine can be found in the sequence of bases determined for an alanine-*t*RNA by Holley *et al.*[11].

The known binding of amino-acid molecules to the C—C—A terminal group of *t*RNA would suggest either that the codon on the RNA only aids in recognition or that in fact the amino-acid is fitted into the codon during transfer. In all successful models listed in Table 1 the —OH of the carboxyl group was not used for bonding to the bases and is therefore available for reactions.

In favour of the second possibility (*b*) is the consideration that the resulting system of protein synthesis would be more compact since ribosome, *m*RNA and the site of addition of amino-acids to the growing peptide would be near together. Bernfield and Nirenberg[12] found that nearly 100 per cent of ^{14}C-phe-*t*RNA was bound to ribosomes charged with poly-U. Since both the trinucleotides UUU and UUC were able to stimulate incorporation of ^{14}C-phe-*t*RNA this result suggests that the affinity of the codon is for the amino-acid and not for the *t*RNA. Experiments of Chapeville *et al.*[13] showed that, if cysteine while bound to *t*RNA is changed to alanine, the amino-acid will still bond to ribosomes with poly-UG as primer. Preliminary trials have shown that alanine, as might be expected from its size, follows a double code and can be fitted to UGC, CGC, AGC, and GGC as well as to the codons beginning with GC— assigned to it. This is in agreement with the experiments of Nirenberg *et al.*[1], who found that UGC, CGC and AGC stimulate the incorporation of alanine into ribosomes. Cysteine can be fitted to UGC, UGU, CGC and CGU. There is, therefore, an overlap and some similarity in the codons, and the results of Chapeville *et al.*[13] may not be so conclusive for the role of *t*RNA in protein synthesis as has been assumed. The available evidence is clearly contradictory. It seems more likely that a coding triplet on *t*RNA aids in recognition (assumption *a*), but a more direct role of the *m*RNA should not be altogether rejected.

The evolution of the genetic code may be reflected in the stereochemical relationship reported here. The structural sequences reported in the preceding article (Fig. 1) are in some instances similar to, and in others different from, the known biochemical and biosynthetic correlations, and a clear distinction between the evolution of amino-acids as chemicals and the evolution of a genetic code must be made. Our evidence rather suggests that the two are not coincidental.

It should be emphasized that, at this stage, our ideas are tentative and that the evidence from molecular model building which we present is in itself not conclusive.

Note added in proof. Recently, Hayashi and Miura (*Nature*, **209**, 376; 1966) found that the rate of formation of phenylalanyl-*t*RNA (phenylalanine code—UUU and UUC) was strongly reduced when oligo-A was added to the reaction mixture, but only slightly reduced when oligo-U, and not affected when oligo-C, were added. Similarly, oligo-U, but not oligo-A or oligo-C, strongly reduced the rate of formation of lysyl-*t*RNA; and oligo-G and slightly oligo-C, but not oligo-U or oligo-A, reduced the formation rate of prolyl-*t*RNA. Hayashi and Miura propose that the amino-acyl synthetase recognition site on the *t*RNA is the anti-codon for the respective amino-acid. We suggest that, if the site recognizing the amino-acid is the codon (our assumption (*a*)), the results can be explained as the formation on this site of Watson–Crick pairs with the oligo-nucleotide equivalent to the anti-codon, which would render recognition impossible. The weaker effects of oligo-nucleotides equivalent to the codons may be due to distortion of the *t*RNA molecules due to pairing of oligo-nucleotide and anti-codon, or to direct competition for the amino-acid.

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OPTICAL ROTATORY DISPERSION OF OLIGOADENYLIC ACIDS AND A CONSIDERATION OF THE FACTORS STABILIZING HELICAL POLYNUCLEOTIDES[1]

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SOME time ago we observed that when adenylic acid (AMP), the optical rotatory dispersion of which is similar to that of adenosine[2], is combined in a dinucleotide, there is a remarkable change of the optical rotatory dispersion[3]. Whereas AMP has only a single weak Cotton effect in the region of the long wave-length absorption band (λ_{max} 260 mμ), adenylyl (3′-5′)-adenosine (ApA) exhibits two peaks (at 277 and 235 mμ) and a deep trough (at 255 mμ). To investigate this effect further, we have examined the optical rotatory dispersion of a series of oligoadenylic acids in buffered salt solutions (0·15 molar) at *p*H 4·86, 6·75 and 7·4 and 20°–25° C. The preparation and purification of the compounds are described elsewhere[4].

Fig. 1 shows the optical rotatory dispersion of AMP, ApA, an oligoadenylic acid of average chain-length 12 (A_{12}), and polyadenylic acid (poly A, average chain-length 200) in potassium fluoride solution over the 200–300 mμ range at *p*H 6·75. The molecular rotation is in terms of mean residue concentration. The biggest change in the form of the curve takes place on going from the monomer to the dimer. The main features of the optical rotatory dispersion curve of ApA have been discussed in an elegant paper by Warshaw, Bush and Tinoco[5]. These authors calculated that the interaction between the two bases in ApA leads to a splitting of the 260 mμ absorption band of each adenine by 14 mμ. The two bands produced must have Cotton effects which are of opposite sign and of approximately equal magnitude. Considering the approximations involved in the calculation, the predicted positions of the two bands (253 mμ and 267 mμ) are in reasonable agreement with the experimental cross-over points of the optical rotatory dispersion curve (247 mμ and 267 mμ). Overlap between the two Cotton effects leads to the deep trough at 255 mμ. It thus appears that the observed optical rotatory dispersion curve consists of a first positive Cotton effect (peak 277, trough 255 mμ) and a second negative Cotton effect (trough 255, peak 235 mμ). There also appears to be a third positive Cotton effect with a peak at about 224 mμ (sometimes as an inflexion on the 235 mμ peak) and a trough at about 215 mμ. (These two extrema are not always observable and the figures are approximate.)

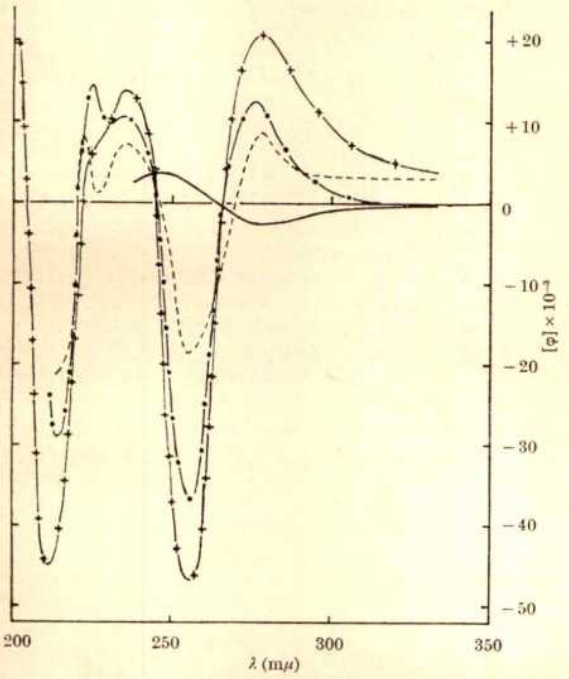


Fig. 1. Optical rotatory dispersion of AMP (———), ApA (– – – –), A_{12} (●—●) and poly A (+—+) in 0·15 M potassium fluoride, *p*H 6·75. [φ] = mean residue molecular rotation

Addition of further adenylic acid residues produces a gradual increase in base interaction which stabilizes the stacked, single-stranded form of polyadenylic acid now believed to exist at neutral *p*H[6–8]. Table 1 shows the increase in the amplitude of the first Cotton effect (first peak to first trough) with increasing chain-length. (The results at *p*H 7·4 were very similar to those at *p*H 6·75.) Investigations of circular dichroism indicate that base interaction at neutral *p*H reaches a maximum when the oligoadenylic acid chain contains about 30 residues[4]. In the case of the second Cotton effect (first trough to second peak) this gradual increase does not appear at all at *p*H 4·86, although at *p*H 6·75 it does for almost all chain-lengths.

No significant change in the position of the extrema or cross-over points with chain-length or *p*H was observed in our investigations, with one exception. This change is associated with a dramatic change in amplitude and secondary structure. Poly A at *p*H 4·86 has a trough at 251 mμ; this is significantly different from the value of 255 ± 1 mμ found in all our other measurements of this extremum. (There is a corresponding shift in the absorption maximum of poly A from 257 to 252 mμ (ref. 7).) The amplitude of the first Cotton effect at *p*H 4·86 increases gradually up to A_{12}, in a manner similar to that at neutral *p*H. However, whereas the increase in amplitude on going from A_{12} to poly A is less than 40 per cent at *p*H 6·75, it is more than 200 per cent at *p*H 4·86. This big change is undoubtedly due to a transition to the protonated, double-helical form of poly A, known to exist below *p*H 5. At *p*H 4·5 the transition appears to begin at a chain-length of seven[4]; it is clear from our results that, at *p*H 4·86, one is approaching the limiting *p*H for the stability of the double helical form, which requires a chain-length >12. The gradual increase up to A_{12}, like the increases at neutral *p*H, is interpreted as being due to a single-stranded helical form; again, the correlation is only with the first Cotton effect.

These results are in agreement with conclusions drawn from other recent studies[4–8]. In the past few years a remarkable change has taken place in theories regarding the stability of helical polynucleotides. It was originally supposed, on the basis of the Watson–Crick structure, that hydrogen bonds between the bases were the main, or indeed the only, factor responsible for the stability of the DNA double helix in aqueous solution, despite the well-

Table 1. Mean Residue Amplitude of the First, Positive, Cotton Effect in Oligoadenylic Acids

	*p*H	
n	4·86	6·75
2	272	272
3	274	319
5	351	330
6	367	388
7	404	423
8	435	441
~12	449	494
Poly A (~200)	1,393	675

known fact that water can itself form strong hydrogen bonds with the nucleic acid bases. That the stability of DNA to heat was proportional to its GC (guanine + cytosine) content was interpreted as being due to the fact that, whereas G + C form three hydrogen bonds, adenine and thymine form only two. It soon became apparent, however, that the hyperchromic effect associated with the heat-denaturation of DNA could not be ascribed to the breaking of the hydrogen bonds between the bases[9]. A number of other results also indicated that hydrogen-bonding could not account for the stability of the DNA double helix. For example, the melting-temperature (T_m) of DNA is unchanged when all the hydrogen atoms are replaced by deuterium[10]. Moreover, the classical hydrogen-bond breaking agents, such as urea, are much less effective in denaturing DNA than are, for example, certain anions like ClO_4^- and Cl_3CCOO^-, which are hydrophobic bond breaking agents. (The concept 'hydrophobic bond' implies that the attraction of the bases for each other is greater than their attraction for water molecules, with which they are surrounded on denaturation.) Recent theoretical studies also support the concept that the main factor stabilizing helical polynucleotides is base interaction[12–14].

Crothers and Zimm[14] still assume, however, that the increase in stability with GC content is due to the extra hydrogen bond between G and C. This assumption is unnecessary. Studies of the optical rotatory dispersion of oligoribonucleotides by Tinoco's group[15] have shown that, of the four RNA bases, uracil interacts less with neighbouring bases than do adenine, cytosine and guanine. If, therefore, stability is mainly due to base interaction, one would expect that DNA with the lowest thymine content (since thymine is very similar to uracil), that is, the highest GC content, would be the most stable. If this explanation is correct, the amplitude of the first Cotton effect in DNA should increase with increasing GC content, and this has indeed been found to be so[16]. (Samejima and Yang actually found a linear correlation between GC content and the molecular rotation at the first peak of DNA. In our investigations we have preferred to use amplitude, as this eliminates errors due to base-line shifts between readings.)

It is not only the DNA double helix which is stabilized by base interaction. Oligo- and poly-nucleotides can exist in single-stranded helical forms, stabilized by base stacking, as shown by some of the papers already mentioned[4–8,15], and by a study of the optical rotatory dispersion of polycytidylic acid, which, like poly A, exists as a single-standed helix at neutral *p*H (ref. 17). The denaturation of these single-stranded structures is accompanied by a hyperchromic effect and by a reduction in optical rotation, just as is the denaturation of double helical DNA. Similar changes accompany the heat denaturation of RNA, and in the past these have been interpreted as being due to separation of double-stranded regions of the molecule[18] but, as is apparent from the foregoing, exactly the same changes can be expected on heating a single-stranded polynucleotide in which the bases are stacked in helical array as a result of base interaction. Consequently there is now considerable doubt regarding the secondary structure of RNA. It is interesting that, in the one type of soluble RNA (*s*RNA) the primary structure of which has been completely determined[19], the base sequence is such that the longest complementary sequence which could give rise to base-pairing between different parts of the chain is only five nucleotides long. Hence double-stranded regions in this molecule must be quite short or contain many imperfections in base-pairing, a conclusion which is in marked contrast to recently discussed models of *s*RNA with more than 80 per cent of base-pairing[20–22].

Note added in proof. Recent studies of the optical rotatory dispersion of *s*RNA suggest that the main factor stabilizing its secondary structure is hydrophobic bonding[23,24].

Measurements of optical rotatory dispersion were made with the Bellingham and Stanley/Bendix-Ericsson Spectropolarimeter 'Polarmatic '62'.

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ROLE OF LYSOSOMES AND OF CELL MEMBRANES IN PHOTOSENSITIZATION

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DAMAGE to cells and tissues can be produced *in vivo* or *in vitro* by the combined effects of a photosensitizing substance and light. Photosensitization occurs *in vivo* in humans or animals exposed to a variety of drugs or noxious substances, or accumulating certain metabolites as a consequence of congenital or acquired metabolic disorders. Among the compounds known to induce photosensitivity in man are phenothiazine tranquillizers, chlorothiazide diuretics, sulphanilamide and the sulphonylurea hypoglycaemic agents[1], demethylchlortetracycline[2] and coal-tar derivatives in pitch[3].

Examples of metabolic disturbances leading to photosensitization are porphyria in man and hypericism, fagopyrism, sporidesmin poisoning and tribulosis in animals[4]. Some of these effects are indirect: thus ingestion of *Tribulus terrestris* causes liver dysfunction and accumulation of phylloerythrin (from ingested chlorophyll) in peripheral blood and skin, and this is the actual photosensitizing agent[5]. A convenient model for *in vivo* photosensitization is provided by experiments in which substances such as anthracene or porphyrin are placed on or injected into the skin of human volunteers or experimental animals. Within a few minutes of exposure to light of appropriate wave-length, erythema or weal formation, associated with an itching or burning sensation, develops[3]. The action spectrum (magnitude of response at

different wave-lengths) parallels the absorption spectrum of the photosensitizer, as expected from the Grotthus–Draper law (with the exception of the furocoumarins[6]).

Isolated cells can also be destroyed by the action of photosensitizing agents and light. Detailed experiments on erythrocytes have been carried out by Blum[7]. It has also been shown that protozoa and other organisms, and mammalian cells in culture, become sensitive to light in the presence of such diverse agents as polybenzenoid hydrocarbons[8,9], neutral red[10,11], acridine orange[12], eosin[13], demethylchlortetracycline[2] and furocoumarins[14]. Blum[7] and Clare[15] have reviewed much evidence showing that both light and oxygen are necessary for photosensitization, and there is general agreement that the damage is due to photo-oxidation. Very little is known, however, about which cellular constituents are affected, although it has generally been assumed that the cell membrane is involved.

In the course of observations on the uptake of fluorescent substances by living cells[16,17], we noticed that many of the classical photosensitizing agents are concentrated in lysosomes whereas others do not enter living cells at all. Further work has shown that two types of photosensitization occur, one due to increased permeability of lysosomal membranes, with secondary effects from released lytic enzymes, and the other resulting from increased permeability of cell membranes. These two types of photosensitization differ in certain important respects, and they provide useful techniques by which selective damage to lysosomes or cell membranes can be achieved. Studies by time-lapse ciné-micrography have revealed a number of features characterizing the two types of photosensitization, and the availability of an efficient monochromator has allowed us to determine action spectra for *in vitro* photosensitization.

Cell types. Red cells were obtained from human or rabbit blood collected into ethylenediamine tetraacetate and washed three times in 0·01 M sodium phosphate-buffered saline, *p*H 7·2. Guinea-pig and rabbit spermatozoa were obtained from the epididymis and suspended in Baker's buffered sperm diluent[18]. Cultures of mouse peritoneal macrophages, monkey kidney cells and human diploid cells (*WI*-38 strain) were prepared on coverslips as previously described[19,20].

Photosensitization. Anthracene, uroporphyrin I, eosin, neutral red, rose bengal, acridine orange and vitamin A were obtained from sources already listed[17]. Highly purified samples of anthracene and uroporphyrin I, dissolved in serum and 0·1 M sodium bicarbonate respectively, were used for most of the work. Fluorescence microscopy[17] and ciné-micrography and interference microscopy with a Baker interference microscope were used to determine the intracellular fate of various substances and to follow the effects of photosensitization.

For determination of action spectra the high-intensity monochromatic light source described by Magnus *et al.*[21] was used. Light from a high-pressure xenon arc passes through a large water prism and system of quartz lenses, focusing mirrors and slits, enabling investigation of any region from 250 to 1,000 mμ. Measurements of light energy were made with a Schwartz linear vacuum thermopile with quartz window coupled to a sensitive galvanometer. Except at very short wave-lengths (340 mμ and less) coverslips with cells were illuminated within glass tubes, but outside the culture medium, to ensure sterility. The slit was adjusted so that part of the coverslip was illuminated, the remaining cells serving as one control. Other controls were provided by illumination of cells without the photosensitizing agent, or after prior exposure to the photosensitizing agent and incubation of the cells at 37° for 30 min in medium equilibrated with 95 per cent nitrogen + 5 per cent carbon dioxide.

Estimates of cell viability. A modification was developed of the eosin dye-exclusion method originally due to Schrek[22]. The technique as usually applied gives erratic results on cells other than certain tumour cells for which it was originally devised, as Black and Berenbaum[23] have recently emphasized. This is chiefly because the high concentrations of eosin present produce variable amounts of photodynamic damage unless care is taken to exclude light in the course of preparing specimens. We added 1 : 10,000 (wt. vol.) eosin to the culture medium and incubated in the dark for 10 min; cultures are then washed in medium without eosin before observation by fluorescence microscopy. Dead cells showed marked uptake of dye and fluorescence, whereas very little or no fluorescence was present in living cells. Another useful technique was incubation of cells for 30 min in the dark with euchrysine (1 : 100,000 in culture medium), washing in fresh medium and examination by fluorescence microscopy. In living cells, lysosomes fluoresce as discrete orange masses in cytoplasm, and nucleus and nucleolus green; in dead cells there is weak, diffuse green fluorescence of cytoplasm. Estimates of viability by the two methods showed good correlation.

Histochemistry. Acid phosphatase staining of unfixed cells was carried out by the Gomori technique as described elsewhere[19].

Observations on localization of photosensitizing agents. Living monkey kidney cells and macrophages incubated with non-toxic concentrations of anthracene and other polybenzenoid hydrocarbons, uroporphyrin I, vitamin A or neutral red showed localization of these compounds in lysosomes when examined by fluorescence microscopy, as described previously[17]. The identification of these organelles as lysosomes was based on the combined use of fluorescence microscopy, light microscopy and acid phosphatase staining in the same preparations. The characteristic distribution of uptake in different cell types, including spermatozoa, also supports the interpretation that lysosomes are mainly—if not exclusively—involved. Acridine orange is taken up in living cells by both lysosomes and nucleus. There was no evidence that eosin or rose bengal in the concentrations used was taken up at all by the living cells which we were investigating.

Photodynamic damage to cell membranes. This was readily demonstrated by following the haemolysis of washed mammalian erythrocytes suspended in phosphate (0·01 M)-buffered saline at *p*H 7·2 in the presence of rose bengal, uroporphyrin or anthracene and light of wavelength absorbed by these agents. More detailed observations were made by phase-contrast or interference microscopy in the presence of rose bengal (1 in 10^5) using blue-violet light from a high-pressure mercury source. After a brief interval, the cells rapidly became spherical and momentarily showed an increased optical density; a sudden drop in optical density was followed by a gradual fading to a density equivalent to that of the surrounding medium. The latter change is due to release of haemoglobin. Corresponding changes in interference colours have been recorded in colour film. These observations show that agents which normally sensitize the lysosomes (for example, porphyrin, anthracene and euchrysine) can also produce photodynamic damage to cell membranes. Further evidence in support of this view came from observations on rat and guinea-pig spermatozoa suspended in Baker's medium. When euchrysine was added (1 in 10^5) the spermatozoa were very rapidly immobilized by blue-violet light. To see how localized these effects could be, Köhler illumination giving a narrow beam of light 10μ in width was applied. It was found that light directed on to the tip of the tail immobilized only that part of the tail. These observations parallel the localized immobilization of the trypanosome flagellum by a narrow beam of light described by Walker[24]. As previously observed with trypanosomes, if the spermatozoa had been incubated with dye for some time before microscopy they were relatively resistant to photo-immobilization.

In other experiments, effects of cell-membrane photosensitization were studied in mouse macrophages with rose bengal in a culture medium lacking serum. Within a few seconds of exposure to blue light, cells were killed, with leakage of potassium, and after a short interval they showed cytoplasmic extrusion in the form of large bubbles (Fig. 1).

Photodynamic damage to lysosomes. Preliminary experiments showed that a number of cell types (HeLa, strain *A* hamster, *WI*-38 human diploid, mouse macrophages and monkey kidney cells) were sensitive to light after they had taken up neutral red, anthracene, porphyrin or acridines into their lysosomes. Detailed investigations were carried out with coverslip cultures of monkey kidney cells and mouse macrophages incubated in the dark for 1–2 h at 37° C in medium containing anthracene or uroporphyrin I (10 μg/ml.). The cells were incubated for a further 30 min in fresh medium without the photosensitizers to avoid cell membrane damage of the type described in the previous section. The photosensitizers were then detectable by fluorescence microscopy only in lysosomes. Cultures were illuminated with monochromatic light (400 mμ in the case of porphyrin and 360 mμ in the case of anthracene) for 1–90 sec, corresponding to energies of 4 to 360×10^4 μW per cm². Control cultures had no photosensitizing agent, no light, or were incubated for 30 min after uptake of the photosensitizing agents in medium without oxygen before illumination. Cultures were examined without further treatment or after incubation for 24 h at 37° C in the dark.

Only with high doses of light (60×10^4 μW per cm²) was there any immediately detectable change in the appearance of cells or loss of viability as judged by eosin staining. However, acid phosphatase staining of lysosomes of unfixed cells was detected after illumination with 12×10^4 μW per cm². Control preparations which had been illuminated in the absence of the photosensitizer showed no lysosomal staining. Since undamaged lysosomal membranes are impermeable to the β-glycerophosphate, substrate, the observations show that a very early change following illumination in the presence of this type of photosensitizing agent is increased permeability of lysosomal membranes.

After incubation for 24 h the changes were much more marked. With high doses of light nearly all the cells were destroyed and many had been shed from the coverslips into the medium. With lower doses of light, many of the cells had become rounded and ballooned, often with cytoplasmic vacuolation and pyknotic nuclei (Fig. 2).

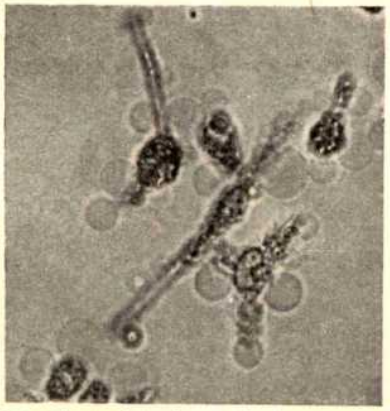
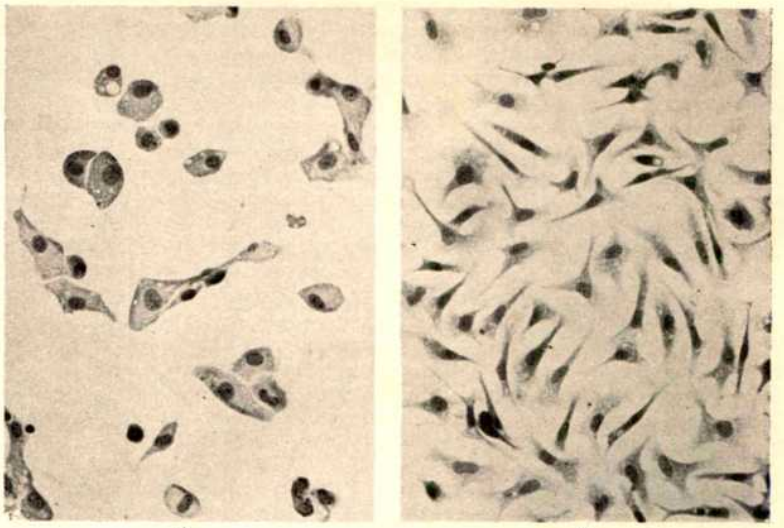


Fig. 1. Phase-contrast photomicrograph of mouse macrophages in culture 5 min after photosensitization with rose bengal, showing cytoplasmic blebs. (× *c.* 430)



A *B*

Fig. 2. *A*, Fixed and stained mouse macrophages 24 h after photosensitization with anthracene (× *c.* 245). The cells are rounded, with cytoplasmic vacuolation and dense nuclei. *B*, Control culture of macrophages (× *c.* 245)

The changes resembled those seen in cells cultured in the presence of high concentrations of oxygen, which were associated with increased permeability of lysosomes[25]. Diffuse acid phosphatase staining in cytoplasm and nuclei showed that lysosomal enzymes had, in fact, been released in the photosensitization experiments. The distinction between these two major types of photosensitization is summarized in Fig. 3.

The viabilities of cells measured at 24 h by the eosin method are plotted in Fig. 4. These show a rather flat dose-response curve which does not depart greatly from linearity. This should not, however, be interpreted as a single-photon-hit mechanism at some critical site. Fluorescence microscopy shows considerable cell-to-cell variation in the number of lysosomes containing the photosensitizer, and even if enzyme has to be released from several lysosomes to damage a cell, the probability of achieving this result might be an approximately linear function of irradiation dose. The counts of killed cells are also underestimated at high doses because of loss of uncontrolled numbers of dead cells into the medium.

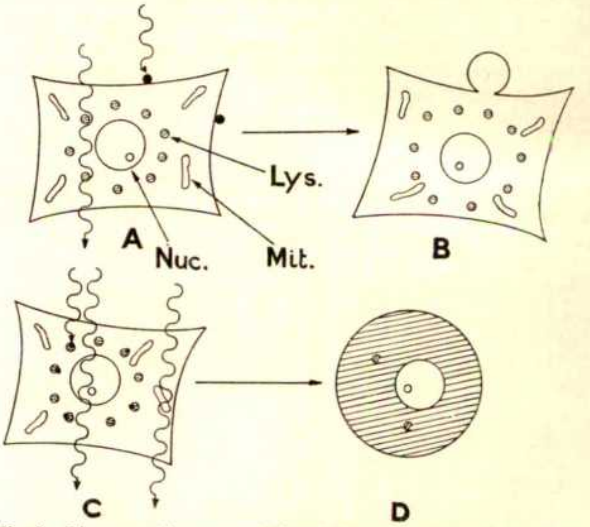


Fig. 3. Diagrammatic representation of the two major types of photosensitization. Nuc., nucleus; Mit., mitochondria; Lys., lysosomes. Some dyes, such as rose bengal, remain at the cell surface (*A*). If light of appropriate wave-length is absorbed, there is immediate photo-oxidative damage to the cell membrane, with loss of potassium and then cytoplasmic extrusion (*B*). Other substances, such as anthracene or porphyrin, are concentrated in lysosomes (*C*). If these absorb light, lysosomal enzymes escape into the cytoplasm and after some delay the cell rounds up and dies (*D*). Photons of these wave-lengths pass harmlessly through cells if no photosensitizing agent is present

These considerations have deterred us from attempting to estimate the quantum efficiency of cell killing.

When cells treated with photosensitizers were illuminated in the absence of oxygen, marked protection was seen. Similarly the same doses of light in the absence of photosensitizing agents had little or no effect. Hence, both light and oxygen are necessary for lysosomal photosensitization, as previously shown for photodynamic damage to red cells. Only cells in the path of the light beam were severely damaged, other cells on the edges of the coverslips appearing healthy. Hence the main effects were exerted on the individual cells themselves, and there was negligible accumulation of toxic materials in the culture media.

In order to obtain action spectra, macrophage cultures similarly treated with anthracene or porphyrin were illuminated with a constant dose of light (120×10^4 μW per cm^2) at different wave-lengths and the proportion of cells killed after incubation for 24 h plotted (Figs. 5 and 6). Short wave-length illumination of this intensity damages cells in the absence of a photosensitizer, as shown in the control curve *D* of Fig. 6, but above 320 mμ it was possible to show the additional effects due to anthracene photosensitization. Thus the action spectra correspond quite closely to the absorption spectra, unlike the action spectrum for anthracene wealing, which falls off below 340 mμ for reasons that will be discussed later.

Photosensitization in vivo. It will be noted that substances known to give rise to photosensitization *in vivo* (for example, porphyrin and anthracene) are of the type producing photodynamic damage to lysosomes *in vitro*. This led us to consider whether lysosomes might be involved in the hyperaemia, wealing and tissue damage associated with photosensitivity reactions in the skin. The problem is of interest because, although it is clear from action spectra that light absorption by the photosensitizing agents triggers off the responses, virtually nothing is known about the underlying mechanisms. Evidence is accumulating that lysosomes contain permeability factors that may be implicated in responses such as the Arthus reaction[26–28]. Moreover, mast cell granules (which have many of the enzymes and properties characteristic of lysosomes) contain histamine which is released when they take up stilbamidine[29]. A basic protein from polymorphonuclear lysosomes can release histamine from mast cells[26].

It therefore seemed possible that photodynamically induced permeability changes in lysosomes of dermal cells—such as endothelial cells in the papillary vasculature, or nearby mast cells—might be the primary event in photosensitization.

In order to obtain more information about vascular responses in photosensitization, we have made some observations on the living mesentery of the rat. Fluorescence microscopy revealed that about 5 min after intravenous administration of acridine orange (5 mg), the dye was present mainly in the endothelial cells of small blood vessels; these showed green fluorescence of nuclei and fine green or yellow-orange granules in the cytoplasm. The latter are almost certainly lysosomes, which have been identified in inflamed endothelial cells by acid phosphatase reactions in electron micrographs[30]. On the other hand, 15 min after intraperitoneal inoculation of acridine orange, the most prominent uptake in the mesentery was by mast cells, the granules of which fluoresced a brilliant orange.

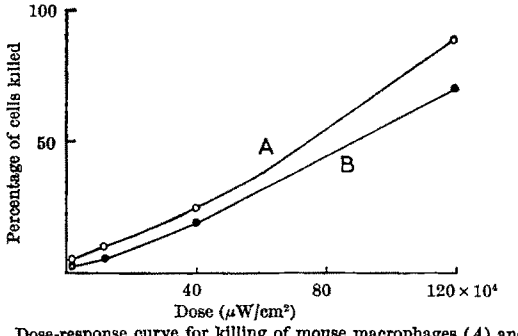


Fig. 4. Dose-response curve for killing of mouse macrophages (*A*) and monkey kidney cells (*B*) sensitized with anthracene and illuminated at 360 mμ. See text for experimental details

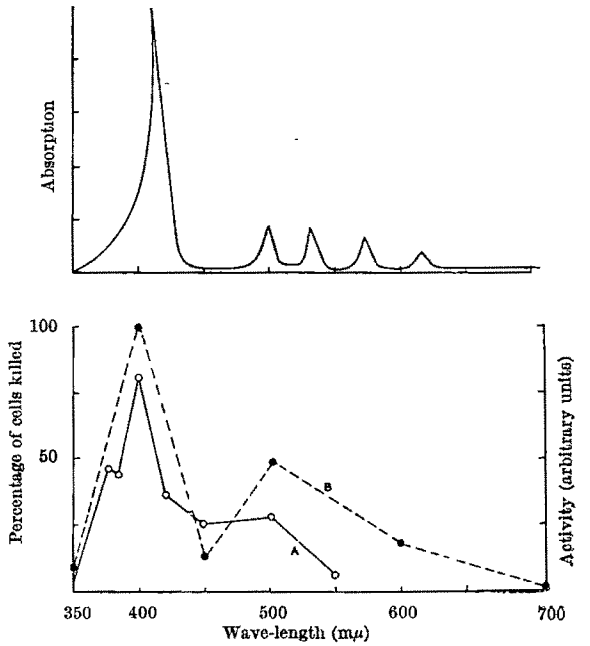


Fig. 5. *Above*, Absorption spectrum of protoporphyrin IX in dioxane (ref. 35). The absorption spectrum of uroporphyrin I is similar. *Below*, Curve *A*, action spectrum for protoporphyrin wealing in human skin (ref. 32). Curve *B*, action spectrum for killing of monkey kidney cells sensitized with uroporphyrin I

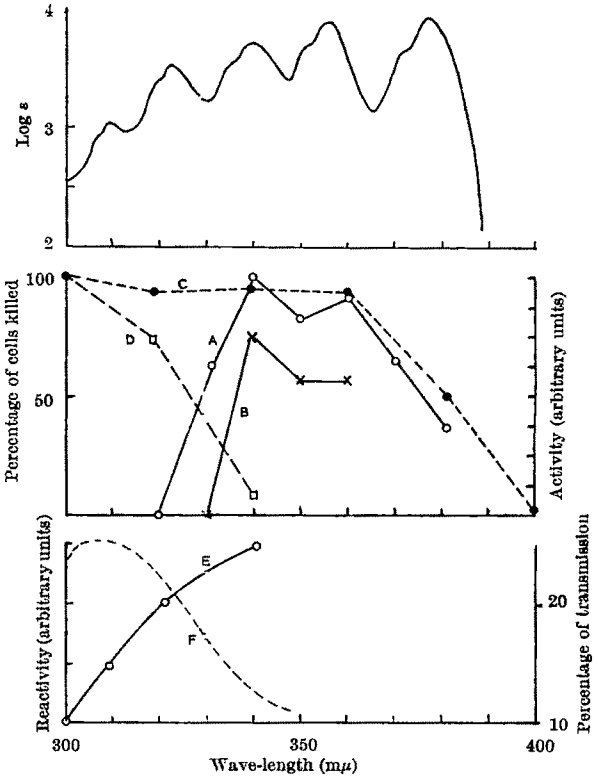


Fig. 6. *Above*, Absorption spectrum of anthracene in acetone. *Middle*, Curves *A* and *B*, action spectra for anthracene wealing in the human skin (ref. 3). Curve *C*, action spectrum for killing of monkey kidney cells sensitized with anthracene. Curve *D*, action spectrum for killing of monkey kidney cells without any added photosensitizer. *Below*, Curve *F*, action spectrum for erythema in normal human skin (ref. 32). Curve *E* shows percentage of transmission of light through normal human epidermis (ref. 34)

There was also nuclear and some lysosomal fluorescence in mesothelial and other connective tissue cells. Thus there is uptake of photosensitizing substances by lysosomes of both endothelial and mast cells, the former being more marked (at least initially) after intravenous administration, which presumably parallels the carriage of blood-borne photosensitizer to the skin in porphyria and other conditions. Mast cell uptake is more prominent with extravascular administration, and this may parallel direct exposure of skin to anthracene or other photosensitizing substances.

Having established the lysosomal location of the photosensitizing substances, we examined the effects of light on the mesentery of the rat viewed with the Leitz 'Ultropak' microscope. Using the frog's tongue, Castellani and his colleagues[31] were able to show that, shortly after intravenous injection of relatively high doses of haematoporphyrin, exposure to light leads to stasis of blood, aggregation of cells, intravascular haemolysis and irreversible damage. We have obtained similar results in the rat mesentery after intravenous injection of uroporphyrin I or acridine orange (5 mg with prolonged illumination for 5 min or more). If pontamine sky blue (0·2 ml. of a 5 per cent solution in saline) was injected intravenously 5 min after acridine orange (5 mg) exposure of the mesentery to a narrow beam of blue light from a carbon arc produced within a few minutes localized vasodilatation and blueing; this did not occur if the acridine was omitted.

Comparable experiments were then carried out in the skin of the guinea-pig. Male albino guinea-pigs (250 g) had their flanks closely clipped and 0·1 ml. of a solution containing 100 μg uroporphyrin I was injected intradermally. Two hours later 0·5 ml. of 5 per cent pontamine sky blue was injected intravenously, and the skin illuminated for 2 min from a carbon arc with heat-absorbing filters. Intense blueing occurred within 2 min around the site of injection of porphyrin followed by illumination; later a weal developed at the site. Injection of porphyrin without illumination, or illumination at another site, had no such effect. This provides a convenient animal system for study of photosensitivity and the effects of drugs on the reaction.

It thus seems that there are two stages in the photosensitization response: first, vasodilatation and increased vascular permeability, which could give rise to erythema and wealing, and secondly, with high concentrations of the photosensitizer, haemolysis, vascular obstruction, haemorrhage and irreversible damage. The first change parallels the relatively mild photosensitization seen in experiments on human skin of the type already mentioned, and also in the common form of erythropoietic porphyria, that is, the so-called erythropoietic protoporphyria[32]. Experimental lesions show only trivial inflammatory changes histologically, but in the natural lesions of porphyria, cutanea tarda changes in the capillary walls occur. In erythropoietic porphyria similar abnormalities, with deposition of periodic-acid–Schiff- and fat-staining material, are found. The more severe reaction we have observed brings to mind the haemolysis and marked histological abnormalities in the rare Gunther's disease.

These observations are consistent with arguments previously summarized by Magnus[32] that photosensitization *in vivo* is due to effects close to the base of the epidermis rather than in the epidermis itself. The rapid onset of erythema following exposure to light suggests the release of a mediator substance very close to or within the blood vessels. In the skin of porphyric subjects (at least in Gunther's disease[32]) porphyrin fluorescence is demonstrable mainly just under the epidermis in the region of the papillary, vasculature. The failure of short wave-lengths (below 330 mμ) to induce anthracene photosensitization in the skin, although they are effective *in vitro*, could be due to loss of light through scattering and absorption before it reaches the deeper layers of the skin (Fig. 5). Failure of anthracene photosensitization to occur if the blood supply is occluded before illumination suggests that oxygen is necessary for photosensitization, *in vivo* as *in vitro*.

In summary, the evidence is consistent with the interpretation that the initial event in photosensitization *in vivo* is increased permeability of lysosomes of endothelial and possibly nearby connective tissue cells, with release of one or more chemical mediators. Antihistamines sometimes reduce urticaria experimentally induced by light in porphyric subjects, so histamine may be one of the agents involved, but the general ineffectiveness of these drugs suggests that other agents are more often responsible for vasodilatation and increased vascular permeability[32]. It is also possible that substances released from lysosomes of epidermal cells, and slowly diffusing down to the papillary vasculature, play a part in the delayed erythema following sunburn or short wave-length illumination, very little of which penetrates through the epidermis (Fig. 5). Damage to lysosomes of epidermal cells by light of these wave-lengths has been demonstrated by Weissmann and Fell[33].

Our observations on photosensitization support the growing body of evidence that lysosomes play a part in early events in the inflammatory reaction. In most experimental situations it is difficult to define which responses come first and which later. In this case there is little doubt that the initial change must be in the lysosomes containing photosensitizing agents. The simplest interpretation of subsequent events would be that endothelial cells, which are flattened and curved around small blood vessels, tend to round up and withdraw their processes (compare Fig. 2); fluid and protein could then escape between cells. The availability of an experimental animal model system showing photosensitization should allow study of the underlying pharmacology.

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COMPARATIVE BIOCHEMISTRY OF PHOTOSYNTHETIC PROCESSES

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INVESTIGATIONS during the past 15 years have revealed important similarities in the molecular mechanisms of photosynthesis in bacteria and green plants. In both kinds of organisms, light energy is converted into chemical energy in the form of adenosine triphosphate (ATP) and cytochromes appear to participate in photochemical electron transfer. The latter is indicated, in particular, by the observation of rapid photo-induced oxidation of reduced cytochrome over a wide temperature range[1]. On the other hand, the overall metabolism of photosynthetic bacteria is clearly distinguished from that of green plants in respect of the source of net reducing power (primarily reduced pyridine nucleotide) for biosyntheses[2–4]. In green plants, water serves as the primary reductant and oxygen is evolved as a consequence. Oxygen is not produced in bacterial photosynthesis[2] and, at first glance, this suggests that the bacteria cannot use water as an electron donor. If this is so, anaerobic photosynthetic growth on carbon dioxide as the sole carbon source would obviously require an alternative donor and, indeed, such growth is observed to be dependent on the presence of substrate quantities of an 'accessory' electron donor such as hydrogen, hydrogen sulphide or thiosulphate[2]. (The absence of oxygen production in bacterial photosynthesis could, however, be rationalized by assuming that water serves as the hydrogen donor for carbon-dioxide reduction and, in doing so, yields an oxidized product (OH) which is not convertible to oxygen; the function of the accessory electron donor, then, would be to reduce the oxidized product. This notion[2] has not received experimental support and seems to be no longer accepted by most of the workers in the field.)

One of the major unresolved, and controversial, aspects of bacterial photosynthesis concerns the pathways through which electrons from accessory donors are transported in the net generation of reduced pyridine nucleotide (PNH). Several kinds of mechanisms can be envisaged. With accessory electron donors of sufficiently low redox potential, such as hydrogen, reduced pyridine nucleotide can evidently be generated through enzymatic dark reactions, as in non-photosynthetic bacteria[5,6]. Accordingly, in photosynthetic growth on carbon dioxide, together with hydrogen, the requirement for light can in principle be entirely attributed to its necessity for production of ATP by 'cyclic' light-induced phosphorylation[7,8]. This type of phosphorylation requires neither external reductants nor oxidants, and is believed to be coupled to a cyclic transfer of electrons within the photochemical system[5,9,10] (see Figs. 1 and 2).

With physiological electron donors of relatively high potential, such as succinate, light is required to effect net reduction of pyridine nucleotide[11]. According to the so-called 'non-cyclic' electron flow hypothesis, the reduction of pyridine nucleotide with such donors requires direct intervention of the photochemical system, that is, the action of light presumably results in transfer of electrons from the photochemical system to pyridine nucleotide and these are 'replaced' by electrons derived from the accessory donor (for example, succinate) by way of a dark process[8]. (In some versions of the hypothesis[12], net formation of PNH is assumed to occur by this mechanism with all accessory donors, regardless of redox potential.) In effect, this implies that one or more components of the photochemical system (for example, bacteriochlorophyll) must act as a redox carrier in the transfer of electrons from donor to acceptor. Thus, in photosynthetic growth on succinate as sole carbon source, or with other accessory electron donors of high redox potential, light is presumed to be necessary both for ATP production and for the generation of PNH by means of non-cyclic electron flow.

The light-dependent production of hydrogen from accessory donors is mechanistically represented in the non-cyclic hypothesis as resulting from a similar transfer of electrons (that is, through the photochemical system) to the hydrogenase enzyme, but a satisfactory physiological rationale for operation of an electron transfer sequence of this kind has not been advanced[8].

Non-cyclic Light-induced Phosphorylation in Plants

In addition to cyclic phosphorylation, preparations from green plants also catalyse phosphorylation coupled to light-dependent net electron transfer. This process, which involves transfer of electrons from water, can be represented by the overall equation[13]:

$$4Fd_{ox} + 2ADP + 2Pi + 2H_2O \rightarrow 4Fd_{red} + 2ATP + 4H^+ + O_2$$

where Fd_{ox} is the oxidized form of the iron protein ferredoxin; Fd_{red}, the reduced form; ADP, adenosine diphosphate; Pi, inorganic phosphate. Subsequent transport

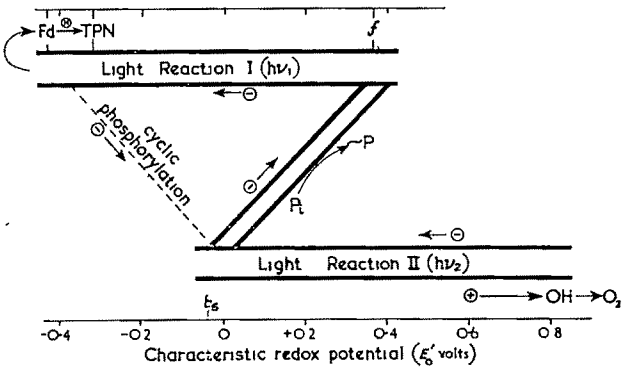


Fig. 1. Working hypothesis for light-induced electron transport and phosphorylation in green plants, after Hill (ref. 14). ⊖, Electron; ⊕, positive hole; Pi, inorganic phosphate; ~P, ATP; Fd, ferredoxin, TPN, triphosphopyridine nucleotide; *f*, cytochrome *f*; b_6, cytochrome b_6. Electrons are transported against the thermodynamic gradient in the light reactions. Light Reaction II results in formation of an electron and a positive hole; discharge of a hydroxyl ion by the positive hole forms OH, the precursor of oxygen

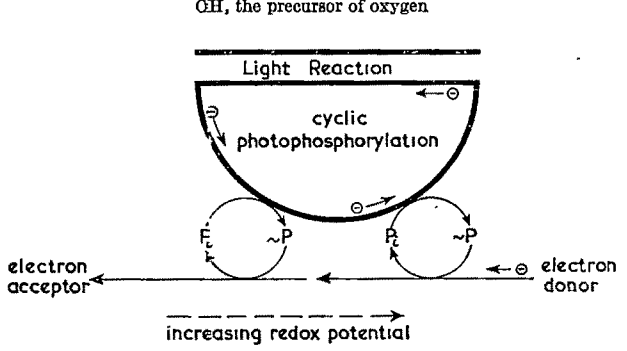


Fig. 2. A 'qualitative' scheme for reverse electron transfer driven by the cyclic phosphorylation system of photosynthetic bacteria. ⊖, Electron, Pi, inorganic phosphate; ~P, ATP or related energy-rich phosphorylated precursors. The heavy curved line represents the pathway of electron transfer in cyclic photophosphorylation; a number of catalysts, including cytochromes have been implicated. It is suggested that reverse electron transfer (horizontal long, solid arrows) may also be promoted by energy-rich non-phosphorylated precursors of ~P; note, however, that the energy coupling could equally well be expressed in terms of Mitchell's chemiosmotic hypothesis (ref. 40)

of electrons (from Fd_{red}) to pyridine nucleotide is mediated by a flavoprotein.

A working hypothesis suggested by R. Hill[14] for light-induced electron transport and phosphorylation in green plants is depicted in Fig. 1.

The scheme is based on the operation of two light reactions which transfer electrons against the thermodynamic gradient. These are connected by a thermochemical reaction (between approximately 0 V and +0·4 V), analogous to reactions characteristic of electron transfer in mitochondria. Phosphorylation coupled to net ('non-cyclic') transfer of electrons from water to Fd_{ox} is visualized as occurring in the thermochemical phase, that is, "photophosphorylation may be regarded as oxidative phosphorylation in the sense used for the mitochondrion"[14]. 'Cyclic' phosphorylation is represented as being associated with an internal cyclic transport of electrons, promoted by Light Reaction I. This light reaction involves oxidation of reduced cytochrome *f* in higher plants, or of an analogous *c*-type cytochrome in photosynthetic bacteria and algae.

There is as yet no evidence for more than one light reaction in bacterial photosynthesis. This, together with the apparent inability of the bacteria to use water as an electron donor, suggests that only Light Reaction I, or its equivalent, is operative in these organisms.

Existence of Non-cyclic Light-induced Phosphorylation in Bacterial Photosynthesis

Pigmented particles from certain photosynthetic bacteria catalyse a number of light-dependent oxidation-reduction reactions. Thus, with *Rhodospirillum rubrum* particles, light-dependent net reduction of diphosphopyridine nucleotide (DPN) is observed when either ascorbate + dichlorophenolindophenol (DCPIP)[15] or succinate[11] is used as the electron donor.

The results of an investigation[16] with *R. rubrum* particles, using ascorbate + DCPIP, have been interpreted[13,16] as demonstrating the occurrence of phosphorylation coupled to a non-cyclic light-induced flow of electrons. In the study cited, it was assumed that phosphorylation observed in the presence of antimycin *A*, a potent inhibitor of cyclic phosphorylation, must necessarily be dependent on transfer of electrons from ascorbate + DCPIP to DPN. It has been shown[17], however, that DCPIP and certain other dyes have the capacity to catalyse an antimycin-resistant electron transfer bypass in the cyclic phosphorylation mechanism. This effect of DCPIP is particularly manifest when the redox conditions are suitably 'balanced'. Over-reduction by ascorbate + DCPIP, or other electron donors, of catalysts participating in cyclic electron flow causes severe inhibition of phosphorylation[15,17–19], and this can be effectively counteracted by addition of oxidants such as DPN or fumarate. Thus, by manipulation of the experimental circumstances, light-dependent antimycin-resistant phosphorylation can be shown to have a putative 'requirement' for an electron donor system, ascorbate + DCPIP, and an electron acceptor, DPN or fumarate, even under conditions where there is no appreciable net electron transfer[17].

Since the cyclic phosphorylation system in pigmented particles isolated from photosynthetic bacteria shows great sensitivity to the redox conditions, especially in the presence of reactive oxidation-reduction dyes such as DCPIP, the interpretation of results of experiments in which exogenous reductants, oxidants and dyes are used is frequently subject to serious ambiguity. The contention[20] that the uncertainties can be avoided simply by using high concentrations of ascorbate + DCPIP as the electron donor supply is contradicted by the results of other studies[17,21].

The light-dependent oxido-reduction reaction between succinate and DPN, that is, succinate + DPN → fumarate + DPNH, can be assumed to have physiological significance and in this respect presents a more suitable system for investigation of the problem under consideration. To my knowledge, there are no published data indicating that a phosphorylation is coupled to this net electron transfer. It is significant (see later) that addition of the phosphorylation reactants (adenosine diphosphate (ADP) + inorganic phosphate (*Pi*) + Mg^{++}) causes inhibition of the oxido-reduction reaction.

In sum, it is concluded that clear-cut evidence for a ('non-cyclic') phosphorylation coupled to light-dependent net electron transfer between exogenous donors and DPN, or other acceptors, in bacterial photosynthesis has not yet been obtained.

Energy-linked Reverse Electron Transfer

Investigations with mammalian mitochondria have established that electron transfer against a thermodynamic gradient can be driven by ATP and energy-rich intermediates associated with oxidative phosphorylation[22]. This (and suggestive discussions[23–25] of photosynthetic electron transport) prompted experimental study in our laboratory[4,6,26] of the possibility that net oxido-reduction reactions dependent on, or promoted by, light in bacterial photosynthesis might also be examples of 'reverse energy-linked' electron transfer. Accordingly, the function of light would be to generate ATP, or energy-rich precursors of ATP, by means of the (anaerobic) cyclic light-induced phosphorylation mechanism; ATP or the related energy-rich intermediates would drive 'dark' transfer of hydrogen (electrons) from donor to acceptor (Fig. 2). Evidence supporting the reverse electron transfer formulation is briefly summarized:

(1) *Light-dependent formation of molecular hydrogen.* Photoproduction of hydrogen by *Rhodospirillum rubrum* requires the presence of oxidizable organic substrates, such as citric acid cycle intermediates[27]. Maximal yields of gas are observed with 'resting cells' and the quantities produced under certain conditions closely approximate those predicted on the basis of complete conversion of the organic compound to hydrogen and carbon dioxide[28], for example:

$$\underset{\text{(succinate)}}{C_4H_6O_4} + 4H_2O \rightarrow 4CO_2 + 7H_2$$

The formation of hydrogen is severely inhibited by 'uncouplers' and compounds which interfere in other ways with cyclic phosphorylation, for example, antimycin *A*, 2,4-dinitrophenol, pentachlorophenol (PCP), *m*-chlorocarbonylcyanide phenylhydrazone (*m*-CCP), *n*-butyl-3,5-diiodo-4-hydroxybenzoate (DIB), and dyes which facilitate over-reduction of catalysts of the phosphorylation system[26,28]. Addition of appropriate concentrations of such agents to illuminated cells not only abolishes hydrogen formation, but usually also induces fermentation of endogenous reserves to fatty acids, a process normally seen only during dark anaerobic metabolism[28]. That is, it appears that inhibition of cyclic phosphorylation leads to suppression of the overall photometabolism of hydrogen-producing cells.

Several lines of evidence clearly indicate that the light-dependent total conversion of succinate and other organic compounds to carbon dioxide and hydrogen occurs through operation of the citric acid cycle, coupled with a light (energy)-dependent process which in some way effects the reoxidation of reduced pyridine nucleotide by liberation of hydrogen[8,28]. The capacity to dispose of electrons in the form of hydrogen makes operation of the cycle possible under anaerobic conditions, that is, in the absence of an external terminal electron acceptor.

Rationalization of the foregoing within the framework of the non-cyclic electron flow hypothesis is difficult and, at best, requires a number of tenuous *ad hoc* assumptions. On the other hand, a reasonable interpretation of the

available data is provided by the reverse electron transfer concept which supposes that the precursor of hydrogen is reduced pyridine nucleotide, generated by the oxidative steps of the citric acid cycle[4,6,26]. Formation of hydrogen from PNH is considered to occur by reverse electron transfer (that is, with the hydrogenase system functioning as the acceptor), promoted by energy-rich intermediates associated with cyclic light-induced phosphorylation. This explanation furnishes, moreover, a plausible physiological rationale for the phenomenon of light-dependent formation of hydrogen. Thus, energy-dependent production of hydrogen can be viewed as a regulatory mechanism which aids in maintaining the pools of reduced pyridine nucleotide and ATP (or precursors) at levels consistent with the demands of the biosynthetic machinery[4,28]. In this connexion, it is worth noting that photoproduction of hydrogen from organic compounds by 'resting cells' is inhibited by addition of NH_4^+ or nitrogen gas, both of which can be readily used by photosynthetic bacteria for endergonic reductive biosynthesis of amino-acids and other cellular constituents[30].

Direct test of the assumption that PNH is the precursor of hydrogen has been hampered by failure, despite numerous attempts[31], to devise a procedure for preparing hydrogen-evolving cell-free extracts from photosynthetic bacteria. It is significant, however, that light-dependent production of hydrogen from reduced triphosphopyridine nucleotide has been observed in extracts made from the alga *Chlamydomonas eugametos*, using cells previously adapted to an anaerobic "H_2 metabolism"[32]; the effects of phosphorylation uncouplers on this activity have not yet been investigated. Relevant also to the present discussion are recent reports[33] which describe an ATP-dependent hydrogen-evolution activity in preparations derived from certain heterotrophic bacteria which, in common with photosynthetic bacteria, have the capacity to fix nitrogen. (According to Bulen, W. A., LeComte, J. R., Burns, R. C., and Hinkson, J., in *Non-Heme Iron Proteins: Role in Energy Conversion* (edit. by San Pietro, A., 261, Antioch Press, Yellow Springs, Ohio, 1965), W. S. Zaugg and W. A. Bulen have observed (unpublished experiments) ATP-dependent hydrogen-evolution by extracts from *R. rubrum*—it appears that the system is very labile.) Conceivably, in photosynthetic bacteria, a phosphorylation step might be required for the formation of a reduced compound other than PNH, that gives rise to hydrogen by way of an ordinary (dark) thermochemical conversion. Although this is considered unlikely, it may be remarked that a mechanism of this kind would, in effect, still represent a promotion of dark electron transfer by photochemically produced ATP or its congeners. (Only light-dependent formation of hydrogen is considered here; several photosynthetic micro-organisms produce hydrogen in darkness, either endogenously or from substrates such as formate and pyruvate, apparently through mechanisms similar to those characteristic of anaerobic heterotrophs[3,29].)

(2) *Light-dependent net reduction of fumarate.* Pigmented particles obtained from hydrogen-producing cells of *R. rubrum* are capable of reducing fumarate with hydrogen when catalytic quantities of certain redox carriers are added[6,26]. The relationship between the effect of light and the redox potential of the mediator can be seen in Table 1; in all instances, the oxidized form of the mediator is readily reduced by the hydrogenase (that is, with hydrogen) in darkness. As expected, mediators effective in the dark have redox potentials between, or overlapping, those of the H^+/H_2 couple ($E_0' = -0{\cdot}42$ V) and the fumarate/succinate couple ($E_0' = +0{\cdot}031$ V). With mediators having potentials in the approximate range 0 to $+0{\cdot}115$ V, the overall reaction is stimulated by light, and with carriers of still higher potential, illumination is required.

The 'light-dependent' reaction with tetramethyl-*p*-phenylenediamine (TMPD; $E_0' = +0{\cdot}26$ V) serving as mediator and the dark process catalysed by Nile blue *A* ($E_0' = -0{\cdot}119$ V) have been examined with respect to the effects of agents which uncouple phosphorylation[26]. Compounds such as PCP, *m*-CCP, and DIB inhibit the 'light-dependent' activity and, at concentrations which completely abolish the overall reaction, these agents do not affect the dark process. In our view[6,26], these observations indicate that the action of light in systems with mediators of relatively high potential is concerned with generation of energy-rich intermediates, associated with cyclic phosphorylation, which drive hydrogen (electron) transfer against an unfavourable thermodynamic gradient, that is, from reduced mediator to fumarate. Consistent with this, oligomycin—a potent inhibitor of net ATP formation by the cyclic mechanism—does not inhibit the TMPD model reaction, and, in fact, appreciably stimulates the net oxidation-reduction process. In mitochondrial oxidative phosphorylation, oligomycin is believed to prevent specifically the formation of phosphorylated intermediates from energy-rich non-phosphorylated precursors (or from ATP), but it apparently does not inhibit utilization of the postulated precursors for driving endergonic reactions[22].

(3) *Light-dependent reduction of DPN with succinate as electron donor.* Although the mechanism of this reaction is popularly discussed in terms of the non-cyclic electron flow hypothesis, it has also been suggested[4,6,26] that the oxido-reduction may occur by reverse energy-linked electron transfer, as in mitochondria. The fact that the succinate-dependent 'photoreduction' of DPN by *R. rubrum* particles is inhibited when ADP + P*i* + Mg^{++} are added[10,34] indeed suggests a competition between the oxidation-reduction reaction and the phosphorylating system for a common reactant. In sharp contrast to the behaviour of the bacterial particles, the photoreduction of pyridine nucleotide by green plant chloroplasts is greatly accelerated on addition of ADP + P*i* + Mg^{++} (ref. 35). This striking difference casts further doubt on the non-cyclic interpretation as applied to pyridine nucleotide reduction in bacterial photosynthesis.

If the reduction of DPN by *R. rubrum* particles occurs by reverse electron transport, transfer of hydrogen from succinate to the pyridine nucleotide might be detectable using tritiated succinate as substrate. Unfortunately, the results of experiments[36] designed to test this cannot be interpreted unambiguously, because the particles catalyse a hydrogen (tritium) exchange between DPNH and water. One alternative type of approach is to test the possibility that net reduction of DPN can be observed in darkness when ATP is provided. According to a recent investigation[37], this can be demonstrated using either succinate or ascorbate + TMPD as the electron donor. Furthermore, the dark reduction of DPN under these conditions is reported to be inhibited if the 'energy supply' (from ATP) is cut off by addition of oligomycin. These observations obviously argue against the non-cyclic hypothesis, but are consistent with and predictable by the reverse electron transfer formulation.

Table 1 EFFECT OF LIGHT ON REDUCTION OF FUMARATE WITH HYDROGEN BY *R. rubrum* PARTICLES; RELATIONSHIP WITH E_0' OF THE REDOX MEDIATOR*

Mediator	E_0' (V)	Light effect (requirements)
Benzyl viologen	−0·359	None
Janus green *B*	−0·256	None
Flavin mononucleotide†	−0·219	None
Nile blue *A*	−0·119	None
Menadione (vitamin K_3)	−0·044	None
Methylene blue	+0·011	Stimulatory
Thionine	+0·063	Stimulatory
N-Methylphenazonium methosulphate	+0·080	Stimulatory
Coenzyme Q_{10} (*R. rubrum*)	+0·089	Stimulatory
Toluylene blue	+0·115	Stimulatory
2,6-Dichlorophenolindophenol	+0·217	Obligatory
Tetramethyl-*p*-phenylenediamine	+0 260	Obligatory
Cytochrome *c* (mammalian)	+0 262	Obligatory
p-Benzoquinone	+0·280	Obligatory
Cytochrome c_3 (*R. rubrum*)	+0 330	Obligatory

* After Bose and Gest (ref. 26)

† Mediator function not observed in the absence of a detergent such as deoxycholate

Two distinct general metabolic patterns can be clearly discerned in photosynthetic bacteria. I propose that these can be understood as follows[4]:

(*a*) '*Autoheterotrophic*' *pattern*. An organic compound serves as the source of carbon and through typical heterotrophic mechanisms directly provides the intermediates necessary for biosynthesis of cellular constituents; quantitatively, reduction of carbon dioxide is not necessarily a major process. ATP and its energy-rich precursors are formed by an autotrophic mechanism, that is, by light-induced cyclic phosphorylation. Reducing power is obtained by dark oxidation of organic substrate (or intermediates), and in some instances this requires an energy contribution from the phosphorylation system.

(*b*) *Autotrophic pattern*. The reductive pentose cycle operates as a major mechanism and together with other pathways enables the conversion of carbon dioxide to all cellular constituents. As in (*a*), the ATP required for these conversions, and energy for other purposes, is furnished by the cyclic phosphorylation system. The inorganic 'accessory' electron donor provides net reducing power through dark oxidation or by energy-linked reverse electron transfer.

The formation of molecular oxygen in green plant photosynthesis is considered to be a specific, and unique, consequence of the utilization of water as an electron donor for processes resulting in net production of physiological reductants. This particular means of generating reductants is absent from photosynthetic bacteria, and there is no compelling reason to assume that the alternative devices used by the bacteria to create reducing power must be 'minor variations' of the green plant mechanism. Emphasis on this important difference, its meaning and consequences, should not be construed as a cryptic argument against the notion of a general 'biochemical unity' in photosynthetic cells. Rather, I subscribe to the view[38] that recognition, and exploration of the basis, of 'biochemical diversity' is no less important for an adequate understanding of the mechanisms and evolution of specialized biochemical functions. An evolutionary transition from anaerobic photosynthesis of the bacterial type to the oxygen-producing photosynthetic mechanism of green plants would have required a significant number of alterations. The acquisition of a new and singular system, Light Reaction II, may well have played a major part in the evolution of photosynthetic cells capable of using water as a primary electron donor[4,14,39].

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INVESTIGATIONS OF LEUCOCYTE KINETICS IN NORMAL AND LEUKAEMIC INDIVIDUALS BY MEANS OF SCINTILLATION COUNTING

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SINCE the introduction of tritiated thymidine in 1955, it has become an important tool for the study of cellular proliferation both *in vivo* and *in vitro*[1]. It is of particular value in the investigation of *in vivo* leucocyte kinetics in normal and leukaemic individuals.

Tritiated thymidine has achieved this importance because it is a specific precursor for DNA and, since its β-emissions are of low energy, autoradiographs with high resolution can be obtained. However, the preparation and reading of autoradiographs are laborious and their interpretation extremely difficult. Accordingly, it occurred to us that liquid scintillation counting might be advantageous. This article presents some examples of the leucocyte kinetic curves after the *in vivo* administration of tritiated thymidine in normal individuals and in patients with leukaemia.

Patients admitted to the National Cancer Institute with a limited life expectancy were studied. So far three patients with localized malignancies have been given tritiated thymidine. These individuals were in good condition without evidence of malnutrition or haematopoietic disorders and were considered the 'normal' group for purposes of this study. Twelve untreated patients with

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chronic myelocytic leukaemia in relapse were studied together with three patients in blastic crisis and two patients with acute myeloblastic leukaemia.

Tritiated thymidine (specific activity 1·9 c./m.mole) was given intravenously at doses ranging from 40 to 100 μc./kg body-weight. At periodic intervals blood samples were obtained and processed by a method previously described[2]. The isolated leucocytes were washed in normal saline and an aliquot counted in an automatic cell counter (Coulter counter, model *B*, Coulter Electronics, Hialeah, Florida). A known number of leucocytes (about 1×10^6) was centrifuged at 2,100*g* for 10 min in a refrigerated International *PR*-2 centrifuge. The cell button was then hydrolysed and an aliquot counted in a liquid scintillation spectrometer with an efficiency for tritium of approximately 20 per cent as previously described[2]. Quenching was monitored by a channels ratio method and internal standards were used as indicated[3].

The leucocyte kinetic curves obtained were consistently reproducible within each group. There were no significant differences in two patients who were studied on two successive occasions. The amount of tritiated thymidine administered within the range used did not appear to influence either the shape of the curve or the degree of labelling. The statistical precision of the technique used will be reported separately.

Fig. 1 is a curve obtained in a 46-year-old patient with an epidermoid carcinoma of the face and illustrates the type of curve obtained in haematologically normal individuals. A low level of radioactivity is present for the first three days, but on the fifth day the level begins to rise, reaching a peak on the seventh or eighth day. As will be reported separately, the low level of activity during the first few days is due to labelled mononuclear cells, particularly lymphocytes from the thoracic duct.

In contrast to the curve obtained in normal individuals, the curve in patients with chronic myelocytic leukaemia is much more complex. Fig. 2 illustrates the results of a study in a 69-year-old woman with a white blood count of 178,500 per mm³ with the usual differential. Cytogenetic analysis of a bone-marrow aspirate revealed the presence of the *Ph*¹ chromosome. Heavy labelling is present immediately after the administration of the isotope. The level of radioactivity then declines in the first 10 h but then rises to an initial peak at 24–40 h. Another peak is present between 50 and 60 h and, finally, a third peak at 5–7 days after administration of the isotope. This last peak generally appeared a little earlier than the corresponding peak in the normal individuals.

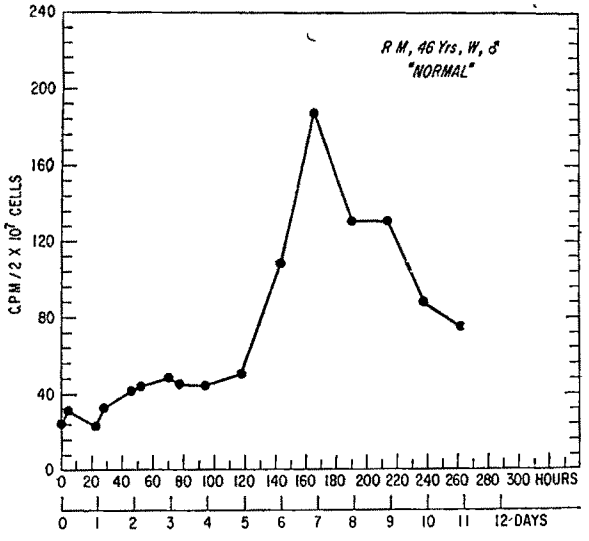


Fig. 1. Results of liquid scintillation counting of peripheral blood leucocytes from a normal individual after the administration of tritiated thymidine

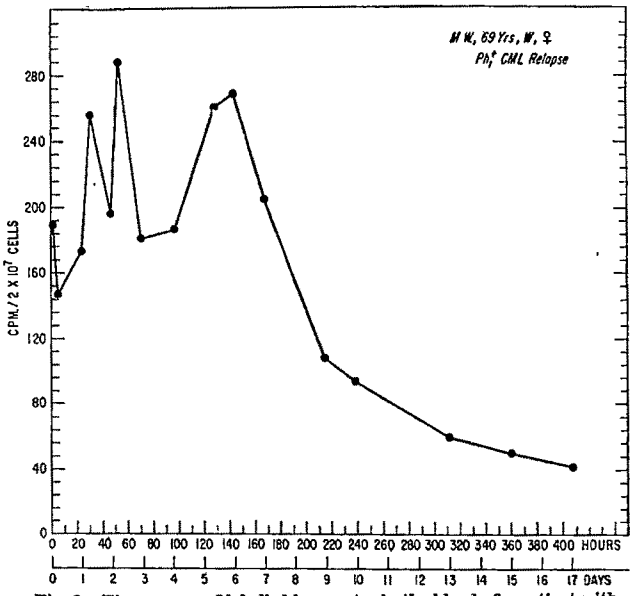


Fig. 2. Time course of labelled leucocytes in the blood of a patient with chronic myelocytic leukaemia

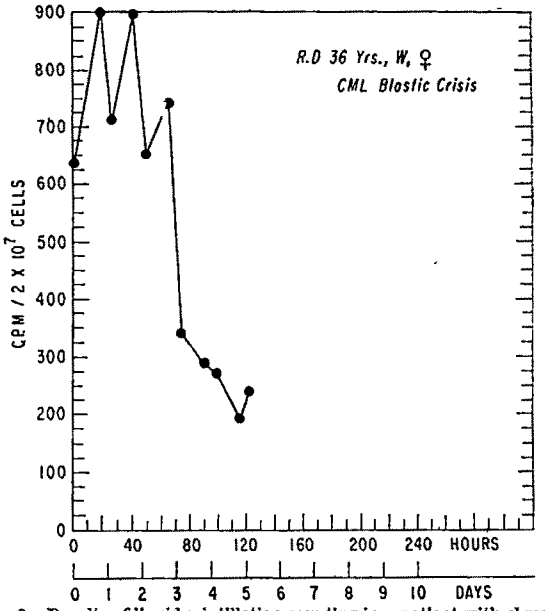


Fig. 3. Results of liquid scintillation counting in a patient with chronic myelocytic leukaemia in blastic crisis

Fig. 3 illustrates the results in a patient with chronic myelocytic leukaemia in blastic crisis. This was a 36-year-old woman with a white blood count of 4,937 per mm³ and 50 per cent blasts in the peripheral blood. Again there is an initially high level of radioactivity, rising rapidly to a double peak within 10–50 h and then declining with another small peak at 65 h. This curve is in marked contrast to that obtained in patients with chronic myelocytic leukaemia in relapse as illustrated in Fig. 2.

The leucocyte kinetic curve obtained in a patient with acute myeloblastic leukaemia is shown in Fig. 4. This was a 55-year-old man with a peripheral blood count of 81,600 per mm³, 76 per cent of the cells being blasts. Leucocyte labelling is high immediately after administration of the label, with a rapid rise to a peak between 20 and 40 h followed by another peak at 60 h. The general shape of the curve is quite similar to that found in the patients with chronic myelocytic leukaemia in blastic crisis as illustrated in Fig. 3.

The leucocyte kinetic curve obtained for a normal individual by scintillation counting is similar to the curve based on the per cent labelled cells in the peripheral blood

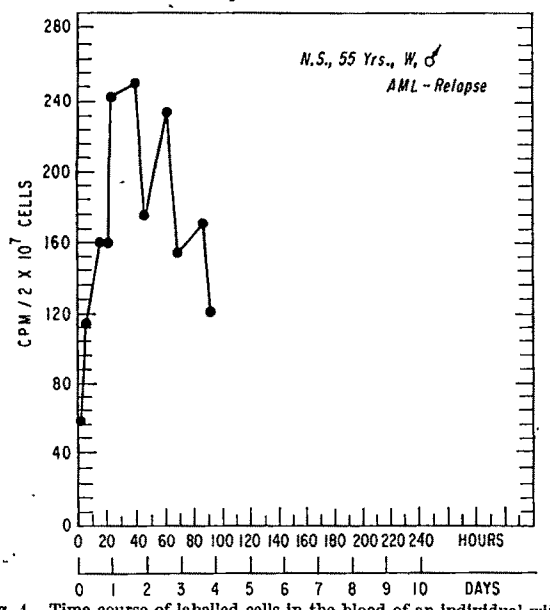


Fig. 4. Time course of labelled cells in the blood of an individual with acute myelocytic leukaemia

after tritiated thymidine reported by Cronkite[4]. In contrast, in patients with chronic myelocytic leukaemia the configuration of the leucocyte kinetic curves is much more complex. There is very early labelling with the appearance of two and often three peaks. These secondary peaks were also observed by the Brookhaven group on analysis of autoradiographs of peripheral blood from patients with chronic myelocytic leukaemia labelled with tritiated thymidine[5]. The secondary peaks were seen only at the lower grain thresholds and were considered to be fluctuations within the range of the theoretical counting error. However, since these secondary peaks are consistent and reproducible by scintillation counting, they are probably not merely random fluctuations. Recently, the Brookhaven group has suggested that the variations may be of physiological significance[6]. The interpretation of these peaks awaits analysis of autoradiographic material.

Finally, it is of interest that, kinetically, patients with acute myeloblastic leukaemia and patients in blastic crisis appear quite similar. In both instances there is early labelling with a rapid rise to peak radioactivity within a short time. It suggests that the cells in these diseases may be entering the circulation at a very rapid rate. Unfortunately, the complete curves have not been obtained in either situation as it has not been possible to continue beyond 4 or 5 days because of medical considerations.

Although tritiated thymidine is the best label for DNA which is at present available, it has a number of disadvantages. These have been considered extensively in previous publications, but the most serious problem in autoradiographs of blood smears is the low and variable geometric efficiency of tritium, which may be less than 5 per cent (ref. 7). This makes precise grain counting difficult and results in inaccurate estimates of cell kinetics. Using the scintillation counting technique, efficiency for tritium is greatly increased. In addition, it is possible to evaluate a greater number of cells than is usually read in autoradiography. Finally, background labelling, which often presents serious difficulties in interpretation of lightly labelled cells, is not a problem in scintillation counting. The principal disadvantage of scintillation counting for the study of leucocyte kinetics is the inability to discriminate among different cell types. However, by using both techniques simultaneously, it may be possible to overcome the most serious problems in the interpretation of leucocyte kinetic data. Such an investigation is now being carried out.

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INDUCTION OF MULTIPLE BRAIN TUMOURS (GLIOMATA AND LEPTOMENINGEAL SARCOMATA) IN DOGS BY ROUS SARCOMA VIRUS

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ATTEMPTS to produce autogenic (primary) malignant tumours in large mammals have usually been unsuccessful. No experimental model has yet been developed in which a predictable host response can be obtained under specified conditions governing exposure to a carcinogenic agent. Since proof of malignancy by transplantation is not feasible in large animals, the clinical-pathological characteristics of malignancy should be carefully followed. They may be briefly summarized: (*a*) histological and cytological evidence of malignancy; (*b*) progression by invasion of the surrounding tissues resulting in the death of the host; (*c*) metastasis from those types of induced tumours which are known to metastasize when occurring spontaneously. In the specific case of viral oncogenesis, cell-free preparations of virus (of known titre) should be used in order to avoid complicating immunological factors such as graft rejection.

In the experiments reported here, young dogs were inoculated intracerebrally with high doses of cell-free preparations of Rous[1] sarcoma virus (RSV). In a relatively short time, the dogs developed multiple brain tumours fulfilling the requirements of malignancy already stated. Oncogenicity of RSV has previously been documented in mice, rats, guinea-pigs and monkeys[2–7].

RSV cell-free pools of Bryan[8] strain (*B*-RSV) and of Schmidt-Ruppin[9] strain (*S*-RSV) were prepared from White Leghorn chicken wing-web tumours by the technique of differential centrifugation[10]. These two strains of RSV were shown to be capable of inducing gliomata in new-born hamsters inoculated intracerebrally[11,12]. The virus was suspended in 0·153 M potassium citrate and preserved at −70° C in sealed ampoules. For inoculation, the virus was concentrated and resuspended in phosphate buffer saline *p*H 7·2 (PBS) as previously described[11]. The preparations were titrated against a known standard in chorioallantoic membrane from embryonated hens' eggs.

Five litters of mongrel dogs (identified in Table 1 by numerals I–V) were used in these experiments. A total of 29 dogs were inoculated in the midportion of the right cerebral hemisphere (at a depth of from 5 mm to 10 mm) using a 20-gauge needle. Different volumes of virus suspension were used depending on the size of the dog. The

titres of these inocula, expressed in focus-forming units (F.F.U.), are shown in Table 1. In addition, two new-born dogs were inoculated subcutaneously in the right flank, one with $8{\cdot}4 \times 10^7$ F.F.U. of *B*-RSV, pool No. 35, and the other with $7{\cdot}5 \times 10^7$ F.F.U. of *B*-RSV, pool No. 39. Of the 29 animals receiving the intracerebral inoculations, three died within the first 72 h after treatment and three were killed accidentally. The results in the remaining 23 dogs are summarized in Table 1.

Neurological symptoms began as early as 15 days after inoculation. The dogs often became somnolent and opisthotonic or occasionally developed ataxia and unilateral hemiparesis. One dog suddenly became paraplegic while others showed marked cachexia. Unilateral proptosis and limb weakness were frequently found.

Five animals were kept until they died, while others were killed when they became moribund. Fourteen were killed in fair to good condition when they were 25–53 days old. Autopsies were performed on all of them. At gross examination, multiple tumour nodules were frequently found on the surface of the brain. They were generally more numerous near the infundibulum and in the posterior fossa. Hydrocephalus was often present and haemorrhagic areas were occasionally noted. Microscopically, multiple tumours were found on the pia-arachnoid and within the brain (Fig. 1*A*).

Table 1. DOGS INOCULATED INTRACEREBRALLY AT DIFFERENT AGES WITH RSV, SCHMIDT-RUPPIN AND BRYAN STRAINS, OBTAINED FROM CHICKEN WING-WEB SARCOMATA. THE VIRAL INOCULUM WAS TITRATED IN CHORIO-ALLANTOIC MEMBRANE OF EMBRYONATED HENS' EGGS. VALUES ARE EXPRESSED IN FOCUS-FORMING UNITS (F.F.U.)

Dog No./ Litter No.*	Inoculum titre/ Vol. (F.F.U. × 10^7/ml.)†	Age at death (days)	Apparent cause of death	Autopsy findings
Schmidt-Ruppin strain, RSV—Pool No. 44: Dogs inoculated less than 24 h old.				
1/I	5·8/0·35	28	Moribund when killed	Leptomeningeal sarcoma and glioma
2/I	7·4/0·45	30	,,	,,
3/I	7·4/0·45	49	In fair condition when killed	,,
4/II	9·1/0·55	31	Cachexia	,,
5/I	10·9/0·60	31	Cachexia	,,
Bryan strain, RSV—Pool No. 19: Dogs inoculated less than 24 h old.				
6/II	4·0/0·45	19	Intracranial haemorrhage	,,
7/II	4·0/0·45	49	In fair condition when killed	,,
8/II	4·0/0·50	18	Intracranial haemorrhage	,,
9/II	5·0/0·60	17	Moribund when killed	,,
Bryan strain, RSV—Pool No. 25: Dogs inoculated less than 24 h old.				
10/III	5·0/0·45	36	In fair condition when killed	,,
11/III	10·1/0·50	36	,,	Negative
Bryan strain, RSV—Pool No. 35: Dogs inoculated less than 24 h old.				
12/I	6·3/0·30	49	In fair condition when killed	Leptomeningeal sarcoma and glioma
13/I	8·4/0·40	49	,,	Negative
14/II	8·4/0·40	25	,,	Leptomeningeal sarcoma and glioma
Bryan strain, RSV—Pool No. 37: Dogs inoculated less than 24 h old.				
15/IV	7·8/0·35	38	In fair condition when killed	Leptomeningeal sarcoma and glioma
Bryan strain, RSV—Pool No. 39: Dogs inoculated less than 24 h old.				
16/V	7·5/0·35	27	In poor condition when killed	Leptomeningeal sarcoma and glioma
Bryan strain, RSV—Pool No. 39: Dogs inoculated when 8 days old.				
17/V	7·5/0·35	53	In good condition when killed	Negative
18/V	7·5/0·35	53	,,	Negative
Bryan strain, RSV—Pool No. 39: Dogs inoculated when 15 days old.				
19/V	7·5/0·35	53	In fair condition when killed	Leptomeningeal sarcoma and glioma
20/V	7·5/0·35	31	Cachexia	Brain abscess
Control—Thermally inactivated virus: Dogs inoculated less than 24 h old.				
21/III	10·1/0·50‡	36	In good condition when killed	Negative
22/IV	7·8/0·35§	38	,,	Negative
23/IV	7·8/0·36¶	38	,,	Negative

* Litters I, II, III, IV, and V refer to mongrel mothers: I—black hound, II—brown collie, III—brown boxer, IV—white collie, and V—brown hound.
† The viral inoculum was suspended in varying amounts of phosphate buffer saline (PBS) *p*H 7·2.
‡ Thermally inactivated virus (Bryan strain, RSV—Pool No. 25) at 75° C for 15 min.
§ Thermally inactivated virus (Bryan strain, RSV—Pool No. 37) at 75° C for 15 min.
¶ Thermally inactivated virus (Bryan strain, RSV—Pool No. 37) at 75° C for 60 min.

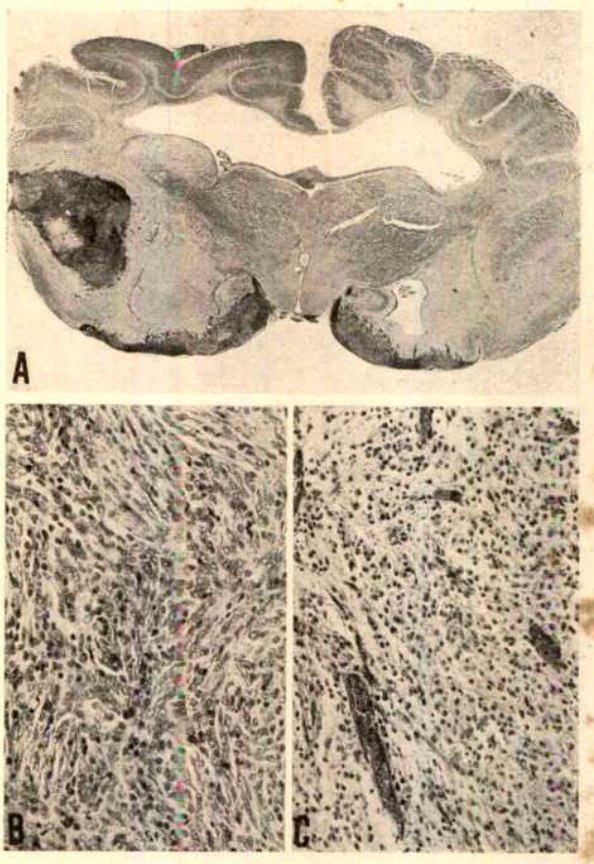


Fig. 1. Dog No. 2/I, 30 days old, injected intracerebrally at birth with $7{\cdot}4 \times 10^7$ F.F.U. of RSV, Schmidt-Ruppin strain. (*A*) Coronal section. Note multiple infiltrating sarcomata located on the cortical surface and the periventricular glioma (× *c.* 20). (*B*) Undifferentiated leptomeningeal sarcoma (× *c.* 235). (*C*) Subependymal astrocytoma (× *c.* 235)

The pia-arachnoid tumours were found to be poorly differentiated meningeal sarcomata, composed of cells which had oval vesicular nuclei and basophilic cytoplasm with thick processes (Fig. 1*B*). The deeply situated tumours were located in the periventricular subependymal tissue. These were made up of round glial cells with basophilic cytoplasm, thin fibrillar processes and numerous mitotic figures. These findings are consistent with the histological diagnosis of astrocytoma (Fig. 1*C*).

In one dog, a neoplasm found in the lungs was interpreted as a primary virus-induced tumour rather than a true metastasis. Subcutaneous fibrosarcoma-like nodules were frequently found over the site of intracerebral injection. These were probably induced by leakage of the virus along the needle tract. The failure of the virus-induced tumours to give rise to any clearly evident metastases is similar to the behaviour of spontaneous human and dog brain tumours, which rarely metastasize.

The two new-born dogs which were inoculated subcutaneously developed nodules which regressed within 30 days. The subcutaneous nodules in the scalps of the dogs which received intracerebral injections frequently grew smaller if the dogs survived for more than 1 month. Similarly regressing subcutaneous fibrosarcoma-like tumours induced by RSV have been observed in rabbits[13,14] and monkeys[15].

Three control animals inoculated intracerebrally with virus suspension thermally inactivated at 70° C for 15 and 60 min did not develop neoplasms during the 38-day

observation period. Chickens were injected intracerebrally in parallel with the dogs, using both active and heat-inactivated virus. Chickens inoculated with the heat-inactivated virus showed no evidence of tumour growth.

To the best of our knowledge, the tumours reported here are the first brain tumours which have ever been induced by viruses in large animals.

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A DEMONSTRATION OF ION-EXCHANGE PHENOMENA IN PHOSPHOLIPID MONO-MOLECULAR FILMS

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SINCE Rb^+ is a substitute for K^+ and Li^+ is a substiute for Na^+ in axonal potentials[1–3], the size and chargetof an ion have been thought by some to determine its pass age through the cell membrane[4]. The ionic radius of Cs^+ is 0·21 Å greater than the ionic radius of Rb^+ and is 1·09 Å greater than the ionic radius of Li^+ (ref. 5). One might expect, therefore, that Cs^+ would substitute for K^+. Experiments indicate, however, that Cs^+ acts neither like K^+ nor like Na^+ (refs. 2, 6). It has also been proposed that La^{+++} can displace Ca^{++} on the outer surface of the cell, since La^{+++} reversibly blocks the excitability of the axon and, when it is used as a stain in electron microscopy, the gap substance between myelinated axons appears densely stained[7,8]. In contrast with the large number of demonstrations of ion effects on the axon membrane, there have been relatively few experiments on the chemistry of these phenomena. The use of monomolecular films as structural models of the cell membrane[9] provides a system the properties of which could, by analogy, give insight into this matter.

Earlier experiments[9,10] indicate that among the molecular components of the cell membrane, phosphatidylserine may provide negatively charged sites for cation exchange. In the present work, therefore, emphasis was placed on phosphatidylserine films. Silicic acid chromatography was used to isolate this phospholipid from 'animal cephalin' (Nutritional Biochemical Co.) and its purity was tested by ascending paper chromatography[9].

The uptake of Ca^{++} from a hypophase containing 0·1 mmole calcium chloride at *p*H 6·00 ± 0·25 was measured by counting the number of β particles emanating from the surface of a hypophase containing calcium-45 before and after spreading of a phospholipid monolayer. An increase in count was interpreted to indicate adsorption of Ca^{++} to the film. The technique has been described in detail elsewhere[9].

The experiments of displacing Ca^{++} from phosphatidylserine monolayers by Li^+, Rb^+ and Cs^+ were designed to show whether these monolayers would discriminate between Li^+, Rb^+ and Cs^+. The amount of calcium-45 adsorbed by the film in the presence of different concentrations of lithium chloride, rubidium chloride and caesium chloride in the hypophase was taken as a measure of the efficiency of displacement of Ca^{++} by Li^+, Rb^+ and Cs^+.

Fig. 1 shows that Li^+, Rb^+ and Cs^+ each displace the same number of calcium ions from the polar groups of the phosphatidylserine monolayer at any given concentration. This experiment substantiates previous findings[9] with Na^+ and K^+ and demonstrates that there is no discrimination among these monovalent cations. Also, it demonstrates that Ca^{++} adsorption depends on competing cation concentration.

The polar head of a phospholipid molecule would have a variable net ionic effect as determined by polar group configuration. It was thought that contaminating the monolayer with an interacting molecule such as cholesterol[11,12] would alter the polar group configuration so as to modify the position of the different ionogenic groups of the phospholipid molecule as previously postulated[13]. If this were so, the contaminated monolayer would show different ion association properties and, perhaps, selectivity. Fig. 1 shows that addition of cholesterol (Sigma Chemical Co.) does not modify the result obtained with phosphatidylserine alone. For example, the number of calcium ions per phosphatidylserine molecule is 0·23 ± 0·04 for phosphatidylserine monolayers and is 0·19 ± 0·05 for 50 per cent phosphatidylserine; 50 per cent choles-

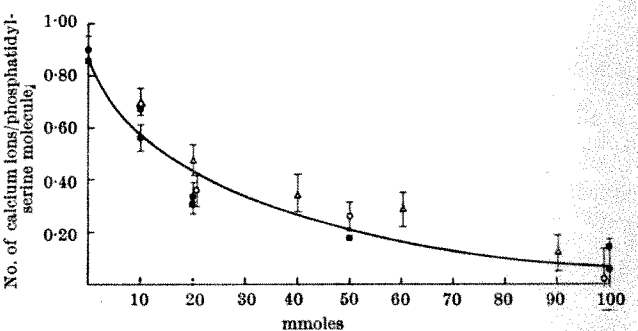


Fig. 1. Calcium displacement as a function of hypophase competing cation concentration. The vertical axis represents the number of calcium ions per phosphatidylserine molecule. The horizontal axis represents the concentration of competing counter ion in the hypophase. Each point represents the average of 3–8 experiments and the vertical bars represent twice the standard error. Area per molecule about 58 Å². Hypophase and monolayer, ●, CsCl, 0·1 mmole $CaCl_2$, PS; ○, LiCl, 0·1 mmole $CaCl_2$, PS; △, RbCl, 0·1 mmole $CaCl_2$, PS; ■, CsCl, 0·1 mmole $CaCl_2$, PS/C. PS, Phosphatidylserine; C, cholesterol

terol monolayers when the hypophase contained 50 mmole caesium chloride.

Thus, it seems to have been established that the adsorption of Ca^{++} by phosphatidylserine monolayer is equally prevented by equal concentrations of any of the following cations: Li^+, Na^+ (ref. 9), K^+ (ref. 9), Rb^+ and Cs^+. There are, however, some uncertainties about whether or not ion exchange occurs. Furthermore, no information is available about the time-course of this ion exchange.

The adsorption of Pm^{+++} (promethium) from a promethium chloride ($PmCl_3$) hypophase at *p*H 6·00 ± 0·25 was measured to determine whether or not ion exchange really occurs between the polar groups and the hypophase and to compare binding efficiency of Ca^{++} and La^{++} by measuring promethium-147 adsorption at different concentrations of calcium chloride or lanthanum chloride ($LaCl_3$).

Fig. 2 shows the time-course of the promethium-147 adsorption when the hypophase contains calcium chloride or lanthanum chloride. It can be seen that time is needed to reach the steady adsorption. Since phosphatidylserine is in its hydrogen form at the beginning of the experiment (as determined by flame photometry), the increase in counts measures the exchange of hydrogen (or Ca^{++} or La^{+++} also present in the hypophase) by Pm^{+++}. It can also be seen that about 500 times more Ca^{++} than La^{+++} is needed to produce the same equilibrium adsorption, starting from the same promethium chloride hypophase. From this experiment it can be concluded that (*a*) ion exchange actually occurs between the polar groups of the phospholipid mono-molecular film and the hypophase and (*b*) that La^{+++} is a strong competitor for the Ca^{++} binding sites. Therefore, the notion that La^{+++} displaces Ca^{++} from binding sites at cell surfaces seems to be well supported and could explain why La^{+++} reversibly blocks the excitable membrane and why the gap substance between myelinated axons appears densely stained when La^{+++} is used as stain in electron microscopy[7,8]. Finally, the idea of an ionic exchanger polar-group phase associated to phosphatidylserine mono-molecular films[9] seems to be supported and is consistent with both the molecular model for the cell membrane[14] and ion-exchange properties postulated for cell surfaces[15,16].

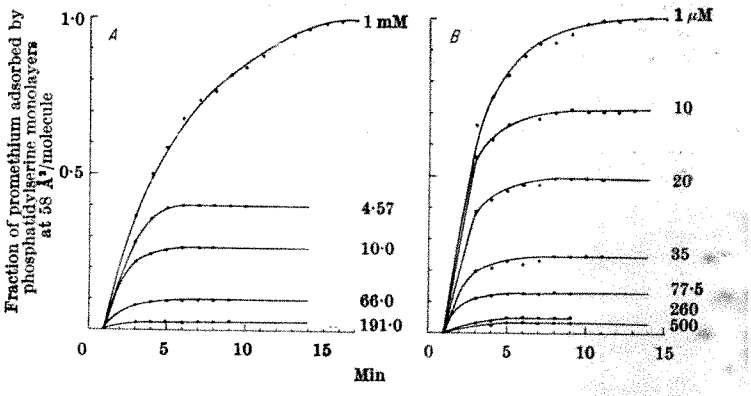


Fig. 2. Vertical axis represents the fraction of promethium adsorbed by phosphatidylserine monolayers, that is, adsorption of promethium when the hypophase contains a given concentration of calcium chloride (Fig. 2*A*) or lanthanum chloride (Fig. 2*B*) divided by the adsorption of promethium when the hypophase contained only 1 mmole lanthanum chloride. Each curve represents one experiment. Horizontal axis represents time in min starting with the solution without the monolayer. The film was spread at time 1 min and the maximum increase in count due to the film was 20,000 c.p.m. *A*, Hypophase, 1 μmole lanthanum chloride and calcium chloride; *B*, hypophase, lanthanum chloride

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A RE-INTERPRETATION OF THE ROLE OF THE MAST CELL

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ALTHOUGH the lung and uterus of rats are known to contain very few mast cells[1], it was found that structures resembling these cells were apparent in sections which had been stained by the histochemical reaction for monoamine oxidase[2]. These tissue elements did not appear to be coloured by the formazan developed in this reaction, but were yellow-brown, as if they had formed an adrenochrome-like pigment from the tryptamine used in the histochemical test. They could not be identified when this substance was excluded from the test, nor could they be found when un-incubated sections were examined fresh, or after staining with methyl green and pyronin. This indicated that the incubation, or the catecholamine, may have induced some change in these elements that caused them to become apparent. To test this possibility, serial sections of the lung of a rat of 5–6 weeks of age were stained with methyl green and pyronin after incubation for 2 h in the medium used in this test, with and without the tryptamine, or with histamine in its place. In all, there was a noticeable increase in the population of mast cells over that seen in sections which were not incubated before they were stained.

However, to test this hypothesis more carefully, pieces of lung from rats, 5–6 weeks old, were maintained in a synthetic, non-proliferative culture system as described by Trowell[3]; each piece weighed about 2 mg. After 24 h of such culture, some pieces were removed and chilled at −70° C in hexane (B.D.H.: "free from aromatic hydrocarbon" grade); the medium in which the other pieces were maintained was changed to a similar solution which also contained 10^{-4} M histamine. In one experiment pieces were taken 5, 15 and 30 min after this change; in the others after only 15 and 30 min; all were chilled in hexane at −70° C. Sections were cut at 8μ in a cryostat refrigerated to about −30° C in which the knife was further cooled with solid carbon dioxide packed around its haft.

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They were stained with a mixture of methyl green and pyronin both to demonstrate the structure of the tissue and to aid the recognition of the mast cells. In other studies it was found that the cells which were identifiable as mast cells by this procedure seemed to correspond with those seen, albeit more weakly, with toluidine blue. The area of the sections was measured by planimetry of the image, magnified by the use of a photographic enlarger.

When fresh tissue sections were studied in this way, those of lung taken direct from the rat showed about two mast cells per cm^2 of section. In two series of experiments, culturing for 24 h alone increased the number to 8 and 4 per cm^2. However, the effect of adding histamine to the medium was more profound. The number increased to 20 per cm^2 after exposure for 5 min to this substance in culture; it became 40 and 66 after contact for 15 min and then declined to 0 and 13 after exposure for 30 min to histamine. These results are mean values for a number of sections. In contrast, this effect was not found if the tissue was kept at $-70°$ C for some days before it was sectioned and stained. This may be related to other histochemical changes found when such chilled tissue is stored at this temperature. Qualitatively, the mast cells observed after contact for 15 min with histamine were large and swollen, with prominent strongly staining granules, whereas after 30 min contact there were many granules lying in what appeared to be burst cells. The mast cells in both the treated and the normally cultured tissue occurred mainly at the periphery; this may be a reflexion of the conditions of culture.

The brown reaction given by the tissue elements suspected of being mast cells was investigated both in rat lung which had been maintained in culture, and in sections of mouse skin which had been subjected to a carcinogenic agent, tri*cyclo*quinazoline (TCQ) or to its various derivatives[4]. The advantage of the mouse tissue was that there was a profusion of mast cells localized in a definite region; they were stained distinctly by the methyl green-pyronin method. In every case, but particularly clearly at the base of the growth produced by prolonged treatment with the carcinogenic agent, the mast cells stained strongly with the histochemical method for monoamine oxidase even when 'Marsilid' was added as an inhibitor of monoamine oxidase activity in normal cells; however, the mast cells were coloured yellow-brown, or purple-brown, and not with the colour usually associated with the formazan of nitroblue tetrazolium. Similarly, in sections of carcinogen-treated mouse skin, a moderate yellow-brown colour was found in the mast cells of sections which had been incubated in the presence of histamine and nitroblue tetrazolium, which gave no reaction in the cells of the rest of the skin.

From these results two main features emerge. First, it would seem that mast cells, in the rat, can be hidden in tissue but that they swell and so become visible in response to these conditions of culture and, more strikingly, to histamine. This seems clear since, because the tissue was isolated and maintained in culture, the increase in numbers could not be due to migration into the tissue. The change in shape and size, effected by exposure to histamine, supports such a view. The second feature derives from the histochemical finding that, although these mast cells do not seem to contain appreciable amounts of amine oxidase activity, they do produce a yellow-brown pigment in response to tryptamine and, more weakly, even to histamine; the presence of a hydrogen-acceptor, here nitroblue tetrazolium, may be beneficial to this process. These results may indicate the presence of a system which can form an adrenochrome-like substance.

At first sight these findings and conclusions seem rather confusing since they appear to be at variance with the usual concept of the function of mast cells. It is generally believed[1,5] that mast cells accumulate in damaged regions and eject histamine into such tissues. This belief is largely based on the facts that mast cells are found in large numbers in damaged tissues, that they contain a bound form of histamine, and that they may become disrupted and apparently lose their granules in damaged tissue which shows the traumatic effects of histamine. In the present investigation it has been shown that exogenous histamine caused an apparent accumulation of mast cells but, since they could not have come from the synthetic culture medium, it is more likely that the histamine merely made them visible. Moreover, it was found that, although exogenous histamine allowed them to swell, prolonged exposure to such a high concentration burst the cells and disrupted the granules. It would appear that catecholamines and related substances are capable of changing the permeability of the membranes of mast cells just as they do those of other tissue cells (unpublished data). Thus it is possible to re-interpret the evidence underlying the concept that mast cells congregate in damaged tissue and contribute to the damage by ejecting histamine: it is more likely, on the data presented in this article, that they are responding to elevated levels of tissue-histamine. The other reason for considering mast cells to be emitters of histamine is that they have a high concentration of bound histamine[1,5]. However, the evidence that they may have a system which is capable of converting tryptamine and histamine into a pigment, like an adrenochrome or a melanin, indicates that they may be 'detoxicating' histamine rather than ejecting it. Only with grossly excess tissue-histamine would they become disrupted and release their normally bound histamine into the tissue. Such concepts may be of considerable significance not only in problems concerning tissue injury generally but also in anaphylactic shock[1].

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LARGE AMOUNTS OF DEOXYPHENYLALANINE IN THE HYDROLYSATE OF MELANOSOMES FROM HARDING–PASSEY MOUSE MELANOMA

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DOPA has never been shown to be a constituent of any type of protein, although it has been found in free form in the seeds of certain plants[1–3]. This article reports our finding that it is possible to isolate and identify dopa from Harding–Passey mouse melanoma by hydrolysis of the trichloroacetic acid (TCA) precipitate of homogenized tumour tissue with strong hydrochloric acid.

Two types of melanoma were investigated: Harding–Passey melanoma (HP) grown in albino Swiss mice, and *B*-16 melanoma grown in *C*-57 black mice. Immediately after excision, 2 g of the tumour to be investigated was

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homogenized in 100 ml. of 10 per cent TCA, centrifuged, again thoroughly homogenized in 10 ml. of fresh 10 per cent TCA and then recentrifuged. The precipitate thus obtained was hydrolysed for 12–18 h in 300 ml. of 6 N hydrochloric acid with refluxing, cleansed of melanin by filtration through a glass funnel and evaporated almost to dryness. Half the residue was used for chromatographic isolation and spectrofluorophotometric assay[4] by a method recently devised in our laboratory. The pooled TCA supernatant was diluted with about 5 volumes of water and placed on a 'Dowex-50-H$^+$' resin column (1·0 × 6·0 cm) for removal of the TCA. The column was then washed with 50 ml. of 0·2 N hydrochloric acid followed by 100 ml. of water. The material still remaining on the resin was eluted with 100 ml. of 0·5 N hydrochloric acid, evaporated to dryness under reduced pressure at low temperature (50°–60° C), and subjected to dopa analysis[4].

The amounts of dopa recovered from 2-g specimens were: 1·0 μg/g and 230·4 μg/g of fresh tissue respectively from the HP supernatant and precipitate; 0·6 μg/g and 8·4 μg/g of fresh tissue from the *B*-16 supernatant and precipitate. In a second series of experiments with 5 g of tumour tissue, the TCA supernatants were found to contain almost negligible amounts of dopa, while hydrolysates of the TCA precipitates of HP and *B*-16 tumours contained approximately 239 μg/g and 11 μg/g of fresh tissue respectively. These findings indicate that neither type of tumour contained an appreciable amount of free dopa, but that relatively large amounts of dopa were present in the TCA precipitates of HP tumour after hydrolysis with strong acid. Dialysis of the TCA precipitates against water and repeated freezing of suspensions of the precipitates failed to release dopa into the suspending medium.

To permit accurate identification of the compound that gave high dopa-like values in chromatographic analysis of the hydrolysate of the TCA precipitate of HP melanoma, three experiments were carried out with large amounts of HP melanoma tissue. In each of these experiments, 100 g of freshly excised HP melanoma were thoroughly homogenized in 200 ml. of 20 per cent TCA and centrifuged until the precipitate separated out. The resulting supernatant was discarded; the precipitate was again homogenized in 100 ml. of 20 per cent TCA and centrifuged a second time. The dark precipitate obtained from this second centrifugation was hydrolysed for 24 h in 1,000 ml. of 6 N hydrochloric acid with refluxing, 100 ml. of concentrated hydrochloric acid being added twice during the 24-h period. After hydrolysation, the insoluble melanin was removed by centrifugation. The supernatant was concentrated to a syrup under reduced pressure at low temperature (50°–60° C) and redissolved in 1,000 ml. of water. An extra step was necessary to remove the excess acid and other extraneous material from this syrup. The syrup was therefore redissolved in 1,000 ml. of water and put on a 'Dowex-50-H$^+$' resin column. After it had passed through the column the resin was washed with 1,000 ml. of 0·2 N hydrochloric acid followed by 2,000 ml. of water. The material still remaining on the resin was then eluted with 2,000 ml. of 0·5 N hydrochloric acid. The eluate was evaporated to dryness under reduced pressure at low temperature. Since dopa is relatively stable in basic borate buffer[4,5], 1,000 ml. of borate buffer (*p*H 9·0) containing 7·63 g of sodium borate and 0·62 g of boric acid were added to the evaporated eluate and the *p*H adjusted to approximately 8·6 with dilute sodium hydroxide solution. For convenience of manipulation, the resulting preparation was divided into ten 100-ml. aliquots, each of which was diluted with 100 ml. of water. These aliquots were applied to ten 'Dowex-2-borate$^-$' resin columns and ten 'Dowex-50-H$^+$' resin columns[4]. The eluates thus obtained were then pooled and evaporated to dryness under reduced pressure at low temperature. The dried residue was dissolved in 25 ml. of aqueous borate buffer (*p*H 9·0) and further purified by gradient-elution chromatography on a 'Dowex-2-Cl$^-$' resin column (2·0 × 15·0), 800 ml. of 0·5 per cent aqueous boric acid solution being used as the mixer and 0·5 per cent boric acid in 0·3 N hydrochloric acid as the reservoir. 6-ml. fractions were collected at a flow rate of 1 ml./min. Before elution started, 20 ml. of 0·5 per cent aqueous boric acid solution was layered over the top of the column to prevent mechanical disturbance. The ultra-violet absorption spectra of the compounds recovered from the column were determined by spectrophotometry.

An unidentified compound (*A*), the ultra-violet absorption of which was maximal at 281 mμ, appeared first in fraction No. 27 and was last present in fraction No. 37; its concentration was highest in fraction No. 28. A very small amount of material (*B*), the ultra-violet absorption of which was maximal at 275 mμ, was found in fraction No. 17—the fraction that theoretically should contain tyrosine. The total quantities of compound *A* recovered in the three experiments were 16, 12 and 11 mg when calculated as dopa from the optical density at 281 mμ of the dopa standard.

The fractions containing compound *A* from all three experiments were pooled and again put on a 'Dowex-50-H$^+$' resin column (2·0 × 5·0 cm) for further purification by gradient-elution chromatography. 400 ml. of water was used as the mixer, 8 N hydrochloric acid was the reservoir, the fractions were each 6 ml., and the flow rate was 1 ml./min. Compound *A* was found to be present in fractions No. 23 to No. 33. These fractions were pooled and evaporated to dryness under reduced pressure at low temperature. After hydrochloric acid had been removed in a sodium hydroxide–calcium chloride desiccator, the compound was dissolved in 4 ml. of distilled water and brought to approximately *p*H 5 by the careful addition of sodium bisulphate. About 1 ml. of ethanol was added before this purified material was crystallized in a deep freeze. Finally, the crystals were centrifuged and washed with 1·0 c.c. of cold distilled water and dried in a desiccator. The amount obtained was approximately 34 mg.

Absorption of ultra-violet light by purified compound *A* was maximal at 281 mμ in 0·5 N hydrochloric acid; absorption of ultra-violet by the known dopa standard was also maximal at 281 mμ in the same medium.

When compound *A* was oxidized by ferri-cyanide (0·05 per cent in 1 M acetate buffer) at *p*H 6·0 and made alkaline with 20 per cent aqueous sodium hydroxide containing ascorbic acid[4], the spectra of the activation and emission of fluorescence were identical with those of a standard dopa control preparation, the maxima for activation and emission being 375 and 480 mμ respectively (Figs. 1 and 2).

When compound *A* and its product were tested by paper chromatography following incubation with tyrosine

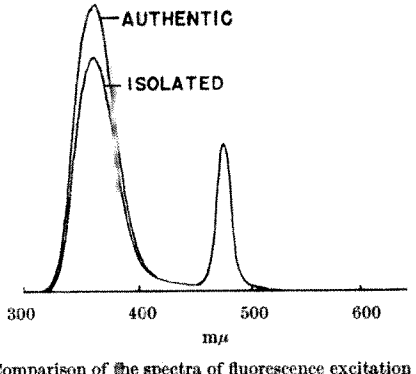


Fig. 1. Comparison of the spectra of fluorescence excitation (emission at 480 mμ) for the compound isolated from Harding–Passey melanoma and the control sample of dopa after oxidation with ferri-cyanide[4]. The curve of excitation for the material isolated from HP melanoma is lower than that for the control solution due to the presence of inorganic impurities. Fluorescence was measured with an Aminco–Kiers spectrofluorophotometer at slit No. 3. The concentration of both samples was 1 μg/ml.

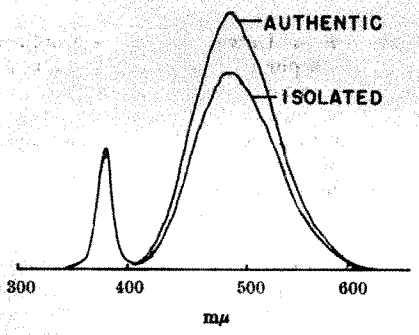


Fig. 2. Comparison of the spectra of fluorescence excitation (excitation at 375 mμ) for the compound isolated from Harding–Passey melanoma and the control sample of dopa after oxidation with ferri-cyanide[4]. The curve of excitation for the material isolated from HP melanoma is lower than that for the control solution because of the presence of inorganic impurities. Fluorescence was measured with an Aminco–Kiers spectrofluorophotometer at slit No. 3. The concentration of both samples was 1 μg/ml.

decarboxylase in the presence of pyridoxal phosphate, spots were produced identical with those produced by dopa and dopamine; compound *A* and the controls had identical R_F values in three different solvent systems under sulphur dioxide and produced the same colour changes in the ferri-cyanide reagent, — red → purple → black (Fig. 3).

When compound *A* was incubated with mushroom tyrosinase in phosphate buffer at *p*H 6·8, the red colour that instantly developed had a broad ultra-violet absorption band with a flat peak at about 475 mμ; this could not be distinguished from that of the red colour of the dopachrome produced in the dopa : mushroom-tyrosinase reaction.

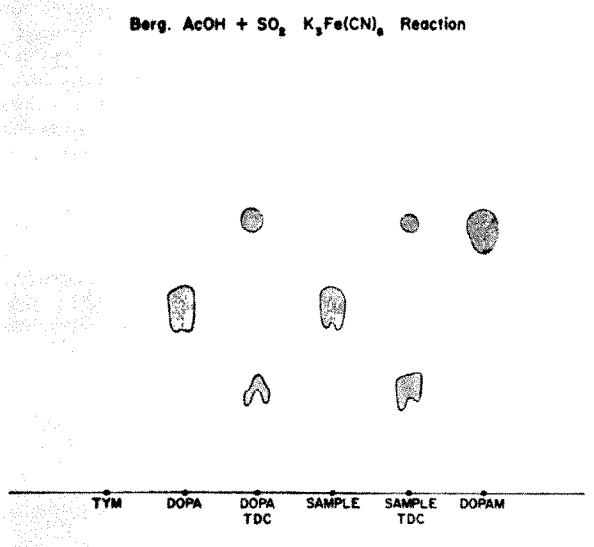


Fig. 3. A paper chromatogram of the compound isolated from HP melanoma and its product after incubation with tyrosine decarboxylase. The solvent system consisted of Berg's solvent (methanol : butanol : benzene : water in the proportion of 2 : 1 : 1 : 1) acidified with 1 per cent glacial acetic acid. The chamber of the chromatograph was filled with gaseous sulphur dioxide. For identification of the *o*-dihydroxyphenolic compounds, the paper was sprayed with 0·05 per cent potassium ferri-cyanide in 0·2 M acetate buffer (*p*H 6·0)

To determine the intracellular localization of compound *A*, which is probably contained in a protein, 10 g of HP melanoma were homogenized in about 9 volumes of 0·3 M aqueous sucrose solution and separated into cell-organelle fractions by the density-gradient centrifugation method[6]. After precipitation by TCA and when dried under an infrared lamp, the proteins in each fraction were hydrolysed for 12–18 h in 300 ml. of 6 N hydrochloric acid with refluxing. The insoluble melanin in each fraction was removed by filtration and the hydrolysates of the different fractions were evaporated to dryness *in vacuo* at low temperature. One-tenth of the dry residue of each fraction was used for dopa analysis.

The amounts of dopa found in the various cell fractions of HP tumours are shown in Table 2. These results indicate that most of the dopa-containing protein is localized in melanosomes where the greatest amount of tyrosinase activity is also found. The fact that various types of treatment other than hydrolysis with strong acid failed to release dopa from the melanosomes may indicate that dopa is present within the protein molecule. To verify this concept, 10 g of HP mouse melanoma were homogenized twice in 50 ml. of 20 per cent TCA; the precipitate was dialysed against distilled water to remove the TCA and then incubated for 12–18 h at 37° C with about 2 g of pepsin in 100 ml. of 0·05 N hydrochloric acid. At the end of the incubation period, the mixture was put into a 'Cellophane' bag and dialysed for 7 h against 1,000 ml. of water acidified with a few drops of hydrochloric acid. Absorption of ultra-violet light by the dialysate was maximal at 275 mμ; its optical density gradually increased during this dialysis. Half the dialysate was frozen, the other half hydrolysed in 6 N hydrochloric acid overnight with refluxing. 100-ml. aliquots of the dialysate were analysed for dopa. The total dopa content of the dialysate was 39·7 μg before hydrolysis, 97·3 μg after hydrolysis. In a similar test made with the large-granule fraction obtained from HP melanoma by density-gradient centrifugation the dialysate contained 29·7 μg of dopa before hydrolysis and 77·9 μg after hydrolysis. In both experiments the dopa content was approximately 2·5 times greater after than before hydrolysis with strong acid.

Table 1. Amounts of Dopa found in the Various Fractions of HP and *B*-16 Mouse Melanoma

	Fractions	HP (μg/g)	*B*-16 (μg/g)
1	Supernatant	1·0	0·6
	TCA ppt.	230·4	8·4
2	Supernatant	—	—
	TCA ppt.	239·0	11·0

Table 2. Amounts of Dopa found in the Various Fractions of HP Mouse Melanoma*

Fractions	Dry weight (mg)	Melanin	Dopa (μg)
Nuclear fraction	332	+ +	204
Soluble protein	571	–	32
Small granules	256	±	57
Mitochondria	306	±	30
Melanosome	940	+ + +	885

* Some fractions contained unhydrolysable melanin.

Some of the dopa released from HP-melanoma protein by pepsin digestion could have been localized at the N-terminal of the protein molecule. It is clear, however, that in addition to liberated dopa, the hydrolysate contains dopa derivatives, probably dopa-peptides. An attempt is now being made to isolate dopa peptides from weakly hydrolysed HP-melanoma protein. The absence of dopa-containing proteins in *B*-16 melanoma might indicate that there is a genetic difference in the melanosomal proteins of HP and *B*-16 melanoma which could account for the characteristic difference in the colour of these tumours.

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LUTEINIZING HORMONE RELEASE FOLLOWING PREOPTIC STIMULATION IN THE MALE RAT

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DIFFERENTIATION of the neural control of gonadotropin secretion depends on sex steroid secretion shortly after birth in the male or female rat. Regardless of the genetic sex of the rat, the non-cyclic male pattern of gonadotropin secretion develops in the presence of androgen or excessive oestrogen early in life. On the other hand, in the absence of sufficient levels of sex steroids the cyclic female pattern ensues[1–3].

Experiments carried out by Harris and Jacobsohn[4] and Martinez and Bittner[5] revealed that the nervous system is the site of differentiation for the appropriate control of gonadotropin secretion, not the hypophysis as supposed by Pfeiffer[1]. Regardless of the sex of the donor, pituitary grafts beneath the median eminence of hypophysectomized male or female animals assumed the functional characteristics associated with the respective sex of the host. Pituitary tissue from either male or female rats grafted beneath the median eminence of hypophysectomized female rats maintained normal oestrous cycles[4]. Identical results were obtained in investigations in mice[5]. Furthermore, in castrated male mice bearing ovarian and vaginal grafts, continuous secretion of gonadotropins occurred when either male or female pituitary tissue was grafted beneath the median eminence.

The cyclicity of gonadotropin secretion appears to depend on sex steroids in circulation as well as on the appropriate differentiation of the neural regulatory systems. Vaginal transplants were used as indicators of rhythmic or continuous gonadotropin secretion in castrated female and male rats bearing ovarian transplants[6]. Concentric layers of cornified tissue interposed with nucleated epithelial cells and leucocytes in vaginal grafts revealed cycling of ovarian hormone secretion and therefore cyclic gonadotropin release. Cyclic gonadotropin secretion was noted only in otherwise intact females with intrasplenic vaginal grafts. When sex steroid release to the peripheral circulation was greatly reduced by grafting ovarian tissue into the spleen of the castrated male or female rat bearing intrasplenic vaginal transplants, evidence of cyclic gonadotropin secretion was absent. Cyclic gonadotropin secretion was also lacking in castrated male rats bearing intrasplenic vaginal transplants with ovarian tissue grafted outside the spleen.

'Electrochemical' stimulation of the preoptic brain in the pentobarbital-blocked rat during proestrus or on the third day of dioestrus in the 5-day cyclic rat induces the normal ovulatory response[7]. In androgen-sterilized female rats the threshold of preoptic stimulation for ovulating hormone release appears to be raised[3,8]. With these experiments in mind, an investigation was initiated in castrated adult male rats bearing ovarian grafts. Ovarian tissue under these conditions remains primarily follicular in nature[9]. It was of interest to determine whether ovulation and/or luteinization could be produced in such experimental animals by stimulation of the hypothalamus. If this were so, it would reflect the release of luteinizing hormone from the anterior pituitary.

Rats were obtained from a locally inbred Osborne-Mendel derivative strain and maintained on Purina 'Laboratory Chow' and water *ad libitum*. From birth they had been exposed to regular daily illumination for 14 h. Male rats were castrated when about 30 days old, and in each animal ovarian tissue (half an ovary) from a littermate was transplanted beneath the kidney capsule. At about 90 days of age further procedures were instituted: (1) 'electrochemical' stimulation of the medial preoptic area under pentobarbital sodium (31·5 mg/kg); (2) simple electrode placement in the medial preoptic area; (3) injection of a known ovulating dose of NIH-LH-B_1 (unpublished observations, 8 μg/100 g body-weight). The electrical parameters of stimulation were 10 μamp d.c. for a period of 20, 40, or 60 sec, and anodic current was applied through the 0·13 mm core of a stainless steel concentric bipolar electrode stereotaxically introduced. Histological procedures for ovarian and brain tissues were described previously[7].

The response of the castrated male animal to preoptic stimulation was much like that which can be expected in the female. Luteinization of the ovarian transplants followed in nearly all rats receiving stimulation by 10 μamp over a period of 60 sec (Table 1). Three animals were autopsied the morning following stimulation and showed numerous fresh corpora lutea. Some of the corpora lutea had entrapped ova, while others did not. The rest of the animals (six) in this group were autopsied 3 days after preoptic stimulation (Fig. 1). Five of six animals had ovaries with marked formation of corpora lutea (Fig. 2).

The threshold for responsiveness in the castrated male rat appears to be slightly higher than in the adult proestrous female rat. A group of animals received low intensity preoptic stimulation (10 μamp d.c./20 sec), comparable to that required to evoke ovulation in all pentobarbital-blocked proestrous rats[7]. This threshold is somewhat lower than that necessary to induce ovulation by preoptic stimulation in late dioestrus of the 5-day cyclic rat (10 μamp d.c./40 sec), and has been correlated with the lower level of luteinizing hormone required to induce ovulation in the former animals[7,10]. In castrated male rats bearing ovarian transplants, preoptic stimulation with 10 μamp/20 sec evoked the release of luteinizing hormone in four out of eight animals. Increasing the duration of the stimulus to 40 sec evoked gonadotropin release and corpus luteum formation in all animals.

Animals submitted only to the stereotaxic and operational procedures with the insertion of the electrode into the preoptic hypothalamus but without passage of electric current demonstrated uniform absence of luteinization (Fig. 3).

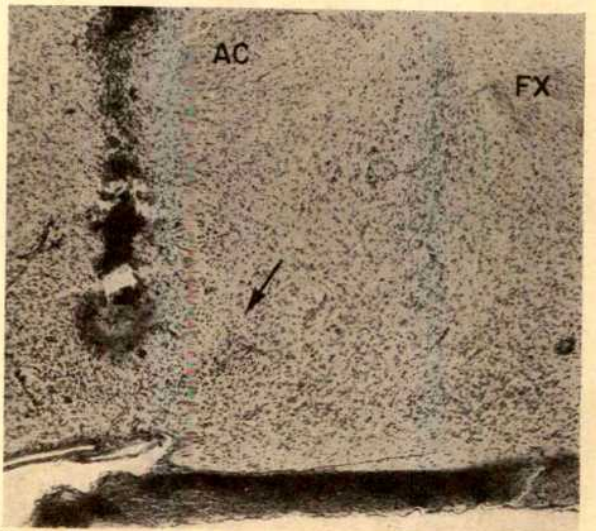


Fig. 1. Parasagittal section of brain illustrating a typical location of the electrode path and nature of the irritative focus established following 'electrolytic' stimulation (10 μamp d.c./60 sec). Arrow indicates approximate outer limit of lesion. *AC*, Anterior commissure; *FX*, fornix. (× c. 35)

Injection of a known ovulating dose of luteinizing hormone into castrated male rats with ovarian transplants produced marked luteinization.

Preoptic stimulation thus abruptly augments the release of luteinizing hormone in the male as it does in the female rat. Ovulation and corpus luteum formation occur in ovarian transplants following stimulation in the castrated male. Apparently the production and release of luteinizing hormone releasing factor by the neural tissue and of luteinizing hormone (LH) by the anterior pituitary are entirely adequate in castrated male animals bearing ovarian transplants to allow for an 'ovulatory surge' of LH following preoptic stimulation. The threshold for this response is comparable with that on the third day of dioestrus in the 5-day cyclic rat and slightly higher than that for the day of proestrus in the 4-day cyclic rat[7]. By contrast, the preoptic threshold for stimulation of LH release in the androgen-sterilized female rat is higher than in the normal female[3,8].

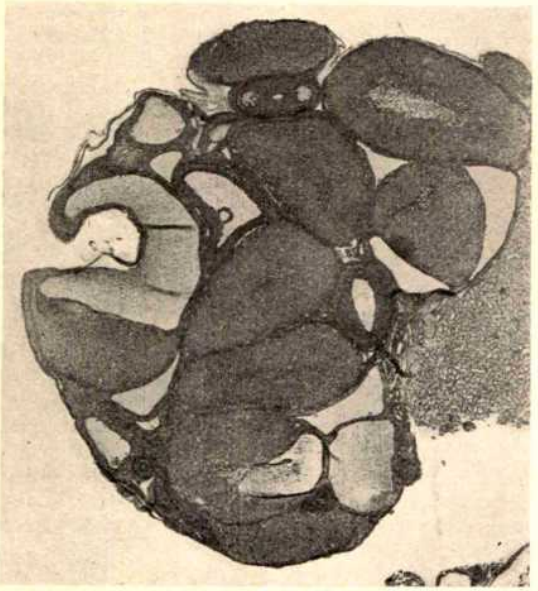


Fig. 2. Transplanted ovarian tissue demonstrating corpus luteum formation following preoptic stimulation of the castrated male rat. (× *c.* 20)

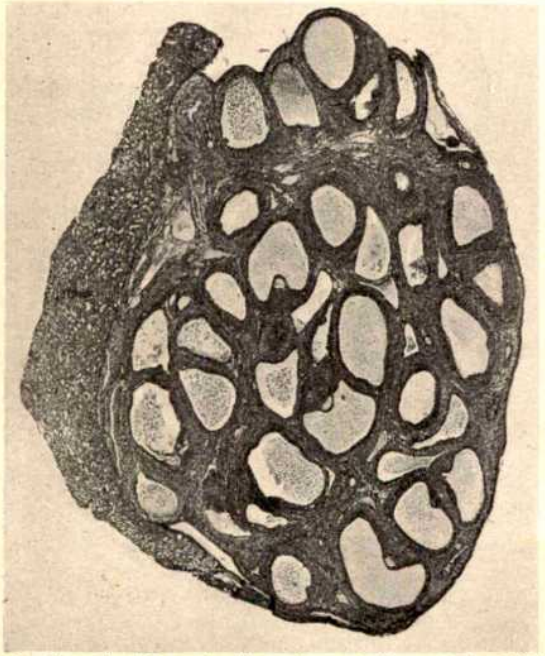


Fig. 3. Transplanted ovarian tissue demonstrating vesicular follicles and absence of corpora lutea following simple electrode insertion into the preoptic area of the castrated male rat. (× *c.* 20)

Table 1. Results following Preoptic Stimulation in Castrated Male Rats bearing Ovarian Transplants

Treatment	Results	
	No. with luteinization	No. without luteinization
10 μamp/60 sec*	8	1
10 μamp/40 sec	3	–
10 μamp/20 sec	4	4
Electrode placement	–	3
LH injection	2	–

* 'Electrochemical stimulation' in the medial preoptic area.

Evidence from other experiments suggests the similarity in the response of the male and female rat with respect to gonadotropin secretion. Progesterone treatment in the castrated male rat bearing ovarian transplants provokes augmented release of luteinizing hormone to such an extent that corpus luteum formation takes place[11]. In the intact female, ovulation can be advanced one day (5-day cyclic rats) by progesterone treatment on the third day of dioestrus[12]. In some types of constant oestrous animals (spontaneous, light-induced, or with anterior hypothalamic lesions) progesterone treatment produces ovulation[13–16]. The failure to obtain ovulation with progesterone treatment in androgen-treated females is difficult to explain, except on the basis that the changes which take place after the treatment may be far more extreme than occur in the normal animal[16]. This situation may be comparable with that in animals with lesions in the suprachiasmatic-medial preoptic area much larger than necessary to induce constant oestrus. These animals also fail to ovulate following progesterone treatment[16].

The analogous responses of the male and female rat to preoptic stimulation or progesterone treatment suggest that the differences known to exist in the neural control of gonadotropin secretion in the two sexes may, indeed, lie outside the preoptic-anterior hypothalamic-median eminence-pituitary system. The difference may exist in the nature of the afferent impulses to this neural region or the inability of the cells in the hypothalamus to interpret the afferent impulses. The fact that the hypothalamic system in the castrated male rat bearing ovarian transplants responds in a way similar to that of the intact female cannot be over-emphasized. Apparently, the necessary quantities of luteinizing hormone-releasing factor and pituitary LH are quite sufficient to induce ovulation when the appropriate stimulus is supplied either to the male or female. Furthermore, the neural components in the hypothalamus required to bring this about are functional in the male rat.

This investigation was supported in part by U.S. Public Health Service fellowship 1 *F2AM*-23, 550-01 from the National Institute of Arthritis and Metabolic Diseases and in part by grant *GB*-1737 from the National Science Foundation administered by Dr. J. W. Everett. NIH-LH-B_1 was kindly supplied by the Endocrinology Study Section, National Institutes of Health.

Note added in proof. While this paper was in the press an abstract by Moll and Zeilmaker[17] appeared, describing experiments comparable with those presented here, but differing in that far greater stimulation was used.

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POLYSOMES ON A NUCLEAR MEMBRANE FRACTION AS INTERMEDIATES IN THE TRANSFER OF RIBONUCLEIC ACID FROM THE NUCLEUS TO THE CYTOPLASM

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THERE is considerable evidence to the effect that messenger RNA (*m*RNA), together with all other forms of RNA, is produced in the nuclei of mammalian cells[1]. This RNA is believed to travel to the cytoplasm, where it becomes the backbone of polysomes which are the subcellular aggregates responsible for protein synthesis[2–4]. However, little is known concerning the manner in which this RNA is conveyed to the cytoplasm, and only small amounts of free *m*RNA can be detected in the cytoplasm[5]. The possibility therefore remains that polysomal aggregates are actually formed in the nucleus or on the surface of the nucleus and travel from it as a unit. This article presents evidence for an association of polysomes with the nucleus and for the role of these polysomes as intermediates in the transfer of newly synthesized *m*RNA from the nucleus to the cytoplasm. This evidence is obtained by relying on the ability of DNA solutions to extract a specific microsome fraction from washed nuclei[6].

HeLa cells were exposed to uridine-^{3}H (U-^{3}H) (4 μc./ml.), while suspended in Eagle's minimal essential medium. Washed cells were allowed to swell in distilled water[7], homogenized with a tight-fitting Dounce homogenizer, and the medium immediately adjusted to the following composition: 0·25 M sucrose, 5×10^{-3} M magnesium chloride, 0·1 M potassium chloride, 0·05 M *tris* hydrochloric acid buffer, *p*H 7·8 (solution *A*), and 0·25 per cent Triton '*X*-100'[8]. Nuclei were removed by centrifugation at 600*g* for 15 min, and the supernatant suspension adjusted to 0·5 per cent with respect to sodium deoxycholate (*p*H 7·8) and used as the cytoplasmic fraction. The nuclei were washed several times in solution *A* plus Triton '*X*-100' and finally centrifuged through a layer of 2·2 M sucrose in solution *A* at 37,000 r.p.m. for 4 h using the '*SW*-39' rotor of the Spinco model *L* ultracentrifuge[9]. The washed nuclei, which were free from any visible cytoplasmic contamination and virtually free from intact cells, were resuspended in solution *A* plus 5 mg/ml. salmon sperm DNA and allowed to stand at 0° C for 30 min. This suspension was then diluted with two volumes of solution *A* and recentrifuged at 12,000*g* for 30 min. The supernatant was adjusted to 0·5 per cent with respect to sodium deoxycholate and will be referred to as the DNA extract of the nuclei. The remaining pellet represented the nuclear residue. When such an experiment was carried out with a short labelling period (5 min) 52 per cent of radioactivity fixed was found in the nuclear fraction and the remainder in the cytoplasmic extracts and nuclear washes. Under such conditions, about 40 per cent of the nuclear radioactivity was extracted by exposure to exogenous DNA, and this material had the physical characteristics of polysomes.

In Fig. 1, the distribution of radioactivity in sucrose gradient centrifugal separations of the cytoplasmic fraction (1*A*) is compared with that of the DNA extract of nuclei (1*B*). In such homogenates the radioactive peak present in the nuclear extract had an S^w_{20} of approximately 110–170 as compared to a nominal 70 *S* for the single ribosome peak. Comparatively little radioactivity was found in the cytoplasmic fraction after such a short labelling period (Fig. 1*A*) compared with the amount found in the DNA extract of the nuclei of the same cells (Fig. 1*B*).

Aggregates in the DNA extract were not destroyed by treatment with DNase (1*C*) while they were extremely labile to minute amounts of RNase (1*D*). This is consistent with the interpretation that these fractions were aggregates having radioactive RNA as a backbone.

The DNA which was used in the extraction did not appear to be bound in this complex in a way which would affect the aggregate size (Fig. 1*C*). This is in agreement with the results of Okamoto and Takanami *et al.*[10,11], who demonstrated that only single-stranded DNA formed complexes with ribosomes. The ability of DNA to effect this extraction was not dependent on the source of the DNA, nor on its molecular weight. Thus salmon sperm DNA was as effective as calf thymus DNA, and a shear-degraded DNA which was no longer precipitable with two volumes of alcohol was still active in extracting the polysomes from the nuclei. On the other hand, some structural specificity is required, since other polyanions such as yeast RNA, heparin, and poly-ethenesulphonic acid were inactive when tested in the same system. It

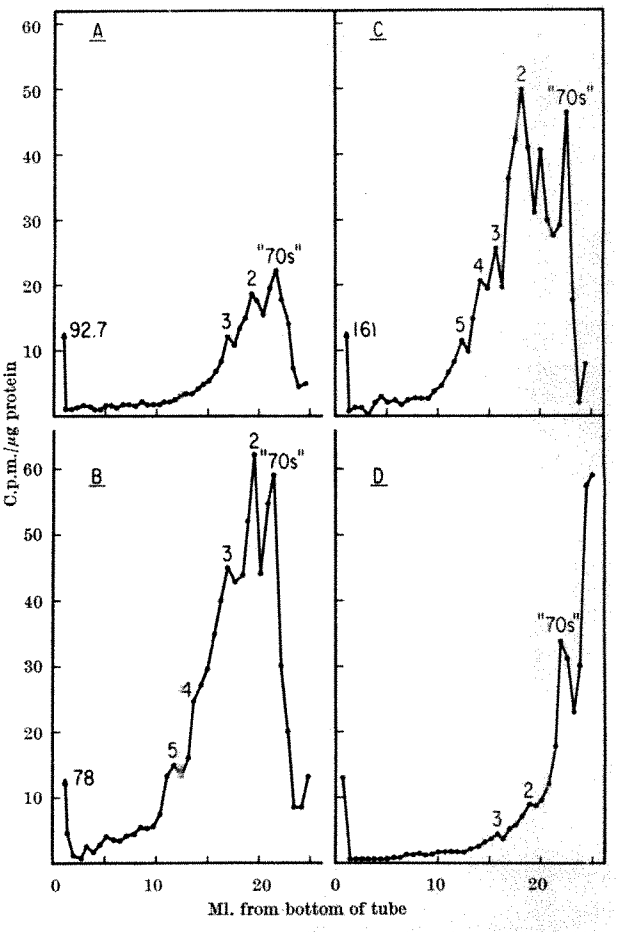


Fig. 1. Sucrose gradient centrifugation of extracts from cells labelled with U-^{3}H for 5 min. Cell fractionation as described in text. Sucrose gradients were linear (15–30 per cent) in solution *A* and contained 15 μg/ml. poly-ethenesulphonic acid mol. wt. 12,900[12]. Centrifugation was at 25,000 r.p.m. for 2 h in the '*SW* 25' rotor of the Spinco model *L* ultracentrifuge. Results represent radioactivity in the acid insoluble fraction expressed as c.p.m./μg protein. *A*, Cytoplasmic fraction; *B*, DNA extract of nuclei; *C*, DNA extract of nuclei after treatment with 2·5 μg/ml. DNase at 37° C for 5 min; *D*, DNA extract of nuclei after treatment with 0·06 mμg/ml. RNase at 37° C for 5 min

must be concluded that the DNA acted as a specific chemical extractant in this procedure.

Further evidence for the polysomal nature of the RNA aggregates found in the nuclear extract came from the examination of this fraction in the electron microscope. Large numbers of 'chains' of particles which corresponded in size to ribosomes were readily demonstrated. Finally, the molecular size of the radioactive RNA species present in the various fractions from labelled cells was estimated by means of the sodium dodecyl sulphate procedure of Gilbert[12]. As can be seen in Fig. 2, the bulk of the radioactivity fixed into each of the three fractions during a 5-min labelling period showed a very heterogeneous distribution of sizes with a peak about 10*S*, corresponding to the characteristic size distribution for *m*RNA. It is interesting that, although most of the radioactivity in the cytoplasmic fraction had been found in the 70*S* region (Fig. 1*A*), the isolated radioactive RNA from this material also corresponded to *m*RNA in size and was indistinguishable from the RNA in the DNA extract of the nuclei. On prolonged exposure to U-^{3}H, the labelling pattern in the nuclei shifted towards larger RNA species which could be ribosomal RNA precursors[13]. The change in the distribution of radioactivity in the other two fractions indicates the incorporation of label into ribosomal RNA. The total amounts of radioactivity in the various fractions also changed markedly (Fig. 2 *D–F*). The large amount of DNA (extractant) present in gradient 2*B* and 2*E* prevented the detection of the characteristic peaks of ribosomal RNA by their absorption at 260 mμ. However, colorimetric

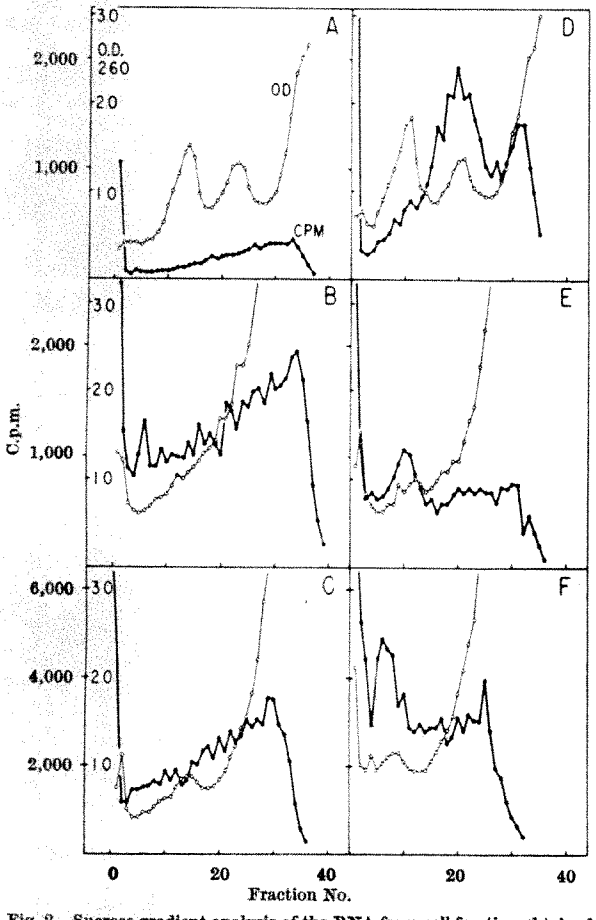


Fig. 2. Sucrose gradient analysis of the RNA from cell fraction obtained as described in text. The isolated fractions were adjusted to 0·5 per cent sodium dodecyl sulphate and layered over linear sucrose gradients (15–30 per cent) which contained 0·5 per cent sodium dodecyl sulphate[9]. Centrifugation was at 20° C, 25,000 r.p.m. for 11 h in the '*SW* 25' rotor. Closed circles, total acid insoluble radioactivity; open circles, absorption at 260 mμ. *A*, Cytoplasmic fraction, 5 min labelling; *B*, DNA extract of nuclei, 5 min labelling; *C*, nuclear residue from same fractionation; *D*, *E* and *F*, same as *A*, *B* and *C*, respectively, but after 90 min labelling

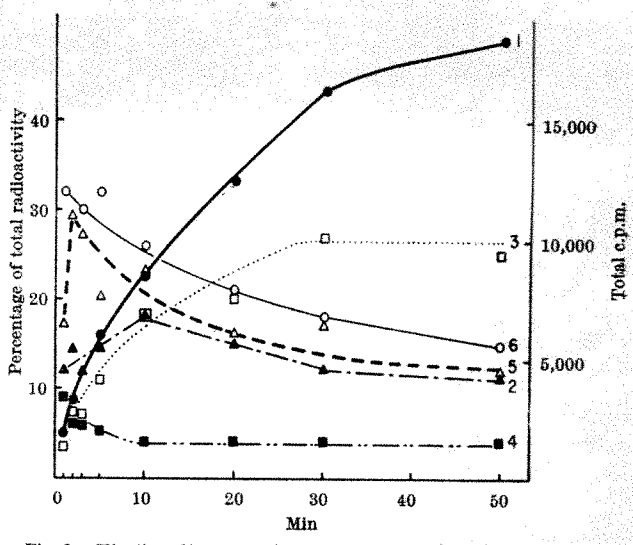


Fig. 3. Kinetics of incorporation of cytidine-^{3}H into various subcellular fractions of HeLa cells. Label was supplied as described for U-^{3}H in the text, and fractionation was as described without addition of sodium deoxycholate. Curve 1 represents uptake of radioactivity into the total fractions. Curves 2–6 represent the percentages of the total radioactivity which were found in: curve 2, cytoplasmic fraction sedimenting at 12,000*g* for 30 min; curve 3, cytoplasmic material sedimenting between 12,000*g* and 105,000*g*; curve 4, cytoplasmic soluble fraction; curve 5, DNA extract from nuclei; curve 6, residual nuclear fraction after extraction with DNA solution

determinations indicated that the RNA/protein ratio in the deoxycholate-treated fractions was approximately 0·8, typical of mammalian ribosomes.

It is questionable whether the polysomes which were associated with the nuclei represented a random sample of the cellular polysome population, as might be expected if they arose by some adsorption artefact, or whether, instead, they represented a special stage in the life-cycle of the polysomes. In order to answer this question the time course of the incorporation of tritiated cytidine into the RNA of the various cellular fractions was followed. The results of one such experiment are shown in Fig. 3. Virtually identical results were obtained on two separate repetitions of this experiment. The most rapidly labelled fraction was the RNA in the nuclear residue fraction (curve 6). The RNA present in the cytoplasmic ribosomes (curve 3) was the last fraction to become radioactive. Kinetically the polysomes of the nuclear extract (curve 5) and those associated with the cytoplasmic membranes (curve 2) behaved as intermediates in the flow of RNA from the nucleus to the cytoplasm. The results are in accordance with the view that *m*RNA is first formed in the nucleus, becomes associated into polysomes in or on the nucleus, after which the polysomes leave the nucleus in association with the membranes of the endoplasmic reticulum and finally end up as free polysomes.

The polysomes which were present after rapid pulse labelling appeared to be primarily membrane-bound. Essentially all the radioactive RNA of these cells was sedimented after centrifugation at 30,000*g* unless the preparations were first treated with sodium deoxycholate. Furthermore, both the DNA extract of the nuclei and the cytoplasmic microsome fractions had similar and typical phospholipid/protein ratios and comparable NADH-cytochrome *c* reductase activities, in agreement with the conclusion that the ribosome aggregates in these fractions were bound to membranes. This is contrary to the results of Penman *et al.*[7], who reported that the use of deoxycholate was not necessary in demonstrating the presence of polysomes in extracts of the *S*-3 clone of HeLa.

Microscopic examination of the nuclei before and after extraction with DNA failed to show any detectable change in their appearance. Furthermore, nuclear counts indicated that essentially all the nuclei were recovered following this extraction. It is thus unlikely that the membranes in

the DNA extract originated inside the nuclei. Because of the pronounced difference in the relative amount of radioactivity which was bound to the membranes in this fraction, it is likely that these membranes originated as portions of the nuclear membrane rather than from the endoplasmic reticulum.

That polysomes which are bound to membranes are more active in protein synthesis than those which are free in the cytoplasm is suggested by the findings of Haselkorn and Fried, Pelmont *et al.* and others[4,14–17]. Thus free polysomes may represent the last stage in polysome development, and the movement of the polysomes from the nucleus to the cytoplasm may take place on the membranes of the nucleus and endoplasmic reticulum. It is possible that this movement is accomplished by movement of the membranes themselves.

We thank Mr. F. LaPlante for technical assistance. Triton '*X*-100' was obtained as a sample from Rohm and Haas Company, Philadelphia, Pennsylvania.

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REGULATION OF CELLULAR AUTOLYSIS BY HYPERBARIC OXYGEN

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INHIBITION of tissue autolysis by hyperbaric oxygen was first described by Paul Bert[1], who ascribed it to an inhibition of "ferments". Few, if any, experimental data have appeared substantiating these observations or attempting to ascertain possible mechanisms of action of the inhibition. Much of this neglect may be due to the unavailability of adequate experimental material. Several desirable features, including relatively uniform and predictable survival times and ease of mechanical handling, make protozoa excellent experimental subjects for investigations of oxygen toxicity, as described by Wittner[2]. Also, ciliate protozoa such as *Paramecium* have well-developed enzymatic mechanisms for intracellular digestion of exogenous as well as endogenous food materials[3]. These attributes combined suggested that at least some of the hydrolytic enzyme systems of these protozoans could serve as test objects for an investigation of the effects of hyperatmospheric oxygen concentrations on intracellular autolytic mechanisms. For our initial observations, we chose a sulphydryl dependent, intracellular enzyme system for which *Paramecium* possesses high levels of activity.

A single line clone of *Paramecium caudatum* was established according to the method of Sonneborn[4]. For experiments, sub-cultures of feeding animals were pooled and washed several times by centrifugation at low speeds (500 r.p.m.) in successive changes of de-mineralized-distilled water.

For exposure to oxygen, aliquots of washed organisms containing 2,000–4,000 animals were placed in small open Petri dishes. The final volume of liquid was kept constant to permit equivalent diffusion paths with respect to depth and cross-section of the Petri dish. The pressure vessel was a 'Lucite' cylinder re-inforced with brass ends, as described previously[2]. Pure oxygen (Linde) was used up to pressures of 7 atm. (abs.) following thorough flushing (5 min) of the tank. Control gas mixtures consisted of a single atmosphere of air (not flushing the tank) with nitrogen to the desired number of atmospheres (abs.). Decompression occurred over a 10-min period to avoid mechanical damage to the organisms.

Our initial observations were made at room temperature (26° C) and consisted of exposing three groups of animals to 3 atm. (abs.) and 7 atm. (abs.) of pure oxygen respectively and a single atmosphere of air with the addition of 6 atm. (gauge pressure) nitrogen. Consistent with the data of Wittner[2], *Paramecia* exposed to 3 atm. of pure oxygen were killed by about 9 h exposure, while under 7 atm. (abs.) they were killed within 3 h. Death was considered to have occurred on cessation of ciliary motion and loss of pulsation of the contractile vacuole. Control animals observed in nitrogen–air mixtures for identical times appeared perfectly normal and, when removed to atmospheric air, remained so throughout a 3-week period of observation.

In the second series of experiments, following death by exposure to hyperbaric oxygen at the pressures and times already outlined, some dishes were returned to normal air while others were kept under pure oxygen for up to 3 weeks at lethal pressures.

Microscopic observations. All organisms were observed by a high-power long-working distance stereomicroscope (Zeiss) which permitted good resolution through the walls of the 'Lucite' pressure chamber. By this method, the time of death as evidenced by cessation of ciliary motion could be determined precisely. It was also possible to follow some aspects of the cytological condition of the protozoa subsequent to death. In all the oxygen-exposed dishes, no evidence of marked cytolysis was detected even after 2 weeks following death of the *Paramecia*, provided these remained under oxygen. The macronucleus, contractile vacuoles and some of the cytoplasmic inclusions ordinarily detectable under the phase-contrast microscope appeared intact. Food vacuoles were not present. Animals exposed to and maintained at 3 atm. (abs.) of oxygen until death showed some signs of cytolysis after 2 weeks; those dead from exposure to 7 atm. (abs.) of oxygen retained their essentially 'normal' appearance for at least an additional 3 weeks. Further details of the cytological alterations of these organisms, including cytochemical observations, are to be reported elsewhere.

Determination of proteolytic activity. We used a modification of the method of Anson[5] with dialysed bovine haemoglobin as substrate. For spectrophotometric determination, approximately 0·1 ml. portions of homogenized *Paramecia* (known number of organisms per unit volume) were added to 0·5 ml. of substrate (*p*H 2·5) at 37° C. At intervals, the reaction was stopped by addition of 1·0 ml. of 0·3 M trichloroacetic acid, centrifuged and the supernatant placed in a specially adapted microwell of a Beckman *DU* spectrophotometer at 280 mμ. Blanks consisted of 0·1 ml. homogenated organisms added directly

to 1·0 ml. 0·3 M trichloroacetic acid followed by 0·5 ml. of haemoglobin substrate. The percentage hydrolysis was calculated by the formula $100\ (E_t - E_0)/(E_\infty - E_0)$ where E_0 is the extinction coefficient of the blank, E_t is the extinction coefficient at time t and E_∞ is the extinction coefficient at complete digestion.

While other dynamics of the enzyme system encountered will be considered elsewhere, a distinct inhibition of catheptic activity by oxygen occurred. Fig. 1 shows the substrate hydrolysis obtained with incubation times up to 16 h. To demonstrate that pressure alone is not a factor, aliquots of homogenized *Paramecia* were exposed to 120 lb./in.^{2} (gauge pressure) using pure 99·8 per cent nitrogen in an unflushed vessel which would thereby contain a normal single atmosphere of air with its normal equivalent of oxygen. These results are also considered in Fig. 1.

Significant inhibition of hydrolysis by the enzyme system under investigation occurred with exposure to 120 lb./in.^{2} (abs.) of oxygen (26° C) during a 45 min exposure (Fig. 2). A 10-min exposure to similar concentrations of oxygen produced no detectable difference in the rate of enzymatic hydrolysis as compared with unexposed control animals killed by thermal shock (10 min exposure to 37° C, Fig. 1). The most marked inhibition of hydrolysis occurred with a 60-min exposure to 120 lb./in.^{2} of oxygen at 26° C (Fig. 2).

The ability of oxygen to inhibit catheptic activity may be dependent on temperature, but our present report deals with a single temperature. The temperature dependence of oxygen poisoning in protozoa was previously described[2], but quantitative data with respect to enzymatic activity have not been reported.

Previous investigations[6] on the effects of hyperbaric oxygen on amphibian embryos have indicated that a limited rate of autolysis occurs. Such an effect, especially apparent during specific early stages of development, could be separated from the oxygen-inhibitions of oxidative enzyme systems which were previously described in tissue homogenates[7–12].

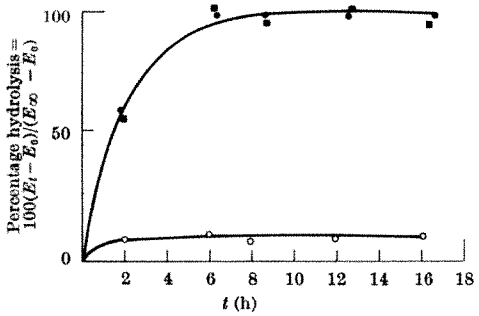


Fig. 1. Hydrolysis of haemoglobin substrate by homogenates of pooled *Paramecium caudatum*. Living organisms had been exposed to 120 lb./in.^{2} (abs.) oxygen (○), or to 105 lb./in.^{2} (gauge) + a single atmosphere of normal air (●), both for 60 min. An additional series consisted of animals killed by exposure to 37° C for 10 min (■). In each case aliquots consisted of 0·1 ml. of a homogenate from a pooled culture containing approximately 16,000 organisms per c.c. Exposure temperature was 26° C while under pressure

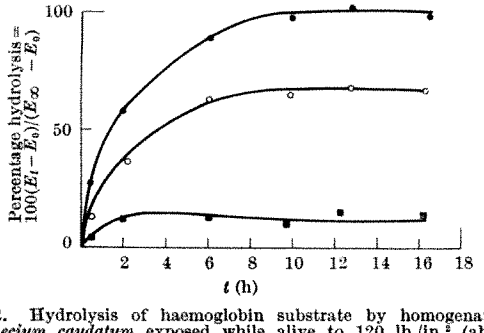


Fig. 2. Hydrolysis of haemoglobin substrate by homogenates of *Paramecium caudatum* exposed while alive to 120 lb./in.^{2} (abs.) of pure oxygen for: ●, 10 min; ○, 45 min; ■, 60 min. Exposure temperature was 26° C. Aliquots consisted of 0·1 ml. of a homogenate from a pooled culture containing 16,000 organisms per c.c.

The effects by hyperbaric oxygen on ontogenesis include production of teratogenic phenomena[13,14] and may reflect a significant role for some hydrolytic enzymes during development. Recently, hyperbaric oxygen has been shown to produce mammalian congenital malformations to some degree[15].

The effect of hyperbaric oxygen on limiting the rate of or totally inhibiting cell autolysis may reflect an inhibition of enzyme by inactivation of the sulphydryl co-factors on which some kinds of 'catheptic' activity are known to depend. However, it is also possible to evoke some recent concepts involving a possible cytochemical basis for control of cell death. In this respect, the subcellular lysosomes, first described as a biochemical entity in mammalian hepatic cells[17], could play a part. In a variety of cell types, these subcellular particles have been described as being composed of a single limiting membrane containing an assortment of hydrolases with acid *p*H optima[17]. The external limiting membrane, composed in great part of a lipoprotein complex, may become stabilized under the oxygen tensions used in the present investigation. Stability of the lysosomal membrane appears to be increased by at least two steroids, oestrogen[18] and hydrocortisone[19]. A stabilization of such a membrane by oxygen could either limit release of enzyme or prevent access of substrate to hydrolases compartmentalized with the subcellular unit. 'Cathepsin' has been described as one of a number of such contained enzymes[17]. However, a recent report[20] has suggested that the lysosomal membrane presents an increased permeability to high atmospheric oxygen concentrations. Furthermore, model lipid membranes appear to become readily oxidized by air and are then unstable[21], but these observations may not be comparable with an *in vivo* membrane system presumably being maintained by higher energy states. With these observations in mind, it is equally likely that in our experiments oxygen did not stabilize the lysosomal membrane but directly inactivated enzymatic protein.

The part played by oxygen in the preservation of mammalian tissue is at present being explored at the cellular level. It has been described, but the mechanism of action has not been explained, for the preservation of tissues for surgical transplantation[22]. It is not unreasonable that a primary action at the cell-level is a limitation of autolysis. Certainly, the effects of hyperbaric oxygen in inhibiting oxidative metabolism at the level of pyruvate oxidase and α-oxaloglutarate dehydrogenase, possibly through α-lipoic acid[11,16], would not permit us to conclude that an increased metabolism was the cause of such tissue preservation.

This work was supported by grants *GM* 05483 and *CA* 07081 from the National Institutes of Health and a contract from the Office of Naval Research.

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PLAQUE FORMATION BY PURIFIED SALIVARY MUCIN *IN VITRO*: EFFECTS OF INCUBATION, CALCIUM AND PHOSPHATE

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DENTAL plaque, which has been defined in a general way as the insoluble organic material which is tightly bound to teeth[1], is implicated in the aetiology of dental caries and periodontal disease because of its content of oral pathogens. It can also become calcified to form salivary calculus which may cause gingivitis by mechanical irritation. Investigation of the origin of plaque thus assumes a high priority among the objectives of dental research.

There is evidence that altered forms of salivary mucin are constituents of plaque. The amino-acid spectrum of the organic matrix of salivary calculus has been found to be qualitatively and quantitatively similar to that of mucin, and there is a qualitative resemblance in the carbohydrate content[2]. Sialic acid[3] and fucose[4] are nearly absent and hexosamine is somewhat low[3] in plaque as compared with mucin, probably as a result of bacterial degradation. Material resembling plaque in its tinctorial properties has been found to result from incubation of precipitated salivary mucin in saliva[5]. The amino-acid spectrum of a certain type of plaque termed 'denture pellicle' has been observed[6] to be quite different from that of salivary mucin. It could, however, be a degradation product of mucin.

It has been suggested that surface denaturation[2], the loss of sialic acid[3], or local *p*H changes[2,7] cause the deposition of salivary mucin on teeth. The evidence is, however, circumstantial and it has not been shown that these changes do, in fact, enable the altered mucin to become attached to the tooth surface. In order to investigate this problem further, we have undertaken a systematic search for various environmental influences which will modify the mucin molecule sufficiently to permit binding to surfaces.

Purified porcine submaxillary mucin ('PSM', Sigma Chemical Co., St. Louis) was dissolved in distilled water (1–2 mg/ml., *p*H 6·7) and subjected to various experimental conditions. Aliquots were then added to depression slides with or without other additions and evaporated in a desiccator over sulphuric acid. The residues were washed twice with five-drop portions of water and the slides were allowed to dry. The material remaining bound to the glass was stained with Ponceau red (200 mg per cent in 5 per cent trichloroacetic acid (TCA)) (Gelman Instrument Co., Ann Arbor) and photographed using green transmitted light.

When aqueous solutions of 'PSM' (1 mg/ml.), either fresh or incubated at 38° C for periods up to 18 h, were treated as described above, no observable residues remained bound to the glass after washing (Fig. 1). If incubation was prolonged beyond 18 h, precipitation occurred in the incubation tube and the precipitate remaining on the slide after evaporation was not removed by washing.

When the 'PSM' was dissolved in water (2 mg/ml.) and an equal volume of sodium phosphate (0·01 M, *p*H 6·5) was added, bound residues always remained after washing, whether or not incubation had been carried out (Fig. 1). The residues from unincubated 'PSM' were more widely distributed over the glass surface, were more flaky, and were more easily washed away. With increased periods of incubation, the residues tended to become concentrated in rings around the periphery and were much more tightly bound; thus, when the number of washings was increased from two to four, the residues from fresh 'PSM' were usually washed away completely, whereas residual material was quite apparent from samples incubated 30 min or more. If incubation was prolonged for periods exceeding 18 h, precipitation occurred in the incubation

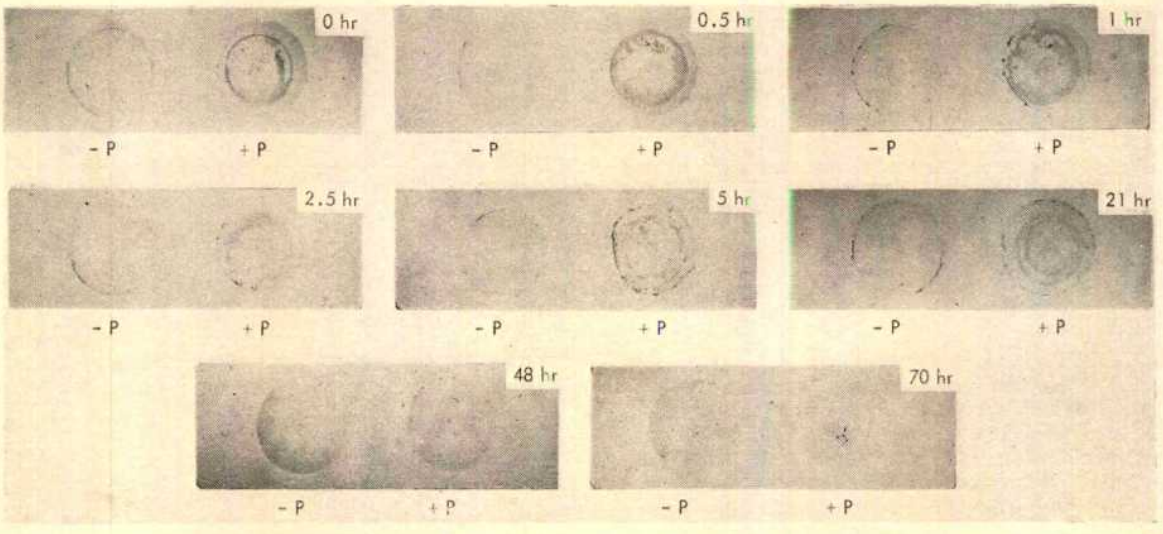


Fig. 1. Effects of phosphate and incubation on the formation of glass-bound residues by porcine submaxillary mucin. The mucin solution (1 mg/ml., *p*H 6·7) was incubated in the presence (+P) or absence (−P) of sodium phosphate (final concentration, 0·005 M) for the indicated periods of time. Aliquots (100 μl.) were evaporated on depression slides, washed, and stained as described in the text

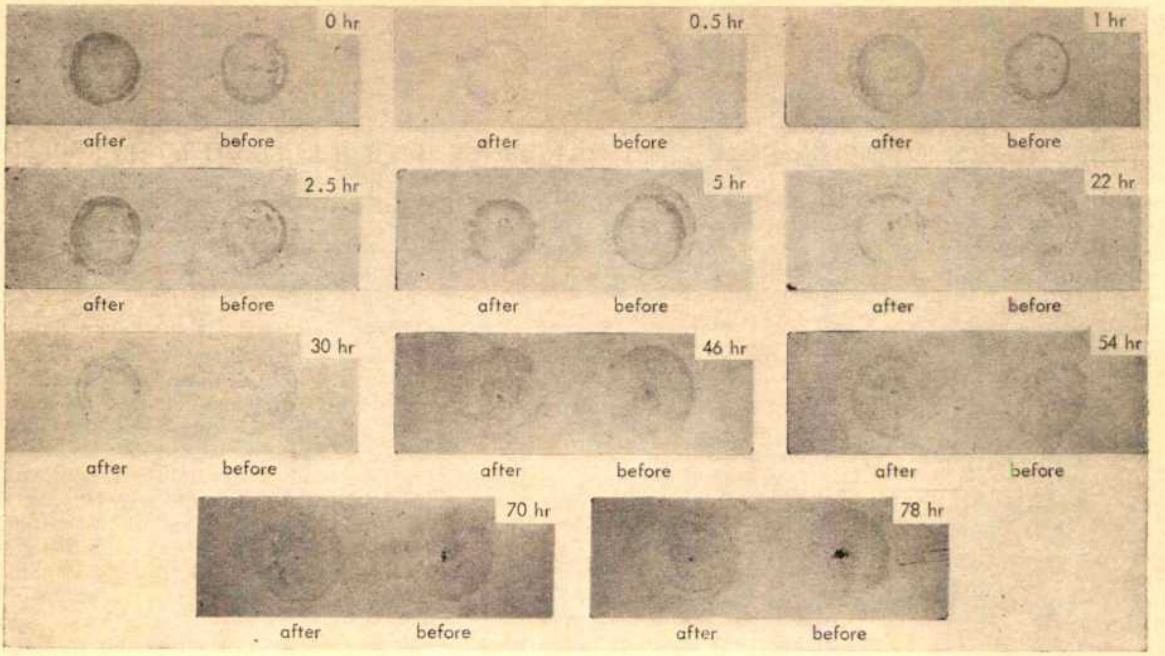


Fig. 2. Comparison of results obtained by adding phosphate before and after incubation of mucin. Where phosphate was added before incubation, the mucin concentration during incubation was 1 mg/ml., the phosphate concentration was 0·005 M, and 100 μl. were evaporated. Where phosphate was added after incubation, the mucin concentration during incubation was 2 mg/ml., and 50 μl. of mucin and 50 μl. of 0·01 M phosphate were evaporated. The amounts and concentrations of mucin and phosphate evaporated were thus kept constant. Evaporation, washing, and staining were carried out as described in the text. Incubation temperature, 38° C; *p*H, 6·7

tubes, but the precipitate coalesced into much larger particles than in the absence of phosphate. These particles settled to the bottom of the incubation tube where they adhered to the glass. In the absence of phosphate the precipitate did not adhere to the glass of the tube. The large particles which formed in the presence of phosphate remained bound to the glass slide after evaporation and washing (70 h incubation, Fig. 1). These observations clearly show that phosphate is necessary for the binding of difficulty soluble forms of porcine submaxillary mucin to a glass surface. It is of interest that after 48 h of incubation, the outer rings were quite apparent after washing with water, but were not visible after staining, having become soluble in TCA. By 70 h, the outer rings were not seen even before staining.

The residues obtained after incubation with phosphate were compared with those obtained when phosphate was added after incubation. Fig. 2 shows that the residues were similar; however, the residues from incubation with phosphate showed much more diffuse, granular material in the middle portion of the slides (not well shown in Fig. 2). Note that the coarse precipitate which had formed after 70 h of incubation with phosphate (centre of depression) was not present when phosphate was added after incubation. Also, there was often more TCA-insoluble material deposited as a thin layer by the latter samples at later incubation times. These results show that the material formed by incubation with phosphate has a nature somewhat different from that of the material formed by incubation followed by addition of phosphate. It is apparent that phosphate is not necessary for the formation of the organic material which binds to the glass, but only for the binding process.

The PSM preparation used contained 5·6 per cent calcium as determined by ethylenediamine tetraacetic acid (EDTA)–calcein titration (unpublished method, Uni-tech Corp., Panorama City, California), but only contained 1·4 per cent total phosphorus as determined by the method of Beveridge and Johnson[9]. It thus seemed possible that the added phosphate became attached to the mucin molecule at the sites of calcium binding. This was tested as follows: calcium was removed from a 10-ml. sample of 'PSM' (2 mg/ml. in water) by a single treatment with large excess of 'Dowex-50-X8' sodium ion-exchange resin (50 mg dry weight). An equal volume of sodium phosphate (0·01 M, *p*H 6·5) was added and incubation was carried out at 38° C for 30 min. Fig. 3 shows that no bound residue was formed by the sample from which calcium had been removed, but that an excellent residue was obtained from a sample to which the removed calcium had been restored. It was shown, by EDTA titration, that 93 per cent of the calcium had been removed from mucin by the resin treatment. These results indicate that the ability of phosphate to promote the formation of surface-bound residues from 'PSM' is dependent on the presence of calcium.

The foregoing results suggest a mechanism for the initial phase of the formation of dental plaque. We have formed the hypothesis that phosphate binds to salivary mucin at sites already possessing bound calcium. This process

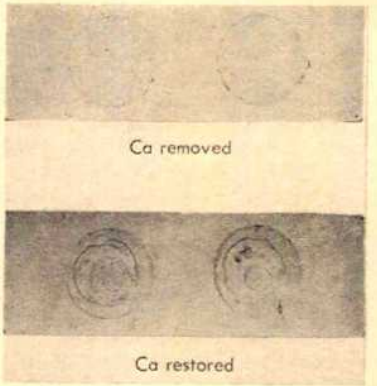


Fig. 3. Effects of removing and restoring calcium on the formation of glass-bound residues by porcine submaxillary mucin. Calcium was removed from the mucin with 'Dowex-50' ion-exchange resin. The solution was divided into two parts and the calcium was restored to one part by adding calcium chloride. Phosphate was added to both of the solutions and incubation was carried out for 30 min at 38° C, *p*H 6·7. Aliquots (100 μl.) were then evaporated, washed, and stained as described in the text. Mucin concentration during incubation, 1 mg/ml.; phosphate concentration, 0·005 M; calcium concentration, 0·0014 M

would form relatively insoluble materials which would adhere to the tooth surface on evaporation of the moisture from thin layers of saliva. It is possible also that bound calcium and phosphate facilitate the attachment of films of saliva to the tooth surface to form a relatively stationary medium capable of trapping oral bacteria. Incubation of the mucin in the oral environment causes its depolymerization, and when the product combines with phosphate the resulting material is less soluble and/or more adherent to surfaces than is the case with fresh mucin. The depolymerization probably involves hydrolysis of peptide bonds. This is supported not only by the formation of TCA-soluble residues in our experiments, but also by the work of Bettelheim[8] who observed the appearance of free carboxyl groups during incubation of bovine submaxillary mucin under mild conditions. Such material would be expected to have a chemical composition different from the original mucin, and this might account for the results of Smith[6], who has found that the amino-acid spectrum of 'denture pellicle' differs markedly from that of salivary mucin. Similar changes could, of course, also occur through bacterial degradation of the mucin[5]; however, bacterial contamination was probably not a factor in our experiments because the results were quite reproducible in all respects. If the mucin remains in the mouth for a sufficient period of time, precipitation of calcium- and phosphate-bound mucin product may occur and, as we suggest, this material would probably adhere to the tooth surface without evaporation.

In contrast with the results for 'PSM', treatment of whole human wax-stimulated saliva with 'Dowex-50' resin did not completely prevent, but only markedly inhibited, the binding of residues to glass surfaces. Experiments are planned to determine whether this resulted from incomplete removal of calcium or from the presence of materials which bind easily in the absence of calcium.

Eilberg, Gould and Sobel[10] have shown that an organic film can form on glass slides as a result of repeated incubation in and evaporation of calcium-free saliva. This film is calcifiable in the presence of calcium and phosphate. It would be of interest to determine whether the deposition of the film is facilitated by these ions.

Finally, the present results fit closely with those of Dawes and Jenkins[11], who have found that both calcium and phosphate are concentrated in plaque with respect to saliva. It would appear that such concentration may occur prior to, and indeed may facilitate, the deposition of the organic matrix of plaque. Calcium phosphate would thereby assume the hitherto unsuspected role of a deleterious 'biological cement'.

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OBSERVATIONS ON THE BIONOMICS OF THE MARINE NEMATODE, *Metoncholaimus* SP.

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COMPARATIVELY scant information exists on the bionomics of nematodes in the marine environment. In conjunction with our biological investigations of sea-grass communities (turtle grass, *Thalassia testudinum*, Konig) in Biscayne Bay (Miami), Florida[1], an undescribed species[2] of the genus *Metoncholaimus* was isolated in large concentrations during the autumn and winter of 1964–65 (ref. 3). At times, the animal comprised the dominant meiofaunal representative in the upper layers of the sediment. Patterns of activity of the species suggested the presence of environmental and biological factors affecting population dynamics as well as the extant ratios of the sexes. Data on various biological characteristics of the animal, including movement, egg deposition and development, are noted here.

Metoncholaimus sp. is a large (4 mm in length) and active animal with characteristic dorso-ventral undulatory movements both in sea water agar (0·5 per cent) and on fungal-cellulose mats. The head moves constantly in a probing manner, as if 'seeking food' or 'testing' the environment. Probably, this movement accomplishes both functions, for numerous sensory organs, labial papillae, cephalic setae and amphids are located at the head. (Disagreement exists on the function of the amphids. In general, the amphid is thought to be a chemo-receptive structure[4,5]. However, it has been suggested[6] that the amphids are mechano-stretch receptors in at least those groups of the Nematoda that possess weak cuticles. While neither hypothesis has been tested experimentally, the former one would appear more acceptable on a morphological/physiological basis.) A withdrawal reaction is noted when contact is made with a needle placed in the path of the nematode. Movement occurs readily in a forward or reverse direction. Frequently during migration on agar, *Metoncholaimus* sp. forms acute angles, resulting in the cuticle becoming twisted and wrinkled. These angles and wrinkles are transmitted down the animal as it passes through the agar. Gray and Lissmann[7] discuss locomotion of other nematodes and describe undulatory waves generated at the anterior portion which travel posteriorly the length of the body. The wave generated by *Metoncholaimus* sp. remains stationary as the nema migrates. The animal does not 'overstretch' on the outer side of the wave but does show an 'accordion-like' appearance on the inner surface.

Individual specimens in sea water apart from a substrate coil into irregularly shaped balls. Often the spinneret is used for attachment to the bottom of the glass dish with characteristic probing action. When many nemas are added to sea water in the same dish, rapid coiling and entwining occur, resembling a Medusa's head. When a cellulosic substrate is placed in the liquid in contact with an animal, the specimen usually uncoils and enters the substrate quickly.

Metoncholaimus sp. dropped into cylinders of sea water exhibit serpentine movements for a short time (<5 sec), quickly coil, and fall through the water column in this position with the anterior region probing. Probably,

their descent in samples with sand and other detritus would be more rapid due to adherence to particles. Striking differences are noted in the rate of fall between viable and freshly preserved specimens of *Metoncholaimus* sp. (Fig. 1). In tests conducted at room temperature the descent of specimens over a distance of 22 cm was recorded at 10 sec intervals. Fifty per cent of the viable animals fell within 60 sec, while 145 sec were required for an equal percentage of preserved nematodes to fall the same distance. Similar striking differences occurred at the 100 per cent level. In general, no differences in the rate of fall were observed between freshly preserved animals and those preserved for 5–10 days.

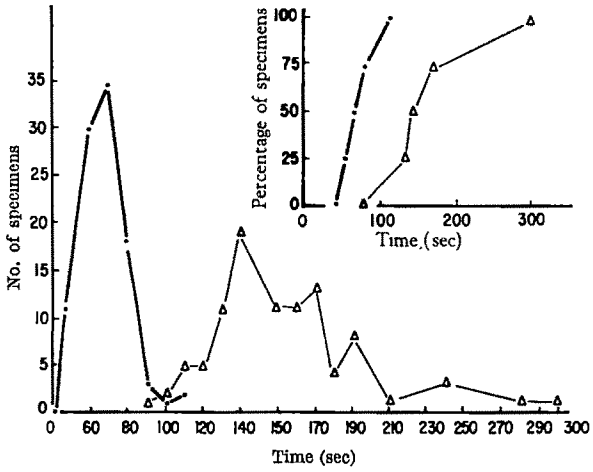


Fig. 1. Rate of fall of viable (•) and freshly preserved (Δ) specimens of *Metoncholaimus* sp.

Swarming or monospecific affinity has been reported for certain plant parasitic nematodes (*Helicotylenthus nannus, Tylenchorhynchus claytoni* and *Criconemoides* sp.) in water agar[6]. Rapid and random dispersal occurred in sea water agar, and in no instance was a monospecific attraction observed. Frequently, freshly collected fungal mats showed large concentrations of *Metoncholaimus* sp. with as many as several thousand gravid specimens on test substrates submerged for periods as short as 72 h. It has not been established whether this aggregation of gravid animals represents a swarming response, as already noted

Terrestrial nematodes differ from marine species in their ability to penetrate and colonize water agar. Marine nematodes do poorly on 1·7 per cent sea water agar (salinity 35 parts per thousand). In tests of various concentrations of agar, that is, 0·5, 1·0, 1·5 and 2·0 per cent, prepared with sea water, specimens of *Metoncholaimus* sp. most readily entered and moved within the 0·5 per cent agar medium. In the latter, specimens have often been observed to travel a distance equal to their body length of 4 mm in less than 30 sec. The 1·0 and 1·5 per cent agars permitted slight penetration of a few specimens, while no penetration and only surface migration occurred in the 2·0 per cent medium. In addition to ease of penetration, the nematodes remained active for a longer period in the 0·5 per cent agar. This latter concentration was used for the majority of our observations on *Metoncholaimus* sp.

Egg deposition. Females commonly carry between 5 and 20 eggs in their uterus, although as many as 51 eggs were counted in one specimen. At deposition, eggs are released in rapid succession, resulting in the formation of egg clusters or packets. Occasionally, single eggs are retained in the uterus after the main mass has been deposited. Death of the female appears to follow egg deposition. Subsequent development of eggs was not observed. Number of eggs in the clusters is comparable to amounts found in the uterus; however, single eggs occasionally are seen.

Clusters of 10 to 20 eggs frequently are observed on fungal cellulose mats. The eggs have a gelatinous coating and adhere together both in agar and on the fungal mat.

Frequently, eggs are deposited within the initial 24 h on sea water agar. In a comparison of egg deposition at 15°, 20° and 25° C, greater egg release occurred at 15° C. Often, eggs were deposited during the short time required for microscopic observation of the 15° C sea water agar plate. The temperature change from 15° C to room temperature during examination may stimulate deposition.

Eggs are deposited unsegmented and undergo several cell divisions during the initial 8 h. In tests in sea water agar and liquid, in the light (25° C) and dark (18° C), with and without agitation, eggs often hatched within six days. Occasionally, hatching may require 8–10 days. Once released, juveniles increase rapidly in length on the sea water agar.

Feeding and development. Limited observations have been made on the feeding of *Metoncholaimus* sp. Animals survive for as long as several months in sea water flasks containing a fungal-cellulose substrate. Adults are able to feed on marine bacteria and yeasts in liquid culture. In agar, juveniles were found ingesting ciliates which had developed adjacent to a dead adult. This feeding was associated with a decrease in the number of ciliates with a concurrent increase in the size of the juveniles. Furthermore, the intestinal coloration was different (darker) from that noted for the non-feeding animals.

Attempts to culture *Metoncholaimus* sp. on the stylet-bearing nematode, *Aphelenchoides* sp., were unsuccessful. In one instance, gravid *Metoncholaimus* sp. fed on the contents of their own broken eggs, whereas adjacent intact eggs remained undisturbed.

Metoncholaimus sp. occasionally swallows air bubbles, often at a rapid rate. The bubbles pass quickly through the oesophagus to the anterior portion of the intestine, where they accumulate. Subsequently, these move posteriorly, decreasing in size, until they are completely absorbed at a distance 1/2 to 2/3 from the anterior end of the intestine.

Both adults and juveniles are active in sea water agar for several weeks, after which the nemas are attacked by bacteria developing in the medium. In one test, *Metoncholaimus* sp. and *Euchromadora* sp. were placed in agar media, following which bacteria attacked the latter animals, decomposing the softer parts of the body and leaving the harder components of the cuticle as 'rows of hoops' through the agar. The bacteria have been identified tentatively as species of *Pseudomonas, Vibrio* and *Agrobacterium*. Initially, *Metoncholaimus* sp. showed resistance to bacterial activity, but after several weeks was also attacked.

Field and laboratory investigations are being continued to determine the life cycle and role of this species in the environment. Present data, based on hatching time, development of juveniles, and seasonal concentration of gravid females, suggest a cycle shorter than one year.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Early Radiation Field and High-energy Electrons in Supernovae

THE discovery of an X-ray source associated with the Crab Nebula[1,2] has prompted an intensive search for X-ray sources associated with other known Type I supernovae, for example, Tycho, and Kepler[3,4]. So far this search has proved fruitless. One is thus led to wonder what essential differences between supernovae of the same type might result in the presence or absence of X-ray emission. Morrison and Sartori[5] have proposed a thermal radiation model to account for this discrepancy. On the other hand, Woltjer[6] has argued that the observed X-ray emission from the Crab Nebula is due to synchrotron radiation. I suggest that if Woltjer's hypothesis is correct, a critical part is played by the intensity of the early post-explosion radiation field; for this field, by way of the inverse Compton effect, limits the energy of the electrons in the supernova. In fact, I estimate that no extreme relativistic electrons ($E > 10^{12}$ eV) can survive the first few weeks after the explosion. The situation is somewhat similar to that which gives rise to an upper bound on the energy of cosmic-ray electrons—the inverse Compton effect from starlight and synchrotron radiation in the galactic magnetic fields forcing a limit on the electron energy[7].

To make my point, I shall discuss a concrete example, namely, the Crab Nebula. Let us take, for the sake of argument, a simple model in which we assume that the currently observed X-ray emission is due to synchrotron radiation by a relatively small number of nearly monoenergetic high-energy electrons trapped in a weak magnetic field. The radiating electrons are assumed not to be significantly accelerated at the present time. Typical parameters appropriate to the foregoing assumptions and consistent with the observed X-ray fluxes from the Crab Nebula are given in Table 1.

Table 1

Half-life	$\gtrsim 10^3$ years
Frequency of X-radiation	$\gtrsim 10^{18}$ c/s
Present electron energy	$\gtrsim 10^{14}$ eV
Magnetic field*	$\gtrsim 10^{-3}$ gauss
Initial energy stored in X-radiating electrons	$\gtrsim 10^{44}$ ergs

* This magnetic field is about one-tenth that normally accepted for the average field of the Crab Nebula. However, consistent with the observations, this smaller field need occupy only a small fraction of the volume of the Nebula. Note that even smaller local fields are necessary to account for recent observations of localized radio-frequency emissions from the Crab Nebula[8].

Recently, Jones[9] has computed the electron energy loss rate due to inverse Compton effect in radiation fields up to 10^4 °K. This corresponds to the radiation energy density ($\simeq 75$ ergs/cm^3) about 10 days after the explosion of the Type I supernova (radiant energy input of about 10^{50} ergs)—it being assumed that the dilution of the radiation field is unimpeded by interstellar matter, and thus, that the radius of the sphere containing the radiation grows nearly at the velocity of light. Jones has shown that in an equilibrium radiation field the electron energy loss rate due to inverse Compton effect is:

$$-\mathrm{d}E/\mathrm{d}t \simeq 2{\cdot}5 \times 10^{-25}\, c\rho \mathrm{F}(E,T) \text{ ergs/sec}$$

where c is the velocity of light; ρ, the energy density in the radiation field (αT^4); E, the electron energy; T, the black-body temperature of the radiation field. All quantities are in C.G.S. units.

The function $\mathrm{F}(E, T)$ is proportional to E^2 for $E \lesssim 10^{10}$ eV, and increases logarithmically for higher electron energies. For electrons with energies in excess of about 2×10^{12} eV and radiation density corresponding to $T \sim 10^4$ °K, we find from Jones's calculation that:

$$\mathrm{F}(E, T) \simeq 10^{12}$$

These electrons will then lose energy at the rate of 4×10^{11} eV/sec. Therefore, the time required for an electron starting with energy E_i to be degraded to a final energy $E_f \simeq 2 \times 10^{12}$ eV is approximately:

$$\Delta t \simeq 2{\cdot}5 \times 10^{-12} E_i \text{ sec}$$

where E_i is in eV.

It is easily seen, then, that if we can characterize the early radiation field in a supernova by an equivalent black-body temperature, electrons with initial energies less than about 4×10^{17} eV will have decreased their energy to 2×10^{12} eV during the first 10 days. But electrons accelerated after some further dilution of the radiation field has occurred may survive with energies sufficiently large to participate in the currently observed X-ray emission. In order for the electrons to retain energy of about 10^{14} eV at the end of a few weeks (as required by our model), their initial energy would have to exceed 10^{20} eV. But then the energy initially acquired by those electrons would exceed the total estimated energy released in the supernova.

Thus, because of the energy requirements, the initial electron acceleration process must have been effective for a time sufficiently long for the radiation field to have been diluted so that electrons with energies $\simeq 10^{14}$ eV did not promptly lose a significant fraction of their energy by the inverse Compton effect.

As seen in the foregoing, in the energy range under consideration losses by inverse Compton effect depend only logarithmically on the electron energy. Thus, the emergent electron energy depends in a weak, albeit non-negligible, manner on initial injection energy. This is unlike the case of losses by synchrotron radiation which depend quadratically on the electron energy.

It is worth mentioning here that, in addition to the limitations on electron energies imposed by the inverse Compton effect, the possible presence of magnetic fields during the early stages of the supernova expansion further limits the efficiency of potential electron acceleration mechanisms. Thus, as is easily shown, an electron injected with an extreme relativistic energy, E_i, traversing a region of typical dimensions R cm containing a magnetic field with energy density $H^2/8\pi$ will emerge with a final energy:

$$E_f \simeq 10^{24}/(RH^2)\text{eV for } E_i \gg E_f$$

Thus we are led to speculate that the absence of sufficiently high-energy electrons, and hence of observable X-rays from Tycho and Kepler, may be due to unfavourable relative phasing of the dilution of the radiation field, the growth of the, as yet, undefined acceleration mechanism, and of the prevailing magnetic field. For as already shown, no significant number of electrons can retain high energies in the face of the initial radiation field of the supernova.

Pending detailed calculations, as well as additional data, it is still an open question whether a favourable relative phasing is at all feasible; or if a continuous but unspecified acceleration mechanism, such as that postulated *ad hoc* by Parker[10] for magneto-active plasmas, is necessary to explain the observation of X-ray emission or lack thereof.

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PHYSICS

Intensity Distribution of the Lyman–Birge–Hopfield Band System of N_2

Recent rocket investigations of the vacuum ultraviolet radiation emitted from the aurora and airglow have shown up the need for laboratory measurements of molecular band intensities in this spectral region. Such laboratory investigations have until now been hampered by a lack of suitable intensity standards. One prominent band system of importance in this region is the Lyman–Birge–Hopfield (LBH) ($a\ ^1\Pi_g - X\ ^1\Sigma_g^+$) system of N_2, which lies between 2000 Å and 1200 Å. It has recently been observed in auroral spectra taken by rocket-borne spectrometers[1,2]. The only laboratory measurements of intensities of bands of the LBH system in emission are estimates of photographic plate blackenings[3], where no account has been taken of variation of instrumental response with wave-length.

Franck–Condon factors $q_{v'v''}$ have recently been calculated for this band system[4] using realistic Rydberg–Klein–Rees potentials. These can be used together with measured band intensities $I_{v'v''}$ to determine the variation of the electronic transition moment $Re(r)$ over the band system and thereby to provide band strengths $S_{v'v''}$, since:

$$\left.\begin{aligned} I_{v'v''} &= K\, N_{v'}\, \nu^4\, R_e^2(\bar{r}_{v'v''})\, q_{v'v''}; \\ S_{v'v''} &= R_e^2\,(\bar{r}_{v'v''})\, q_{v'v''} \end{aligned}\right\} \qquad (1)$$

where K is a constant including units and geometry, $N_{v'}$ is the population of the upper state, and ν is the frequency of the band and $\bar{r}_{v'v''}$ is the r-centroid of the band. $R_e(r)$, the electronic transition moment, is assumed to be a smoothly varying function of internuclear separation.

This communication reports the results of relative intensity measurements of fifty of the major bands of the LBH system excited in a high-voltage a.c. discharge through nitrogen. A Hilger 3 m vacuum spectrograph with a photoelectric detector was used and the relative sensitivity of the instrument as a function of wave-length was established using a combination of standards including a carbon arc and a number of atomic lines the intensities of which could be calculated. Details of the calibration techniques will shortly be published.

From (1) it is seen that a plot of

$$\left(\frac{I}{q\nu^4\ N_{v'}}\right)^{\frac{1}{2}}_{v'v''}$$

versus $\bar{r}_{v'v''}$ or wave-length (to which it is monotomically related[5]) for each band delineates the variation of $R_e(r)$ over the band system. This plot was made for bands from the first four levels of the upper state ($v' = 0$ to 3) with the appropriate Franck–Condon factors[4] and population factors determined by inspection of the relative separation between the plots of $(I/q\nu^4)^{\frac{1}{2}}$ versus $\bar{r}$ for $v' = 0, 1, 2, 3$. No significant variation of $R_e(r)$ over the band system was observed. Hence to within the accuracy of the intensity measurements ($\pm$ 20 per cent) the electronic transition moment can be considered constant over the LBH system, and thus the calculated Franck–Condon factors directly indicate the relative band strengths. This conclusion had previously been tentatively suggested from an empirical observation on eye estimates of photographic blackening[6]. It is of considerable significance in identifying LBH bands in the spectra of such sources as the aurora and in determining source conditions.

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Simplified Bulb Tracer System for Argon Analyses

This communication describes a simplified and inexpensive bulb-tracer system used for argon analyses in the potassium–argon age-determination laboratory of the United States Geological Survey. The system is easy to build and operate, and is portable. The cost of the tracer system, including the argon-38, is less than 50.00 dollars, so the unit cost per tracer is low. In the past year we have made more than 300 tracers, and we expect to make at least 300 more before refilling the bulb.

The tracer system (Fig. 1) consists of three 2-mm bore mercury-seal stopcocks connected to a 2-l. 'Pyrex' boiling flask. The gas pipette, D, is the volume between control stopcocks B and C. Stopcock A is a safety stopcock that allows the system to be removed from a vacuum line without exposing stopcock B to the atmosphere. The stopcocks are greased with high-vacuum silicone grease, which has a vapour pressure of about 10^{-7} mm Hg, to which we have added a very small amount of molybdenum sulphide. The molybdenum sulphide helps to prevent the

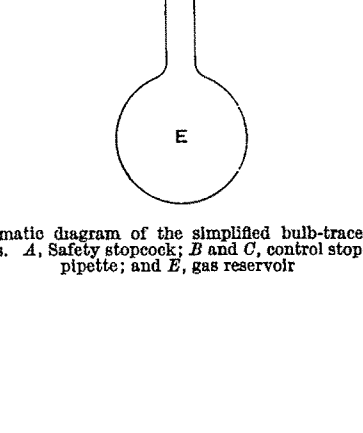


Fig. 1. Schematic diagram of the simplified bulb-tracer system for argon analyses. *A*, Safety stopcock; *B* and *C*, control stopcocks; *D*, gas pipette; and *E*, gas reservoir

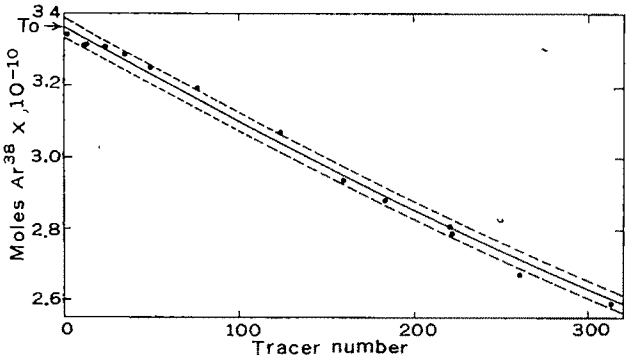


Fig. 2. Analyses of an intralaboratory standard mineral relative to the calculated depletion curve (solid line) for the simplified bulb-tracer system. The broken lines are 1 per cent above and below the calculated depletion curve

stopcocks from 'freezing up' with time. The apparatus is constructed inside a wooden case so that the system can easily be moved or stored when it is not in use.

The volume of the gas pipette, V_D, was determined by weighing the amount of mercury required to fill the pipette, and the volume of the gas reservoir, V_E, was determined by weighing it empty and full of water. The uncertainty in these measured volumes is about $\pm$ 0·1 per cent (standard error of the mean). The depletion, λ, of the reservoir per tracer release is given by:

$$\lambda = \frac{V_D}{V_E + V_D}$$

which for our system is equal to $0{\cdot}826 \times 10^{-3}$.

The amount of argon-38 in any given tracer, T_x, is found from:

$$T_x = T_0 \exp(-\lambda x) \qquad (1)$$

where T_0 is a constant and x is the tracer number (that is, if the tracer is the one hundred and forty-fifth one taken from the bulb, $x = 145$). T_0 is the mean of several values of T_0', which are calculated from formula (1) using experimentally determined values of T_x. These experimentally determined values of T_x are found by calibrating several individual tracers either by comparing them with a volume of atmospheric argon measured with a McLeod gauge or by comparing them with a known inter- or intra-laboratory standard mineral.

For routine operation we attach the tracer system to an ultra-high vacuum line that has two bakeable metal valves between the tracer and the pumping systems. Tracers are collected in individual tubes that consist of a breakseal tip and a side-arm containing activated charcoal. About twenty-five of these tubes are attached to the vacuum line at a time between the tracer system and the two valves, and the entire system, exclusive of the tracer system itself, is baked overnight at 300° C. An ionization gauge between the valves is used for measuring pressure after bakeout and for checking leaks.

If the system is vacuum-tight, the tracers are prepared in the following way. With stopcock B and the valves closed, stopcock C is opened and the gas pipette D and reservoir E are allowed to equilibrate for 20 min. Then C is closed, B is opened, and the argon in the pipette is adsorbed on a charcoal side-arm of a tracer tube cooled with liquid nitrogen. After collecting the argon for 30 min, the tracer tube is sealed off, the valve to the pumping system is opened, and the manifold and gas pipette are pumped for 10 min. The procedure is repeated for each tracer. Pressure measurements made with an ionization gauge indicate that more than 99·9 per cent of a prepared tracer is collected by this procedure. The results of tracer calibrations also indicate that quantitative collection of each tracer is obtained.

The tracer system may also be directly attached to the argon extraction line and a tracer prepared at the time of each experiment, but the procedure described here enables us to prepare and calibrate tracers before they are used in isotope dilution analyses. Should an accident occur to the tracer system between calibrations, no data are lost.

Plotted on Fig. 2, to illustrate the consistent behaviour of the tracer system, are fourteen analyses of a biotite that we use for an intra-laboratory standard. We analyse this biotite at least once in every fifty tracer releases in order to check for any relative changes in tracer concentration other than normal depletion. The standard deviation of these fourteen determinations is 0·6 per cent (relative to the calculated depletion curve) and represents not just the error contributed by the bulb-tracer system but the net result of all the errors involved in an isotope dilution determination of the quantity of radiogenic argon in a mineral. Our experience with a variety of bulb and manifold tracer systems indicates that this simplified system is as precise as any known system.

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A Peak in the Tritium Content of Atmospheric Hydrogen following the Accident at Windscale

IN October 1957 Gonsior and Friedman[1] observed a strong peak in the tritium content of molecular hydrogen for air samples taken at Hamburg, Germany. They attributed this peak to the direct admixture of tritiated hydrogen produced on October 7, 1957, by a large thermonuclear explosion in the U.S.S.R. However, the mean monthly concentration of long-lived fission products in surface air showed no unusual increase during October 1957 (ref. 2). Additionally, no other such peaks have been observed to occur following the other thermonuclear test explosions during that period.

In view of the time of onset and the brief duration of the tritium peak it seems far more likely that the tritiated

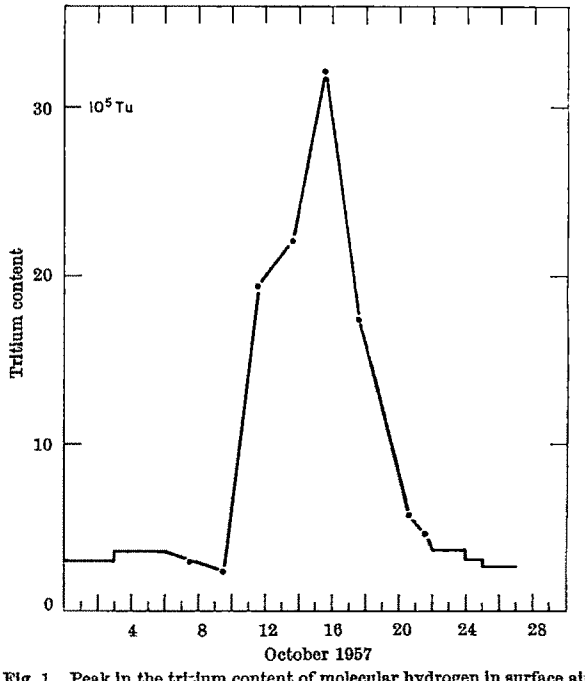


Fig. 1. Peak in the tritium content of molecular hydrogen in surface air during October 1957. The samples were taken at Hamburg and measured by Gonsior[1] (comp. his Table 1, samples 11–23)

hydrogen was released during the reactor accident at Windscale on October 10 and 11, 1957. Since the samples were taken two days apart, the initial rise in Fig. 1 is not well defined, but obviously the increase began either on October 11 or on October 12. The final rise to full maximum occurred between October 14 and 16. Thereafter the tritium content dropped steadily to its former value, which was reached about October 23.

This peak in tritiated hydrogen coincides well with the mixing pattern observed for the radioactive cloud injected by the Windscale accident, as illustrated by the distribution of the Windscale iodine-131 activity in air. From Figs. 3, 4 and 5 of the paper by Crabtree[3], it can be seen that the cloud arrived in the vicinity of Hamburg on about October 12 and extended farther north-east on subsequent days, giving rise to a continuous increase of the iodine-131 activity in air at Hamburg. No data were reported after October 15. By October 15 the horizontal gradient of the iodine-131 activity was already so small that subsequent horizontal and vertical mixing would be expected to cause a dilution rather than a further increase of activity.

The total amount of tritium released in the form of tritiated hydrogen cannot be estimated but cannot have contributed significantly to the global inventory of tritiated hydrogen, considering that the tritium concentration returned to its former value within 8 days.

Part of the released tritium must have been produced by ternary fission. A production of 0·8 tritium atoms per 10^4 fissions has been reported[4]. Another source is the reaction $^6Li(n\ \alpha)T$, due to irradiation of cartridges containing lithium–magnesium alloy which were present in the pile[5].

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METEOROLOGY

Estimating Severity of Drought

In central Australia, the red kangaroo, *Megaleia rufa* (Desmarest), breeds continuously during favourable weather when the green herbage, which it prefers to eat[1,2], is abundant; but progressively more and more females become anoestrous during prolonged drought[2–4].

Pastures inhibited by drought respond to falls of rain ranging from about 1·16 in. in January to about 0·29 in. in July, and they respond well should enough rain fall later to keep the soil moist for about 4 weeks[5]. Since water evaporates from the soil about 0·2 times as fast as from a standard evaporimeter, an extra 2·31 in. of rain is needed in January and about 0·58 in. in July[5]. But the climate of the area is arid, the mean annual rainfall and evaporation at Alice Springs being 9·93 in. and 95·18 in. respectively. Therefore, adequate rain to stimulate general growth of pastures is uncommon, and droughts are frequent and often prolonged.

In order to compare droughts in different places and at different times during the course of the investigation of red kangaroos, a formal method of measuring the severity of a drought was needed. The 'drought index' described here served this purpose quite well.

When rain sufficient to cause pastures to respond fell in any month, the index was set to zero. The estimated rate at which moisture evaporates from the soil was then subtracted day by day from the amount of rain that fell, until the remainder reached zero. Throughout that period of time the drought index was kept at zero, that is, any drought experienced in that time was considered to be ineffective. Thereafter, the estimated evaporation from the soil was accumulated day by day to provide a measure of the aridity of a given period. If any rain falling in that time was insufficient to cause a general response in pastures, then the amount falling was subtracted from the index on the day it fell. But, if the rain falling was sufficient, then the index reverted to zero. It was found that the periods during which the index was set at zero corresponded well with the periods during which green herbage was abundant in the countryside. Because the index made allowance for the average drying power of each day in each month, it provided a quantitative estimate of the stress imposed on the physiology of plants, and of animals grazing them.

When the proportions of anoestrous female kangaroos and of the pouch-young which die in the pouch were plotted against the values of the drought index, *S*-shaped curves resulted[6]. This is the classic response obtained when groups of animals are experimentally exposed to increasing doses of a drug, and the appropriate model for testing this response is the probit analysis[7], which transforms such data so that a linear regression results. Probit analyses of the data for the kangaroos proved to be quite satisfactory. They showed that kangaroos on two areas of central Australia, separated by only 15–20 miles, responded differently ($P < 0{\cdot}05$) to droughts of the same severity, thus highlighting the different nature of the two areas, as one area was better grassed than the other[8].

The drought index, calculated in this manner, is therefore likely to be a sensitive way of describing the prevailing weather. It would do so more accurately if the actual evaporation of any day or month were to be used instead of the average, but none the less it seems quite satisfactory.

This method of describing the weather is an improvement on older methods which used the ratio of rainfall to evaporation, or some function of it[8–12], because it has a biological foundation (the response of native pastures), allows for the effectiveness of individual falls of rain and the severity of individual droughts, and offers a continuous record from day to day.

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GEOLOGY

Allometric Growth of Ammonoid Shells : a Generalization of the Logarithmic Spiral

The fact that in many ammonoids the shape of the shell is only approximately represented by a logarithmic or equi-angular spiral curve is now fairly well known[1–4]. From the work of Currie[4], it is clear that the spiral angle of the curve traced by the umbilical seam in two species of *Cadoceras* undergoes changes during growth which have a fairly complicated pattern, so that a single continuous mathematical function may not fit any better than would two or more equi-angular spirals having different values of the spiral angle (φ). Moreover, the proportions of the cameral cross-section may vary during growth, with the result that the venter and the umbilical seam will follow different spiral curves. Also, in many ammonoids there

are rather clearly marked discontinuities in growth after the first or second complete whorl, and again at the commencement of the body-chamber.

Nevertheless, it may be useful to set out some results relating to the type of spiral curve to be expected when the Huxley–Teissier law is obeyed by the growth of the shell radius (R) and peripheral length (P), that is:

$$P = \beta R^{\alpha} \qquad (1)$$

If the proportions of the cameral cross-section remain constant, a similar relation will hold between the radius and the shell volume. Thus, Trueman[2] found that in *Dactylioceras commune* the volume of the shell is proportional to the diameter raised to the power 2·7. If we denote the cross-sectional area of a camera by A, it follows from our assumption that A is proportional to R^2. Then the volume V is given by.

$$V = \int A\,dP = k\int R^2 dP$$

From (1) we have:

$$dP = \alpha\beta R^{\alpha-1}dR$$

and therefore:

$$V = \alpha\beta k\int R^{\alpha+1}dR$$

whence we get.

$$V = \frac{\alpha\beta k}{\alpha+2}R^{\alpha+2} + \text{const.} \qquad (2)$$

The shell volume is thus proportional to the diameter (or radius) raised to the power $\alpha + 2$. We conclude that the spiral curve traced by, say, the venter of *D. commune* is that given by equation (1), with allometric index α approximately equal to 2·7 − 2 = 0·7.

It is not very convenient to measure the peripheral length directly on the shell of an ammonite. What we need is an equation relating the radius to the semi-whorl number: in other words, to the angle θ swept by the radius vector. I have shown elsewhere[5] that the polar equation can be derived by straightforward methods, by making use of the fact that $dP/dR = \sec\varphi$. We obtain:

$$\theta = \frac{1}{\alpha-1}(\tan\varphi - \varphi) + \text{const.} \qquad (3)$$

When $\alpha = 1$, $\sec\varphi$ is constant, and we then have:

$$\theta = \tan\varphi.\log R + \text{const.} \qquad (4)$$

which is the familiar result for the equi-angular spiral. Expressing (3) in terms of R instead of φ gives:

$$\theta = \frac{1}{\alpha-1}[\{(\alpha\beta R^{\alpha-1})^2 - 1\}^{1/2} - \text{arcsec}\,(\alpha\beta R^{\alpha-1})] + \text{const.} \qquad (5)$$

Suppose our data consist of a series of measurements of the radii of consecutive semi-whorls of the shell, that is, values of R at constant increments of θ equal to 180°. If we assume φ to be approximately constant over the interval $\theta_3 - \theta_1 = 2\pi$, then from equation (4) we obtain:

$$\tan\varphi_2 = \frac{2\pi}{\log\left(\frac{R_3}{R_1}\right)} \qquad (6)$$

at $R = R_2$. If we compute consecutive values of $(\tan\varphi - \varphi)$, and plot them against corresponding values of θ, then from equation (3) we may expect to find that the points lie on a straight line having slope $(\alpha - 1)$.

This procedure will thus yield an estimate of the value of α. To complete the process of curve fitting, we have to find the value of β, and also to find the value of a 'nuisance' parameter θ_0, which is the arbitrary constant in equations (3), (4) and (5). One method of estimating β is the following. For the jth measured point on the spiral, compute the geometric mean of R_{j-1}, R_j, and R_{j+1}. (We are assuming that over a short portion of the curve the trend of log R is linear.) We also find the arithmetic mean of a group of values of $(\tan\varphi - \varphi)$ centred on the jth point, and from it find the 'smoothed' value of φ_j from a table of arctangents. We can then compute β from the relation $\sec\varphi = \alpha\beta R^{\alpha-1}$. The process may be repeated at other points on the curve, and the individual values of β averaged. They should not show any systematic trend of increase or decrease: if they do, the allometric growth model may not be appropriate.

Finally, from (3) we obtain:

$$\theta_0 = \theta - \frac{1}{\alpha-1}(\tan\varphi - \varphi) \qquad (7)$$

In order to 'predict' values of R for specified θ, given the values of the parameters α, β and θ_0, we may re-arrange equations (5) and (7) as follows:

$$R = (\alpha\beta)^{1/(1-\alpha)}[\{(\theta - \theta_0)(\alpha - 1) + \varphi\}^2 + 1]^{1/2(\alpha-1)} \qquad (8)$$

We have to take an assumed value of φ in order to compute R from the foregoing expression, and if necessary iterate once or twice, correcting φ from the value of R obtained. In practice this is not a serious inconvenience, as convergence is rapid.

It follows from the foregoing results that when α is less than unity, φ decreases as R increases, and finally becomes zero at a finite value of R given by:

$$R_{\max} = \alpha\beta^{1/1(1-\alpha)}$$

There is thus a distinct possibility that the uncoiling that occurs in the final growth stages of certain ammonoids might indeed be the direct consequence of allometric growth (Thompson[1], p. 809).

The curve fitting process may be illustrated with an example of some data quoted by Thompson (*ibid.*, p. 797) for the shell of *Arcestes tornatus*. Thompson has already computed 'instantaneous' values of φ at successive semi-whorls of the shell, and we can proceed at once to plot θ against $(\tan\varphi - \varphi)$. (Values of θ and φ must, of course, be in radians.) The points lie on a more or less straight line with the exception of the first one (at R = 0·30 mm). As explained earlier, we would expect some deviation at the extremes of early and late growth; it is probably best in this case to exclude the first point from the fitting process. Since θ is virtually error-free, it would be appropriate to compute the regression of $(\tan\varphi - \varphi)$ on θ: however, the first-moment method due to Wald[6] is much quicker and is reasonably efficient[7]. The data are shown in Table 1.

Table 1. ALLOMETRIC GROWTH IN *Arcestes tornatus*

Semi-whorl number (θ/π)	Shell radius, (R) (mm)	Spiral angle, φ	Values of $(\tan\varphi - \varphi)$	Computed values of R (mm)
1	0·25			0·213
2	0·30	86° 56′	17·149	0 291
3	0 35	85 21	10·805	0·378
4	0·50	83 42	7·597	0·509
5	0·70	83 42	7·597	0·708
6	1·00	83 42	7·597	0·995
7	1·40	83 16	7·017	1·41
8	2·10	82 56	6·620	2·06
9	3 05	82 42	6·363	3·05
10	4·70	81 44	5·457	4·65
11	7·60	81 27	5·230	7·28
12	12·10	81 33	5·308	11·6
13	19·35			19 5

The data, consisting of measured shell radii and computed 'instantaneous' spiral angles, are from Thompson, p. 797, quoted from A. H. Grabau. The computed shell radii in the right-hand column are for a spiral curve having allometric index 0 8519, scale parameter 10·74, and location constant 70·55 (radians).

We divide the measurements into two groups, (1) for which $3\pi \leqslant \theta \leqslant 7\pi$, and (2) for which $8\pi \leqslant \theta \leqslant 12\pi$.

(1) Mean $\theta = 5\pi = 15{\cdot}708$; mean $(\tan\varphi - \varphi) = 8{\cdot}1226$, whence $\varphi = 1{\cdot}466$, $\cos\varphi = 0{\cdot}1046$; $R = 0{\cdot}7047$ (geometric mean of three values).

(2) Mean $\theta = 10\pi = 31{\cdot}416$; mean $(\tan\varphi - \varphi) = 5{\cdot}7956$, $\varphi = 1{\cdot}434$, $\cos\varphi = 0{\cdot}1364$; $R = 4{\cdot}776$ (geometric mean of three values).

We thus have:

$$\alpha - 1 = \frac{5{\cdot}7956 - 8{\cdot}1226}{31{\cdot}416 - 15{\cdot}708} = -0{\cdot}1481$$

$$\alpha = 0{\cdot}8519$$

For the first group, the estimate of β is given by:

$$\beta = \frac{(0{\cdot}7047)^{0.1481}}{0{\cdot}8519 \times 0{\cdot}1046} = 10{\cdot}66$$

and for the second group, β is found similarly to be 10·86: the mean of the two values is thus 10·76.

Finally, we have:

$$\theta_0 = \tfrac{1}{2}[15{\cdot}708 + 31{\cdot}416 + (8{\cdot}1223 + 5{\cdot}7956)/0{\cdot}1481] = 70{\cdot}55$$

In the right-hand column of Table 1 are shown the 'predicted' values of R computed from equation (8), inserting the foregoing values of α, β and θ_0. The fit is reasonably good, even at the extremes of the range of θ.

Doubtless it will not always be profitable to complete the fitting process by computing β and θ_0. The first step would normally be to plot θ against log R, to test whether the data can in fact be fitted by an ordinary logarithmic spiral. If a straight line is not obtained, a plot of θ against (tan $\varphi - \varphi$) will indicate whether the Huxleian growth model is likely to be useful.

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Historical Marine Levels in Italy

In 1964, Gunther reported[1] *Lithodomus* borings in the columns of the Temples at Paestum at a maximum elevation of 18·5 m above sea-level. He explained them by a "fall of the land" and by subsequent uplift, and corroborated his hypothesis with numerous observations along the coasts of Italy, Sicily and Malta. His account of the evidence in the *Illustrated London News* of the same year prompted me to visit the site; I later discussed my findings with N. C. Flemming at Cambridge. I have now seen the letters written by Flemming[2] and A. N. Burton[3] to *Nature* in reply to Gunther's communication. Flemming considers that if there has been any earth movement it must have been very localized, which is possible in a seismic coast like that of west Italy; Burton states that, to judge from his own work on raised beaches in Calabria, uplift at the rates implied by Gunther is impossible.

I feel I must enlarge on a point hinted at by Flemming in his phrase "the supposed presence of *Lithodomus* borings in the columns". Gunther himself mentioned that the columns were made of travertine; this is the term generally used in Italy for what (in the present case) should more properly be described as tufa, a porous, spongy limestone deposited by running water on growing plants and thus bearing the imprint of leaves and stems[4]. The columns are thus riddled with holes which need not be attributed to *Lithodomus* and which could provide a determined investigator with evidence of former sea-levels at any height he pleased. The fault lies in the original observations rather than in their interpretation.

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CHEMISTRY

Catalysis by Alkali Metal Ions of the Racemization of the *Tris*-oxalato Chromium-III Anion

The rates of racemization of the complexes $M^I_3Cr(oxalate)_3$ have been found to depend on the nature of the alkali metal ion M^I as shown in Table 1.

Table 1

M^I	Li	Na	K	Cs	
$k_1 \times 10^3$ min^{-1}	29·26	30·85	26·91	23 62	all ± 1 per cent

(pH 6·5; temperature 21·6° C; $[M^I_3CrOx_3]$ = 2·3 × 10^{-3} M; no added salt.)

It can be seen that the different cations affect the rate in the order Na > Li > K > Cs—an order of increasing size (if Li^+ is assumed hydrated) and of decreasing co-ordinating power. Beese and Johnson[1] have shown that Li^+ accelerated this racemization more than K^+, but their results were obtained with a large concentration (0·5 M) of added salt (lithium chloride or potassium chloride).

Addition of sodium perchlorate increases the rate of racemization, but at concentrations greater than about 0·1 M added salt the rate of increase in rate against concentration falls off, as shown by the data in Table 2.

Table 2

[sodium perchlorate]	0·0	0·1	0·47	0·98	1·98	4·7	M
$k_1 \times 10^3$ min^{-1}	4 15	4·71	5·59	6·50	7·14	8·13	

(pH = 5·8–6·6*; temperature = 0·1° C; (Na_3 $CrOx_3$) = 0·003 M.)
* The rate of racemization is independent of pH in this region.

This is similar to the acceleration, although much smaller, produced by addition of cupric ions, where it is clear that the initial acceleration is due to co-ordination of one copper ion, and the co-ordination of a second copper ion gives a species which racemizes at a very much slower rate[2]. A similar phenomenon has been observed by Hay and Harvie[3] for the nickel ion catalysed decarboxylation of dihydroxy fumaric acid.

The recent work of Ashley and Hamm[4] on the acceleration of the *trans-cis* isomerization of diaquo *bis* oxalato chromium(III)anion supports the 'one-ended' dissociation hypothesis[5], with the cation co-ordinating to the free end of the half-dissociated oxalate. Such a mechanism probably also operates in the alkali metal catalysed racemization of the *tris* oxalato complex.

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Measurement of Graphitic Acid Surface with Polar Molecules

We have been carrying out sorption of polar molecules by graphitic acid, measuring the quantity sorbed in order to determine the interlamellar surface. The following experimental methods were used: (1) volumetric Brunauer–Emmett–Teller (B.E.T.); (2) gravimetric Joly balance[1]; (3) X-ray diffraction. The first two give a measure of the surface, the third technique gives a measure of the increase of space between the layers.

Table 1. SPECIFIC SURFACE OF GRAPHITIC ACID DETERMINED WITH POLAR MOLECULES

Sorbed molecule	Molecule S (Å²)	Technique	Temperature	g/g GA	V_m (cc/g)	Specific surface (m²/g)	d_{GA}	Type of complex
Water	10·8	B.E.T.	20° C		102	298 × 2	9·2	α
Ammonia*	15	B.E.T.	−47° C		147·5	592 × 2	9·2	α
Ammonia*	15	B.E.T.	0° C		154	619 × 2	9·2	α
Ammonia†	15	B.E.T.	0° C		207·4	833 × 2	9·2	α
Acetone	25	B.E.T.	20° C		89·5	550 × 2	9·0	α
Ethylene glycol	33	Joly balance	room temperature	0·2558		1,650	9·06 9·6 (ref 2)	α
n-Butylamine	20 ?	B.E.T.	20° C		136		14·97	β
n-Octanol	20 ?	Joly balance	room temperature	0·791			23 5	β

* Samples oxidized once

† Samples oxidized three times.

S = Surface; V_m = volume of vapour for monolayer; d_{GA} = (001) spacing of graphitic acid in Å.

The following molecules were sorbed: water, ammonia, acetone, ethylene glycol, *n*-butylamine and *n*-octanol.

The experimental results are shown in Table 1. The specific surface of the graphitic acid was calculated according to the formula C_4O_2H of molecular weight 81·05, leading to a value of 1,550 m²/g.

With water, ammonia, acetone and ethylene glycol, the sorbed molecules lie with their axes parallel to the surface of the lamellae giving an α complex[2].

The measurement of the surface in this case presents no difficulty, and with ethylene glycol a value is obtained for the total surface (S_{int} + S_{ext}).

The problem is more complicated if one tries to make a quantitative study of the β-complexes. In these, the molecules are more or less perpendicular to the layer, although some of the terminal carbon atoms may be in contact with the layer[3,4].

The completely erect position of the molecular chain is a limiting case, which is favoured by low temperatures[5].

Thus, the surface covered by butylamine and octanol cannot be calculated until the configuration of these molecules in the sorbed state is known. A calculation of the number of Å² corresponding to each molecule of octanol results in a value of 42 Å² (for specific surface 1,550 m²/g). A calculation of the density of the interlamellar liquid (using the X-ray value of the interlamellar spacing) gives $d_{20°} = 0·54$. The sorbed layer is thus much less dense than the liquid ($d_{20°} = 0·84$).

We intend to make further investigation of the density and heat of sorption of polar molecules with graphitic acid.

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Formation of Mercuric Iodo-acetate in Glacial Acetic Acid and its Reactions with Olefines

THERE are many examples in the literature referring to mercury salts of the type $Hg.R_1.R_2$, where R_1 and R_2 are different anions. In aqueous solution it has been shown by Raman spectroscopy that a mixture of mercuric chloride and bromide gives rise to the molecule HgCl.Br (ref. 1), while HgClI exists in the gas phase[2]. Molecular weight and conductance measurements of a mercuric bromide solution of mercuric sulphide indicates, however, that $2HgS.HgBr_2$ is formed[3].

The red polymorphic form of mercuric iodide is sparingly soluble in hot glacial acetic acid but dissolves readily in a solution containing mercuric acetate such that one mole of mercuric acetate just dissolves one mole of mercuric iodide[4]. On cooling the solution yellow needle-like crystals are obtained which, on standing for a few hours, turn red with separation of mercuric acetate. The driving force for solution is the formation of Hg(OAc)I or HgI_2. $Hg(Ac)_2$. To distinguish which of these hetero-anion salts may be formed an ebullioscopic determination of molecular weights was carried out using a conventional all-glass Cotrell pump apparatus. It has been suggested that mercuric acetate in aqueous solution probably consists of indefinitely long chains[5]. Using A.R. glacial acetic acid throughout, recrystallized mercuricac etate and resublimed mercuric iodide, the apparatus was calibrated using triphenyl methane. Over the concentration range 0·025–0·1 M, mercuric acetate was found to be monomeric (found, 335, 330, 324, 312; calculated, 318·7). Recently the molecular weights of mercuric halides in acetone were measured and indicated that they are monomeric in this solution[6]. The observed elevations over a similar concentration range, but containing mercuric iodide, indicated that monomeric Hg(Ac)I was formed (found, 346, 370, 376, 382; calculated, 386·6).

In order to assess the consequences of forming a hetero-anion salt the reaction of mercuric iodo-acetate with olefines was studied. Hugel and Hibou[7] investigated the olefine complexes formed by mercuric acetate and noted that mercuric iodo-acetate does not react directly with 1,2-hexadecene to give $C_{16}H_{32}Hg(Ac)I$. Ethylene was passed into a refluxing solution of mercuric iodo-acetate (3·87 g). After 3 h, mercuric iodide was obtained unchanged from the solution (2·20 g) and the complex $C_2H_4.Hg(OAc)_2$ recovered after evaporation of acetic acid ($C_8H_{10}O_4Hg$ requires C, 14·9 per cent; H, 2·0 per cent—found, C, 14·1 per cent; H, 2·1 per cent). The reaction with 1-dodecene led to quite different products. From a reaction mixture of mercuric iodo-acetate (30·8 g) and 1-dodecene (13·6 g) in refluxing glacial acetic acid mercurous iodide was obtained (9·92 g). On cooling, mercuric iodide (3·36 g) was obtained and, after removal of acetic acid, a heavy unstable oil. Vacuum distillation resulted in the formation of large drops of free mercury; gas chromatographic analysis showed that the distillate consisted of dodecene and two other high boiling products neither of which contained iodine. The resolution of this mixture is the subject of further work.

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Photolysis of Organic Sulphides

Irradiation of solutions of diphenylamine with ultraviolet light effects cyclization to carbazole in good yield[1]. It was of interest to determine if diphenyl sulphide would similarly afford 9-thiafluorene, some polymethyl derivatives of which were required. In the event, reaction took a different course, and benzene and diphenyl disulphide were produced. Trace amounts of diphenyl were also detected by gas-liquid chromatography but none of the desired 9-thiafluorene was found. The diphenyl disulphide (m.p. and mixed m.p. 57°–59°) was separated from unchanged sulphide by preparative gas-liquid chromatography; benzene was detected by gas-liquid chromatography but was not isolated. The following mechanism is adequate, assuming initial cleavage into free radicals as postulated in another case[2]. Free radical formation is energetically possible with light of wave-length less than 3900 Å taking the energy of the carbon–sulphur bond to be about 73 kcal/mole (ref. 3).

$$\begin{aligned} \mathrm{PhSPh} &\rightarrow \mathrm{PhSPh}^* \\ \mathrm{PhSPh}^* &\rightleftarrows \mathrm{Ph.} + \mathrm{.SPh} \\ 2\mathrm{PhS.} &\rightleftarrows \mathrm{PhSSPh} \qquad (1) \\ \mathrm{Ph.} + \mathrm{solvent} &\rightarrow \mathrm{C_6H_6} \qquad (2) \end{aligned}$$

Reactions of type (1) and (2) are well known[4]. Lyons has reported[5] that irradiation of diphenyl disulphide in *n*-hexane and other solvents gave thiophenol by dissociation and abstraction of hydrogen from the solvent by thiophenoxyl radicals. No thiophenol could be found among the products obtained in the present experiment using light petroleum (b.p. 40°–60°) as solvent.

A number of other sulphides behaved similarly on irradiation. Thus di-*n*-butyl sulphide was smoothly converted into the disulphide, isolated by preparative gas-liquid chromatography and identified by its infra-red spectrum, and di-*n*-octyl sulphide gave the disulphide and *n*-octane (detected by gas-liquid chromatography). With benzyl phenyl sulphide, which could theoretically cleave in two ways to give either dibenzyl and diphenyl disulphide or benzene and dibenzyl disulphide, only the former reaction occurred, through the most stable pair of radicals. Similarly benzyl *n*-butyl sulphide gave mainly dibenzyl and di-*n*-butyl disulphide. In each case small amounts of phenanthrene and *trans*stilbene were detected by gas-liquid chromatography. Diallyl sulphide was decomposed and sulphur was precipitated, but the only other product was a high boiling liquid shown by gas-liquid chromatography to contain at least four components.

Dibenzyl sulphide predictably gave dibenzyl and dibenzyl disulphide, but an unexpected product was phenanthrene, which was isolated by chromatography on alumina and identified by mixed melting point. The exact sequence of reactions leading to the formation of the phenanthrene is not clear. It seemed likely that it might have been produced by photo-cyclization of stilbene[6], itself formed by dehydrogenation of dibenzyl by benzylthiyl radicals[7]. In agreement a small amount of benzyl mercaptan was detected among the reaction products by gas-liquid chromatography. On the other hand, no more than a trace of 1,8-dimethylphenanthrene was formed when the reaction was carried out in presence of an equimolecular amount of 2,2′-dimethyldibenzyl, suggesting that there may be another route from the sulphide directly to stilbene or phenanthrene. Phenanthrene was also produced, along with some dibenzyl, by irradiation of dibenzyl disulphide, but, surprisingly, the main product of this reaction was dibenzyl sulphide. Prolonged irradiation of either the sulphide or the disulphide resulted in complete destruction of the sulphur compound and formation of a mixture of dibenzyl and phenanthrene in the ratio of approximately 2·5 : 1.

Dissociation of disulphides into radicals on irradiation with ultra-violet light is well known[7,8], but there has apparently been no previous instance of sulphur extrusion from a disulphide by light. The reaction is not general, for both diphenyl disulphide and di-*n*-butyl disulphide were unaffected under the same conditions. Measurements of bond dissociation energies show[3,9] that the carbon–sulphur bond in benzylic sulphides is considerably weaker than that in simple dialkyl sulphides, and it is reasonable to suppose that in dibenzyl disulphide, but not in the other disulphides examined, the usual dissociation into thiyl radicals is accompanied by cleavage of the carbon–sulphur bond, the sulphide then being formed by combination of benzyl and benzylthiyl radicals.

Reactions were conducted for 12–20 h under nitrogen (in the case of diphenyl sulphide oxygen was also used) in a quartz flask with light from a 125 W Mazda mercury vapour lamp from which the outer glass envelope had been removed, using dilute solutions (0·1–0·2 molar) in light petroleum (b.p. 40°–60°). In 'Pyrex', di-*n*-butyl and dibenzyl sulphides were unchanged, but diphenyl sulphide again gave the disulphide.

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Complex Formation between Transition Metal Ions and Organic Solvents : Electron Spin Resonance Investigations

The electron spin resonance (ESR) technique provides a powerful tool for investigating transition metal complex formation in non-aqueous solutions. In recent work we have shown[1] that ESR and optical absorption spectra of different paramagnetic ions are changed when glycerol replaces water as a solvent. Both the variation in line width and the optical 'red shift' obtained suggest a change in crystal-field energy levels by spin-orbit mechanisms. Newer theories have linked octahedral–tetrahedral changes to these ligand-field effects on non-bonding *d* electrons[2]. Thus, it might be of interest to investigate the ESR spectra of the chlorides of Mn^{2+}, Fe^{3+} and Cu^{2+} in different organic solvents including dimethylsulphoxide (DMSO), dimethylformamide, formamide, acetone, glycerol and ethanol. These solvents were chosen to provide a varying dielectric constant and base strength since such parameters are known to affect the equilibrium between the octahedral and tetrahedral states.

The ESR spectra were determined with a Varian *V*4500 100-kc ESR spectrometer using a liquid sample accessory. A DPPH standard ($g = 2{\cdot}0036$ as indicated by the arrow in Fig. 1) was used as a reference for marking resonance positions. The first derivative of the absorption curve was recorded. All solvents and hydrated salts used were of reagent-grade quality.

The spectra obtained are shown in Fig. 1. In formamide a Mn^{2+} spectrum (six-line *hf* pattern) similar to that obtained with water can be observed, while all other solvents result in a change of the spectrum. A splitting of the two outer lines and a reduction in intensity is observed with dimethylformamide and DMSO, probably due to the

influence of the two methyl groups present in both solvent molecules. A very broad signal with the normal six-line *hf* pattern superimposed was obtained for Mn–glycerol and Mn–ethanol solutions. Manganese chloride is very slightly soluble in acetone (yellowish-brown); therefore, the ESR spectrum of a saturated solution was recorded. All other solutions were colourless except for water and glycerol, which were pink.

Considerable changes in Cu^{2+} *hf* pattern were found with different solvents. In contrast to Mn^{2+}, Cu^{2+} in formamide gave a very unusual *hf* pattern. Copper chloride was found to be quite soluble in all solvents giving a blue colour in formamide and water, yellowish-green in DMSO and dimethylformamide, slightly green in glycerol, green in ethanol, and amber in acetone.

The Fe^{3+} spectra for water and ethanol are very broad, which, in the case of water, is probably due to the hydration effect. The high dielectric constant of water and its base strength are responsible for a complete dissociation of the tetrahedral form. While all the spectra obtained were near the free-electron value, as expected for *S*-state ions, the Fe^{3+}-glycerol signal is at a considerably higher *g*-value[1]. No signal could be obtained with formamide as a solvent, a result also observed by Swanson *et al.*[3], who assumed a breakdown of the tetrachloroferrate complex, $(FeCl_4)^-$, in solvents of high dielectric constant. The tetrahedral complex of iron has been investigated spectrophotometrically by Katzin[4] and was attributed recently by Swanson *et al.*[3] as being responsible for the ESR signal obtained. Since only halide anions seem to form the tetrahedral complex, an iron salt of other anions should not give a signal. Ferric nitrate or ferric sulphate, dissolved in DMSO, confirmed this prediction. Addition of small amounts of magnesium chloride or hydrochloric acid to this solution, however, resulted in a strong iron signal similar to the one obtained with ferric chloride–DMSO solutions. Addition of other halide anions (Br^-, I^-, F^-) in the form of their potassium salts to the ferric nitrate–DMSO solution did not produce an iron ESR signal. Nevertheless, distinct colour changes in the solutions on the addition of these anions suggest alterations in the electronic energy-levels of the ferric ion. Optical absorption studies are at present being undertaken to obtain more information. All Fe^{3+} solutions were yellowish with the exception of formamide which gave a brown colour.

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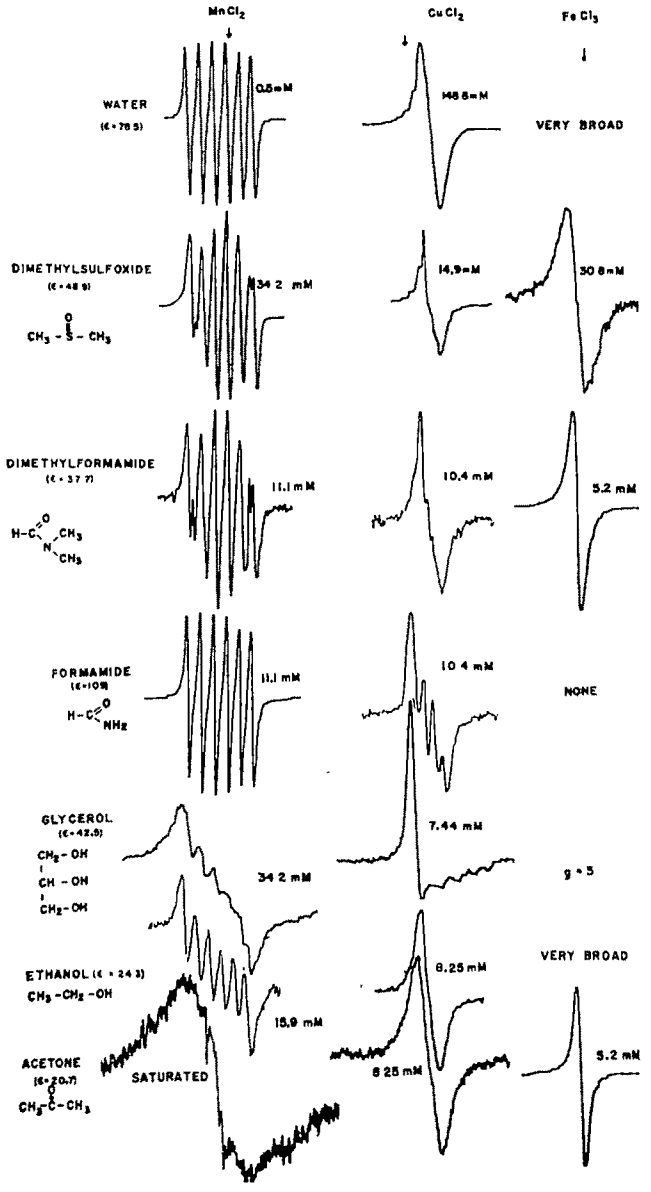


Fig 1. Electron spin resonance spectra of different paramagnetic ions in organic solvents

BIOCHEMISTRY

Kinetics of Ribonucleic Acid Synthesis on Deoxyribonucleic Acid Template

The purpose of this communication is to propose a mechanism, and to establish on its basis a system of equations, for the description of the kinetics of the synthesis of ribonucleic acid (RNA) templated by deoxyribonucleic acid (DNA). The models proposed[1] for the kinetics of the DNA synthesis cannot be extended to the RNA synthesis, since they assume that the uncoiling of the DNA double helix is the rate determining step. The time required for the unwinding of a DNA molecule with a length of 10^4 nucleotide pairs is of the order of about 30 sec (ref. 1). The *in vivo* synthesis rate for RNA is experimentally found to be much larger. It is therefore reasonable to assume that, in contrast to the replication of the DNA molecule, the RNA synthesis does not require the unwinding of the whole DNA double helix but only of a short part of it.

The essential characteristics of the RNA synthesis process seem to be (for a detailed discussion see ref. 2): (i) the formation of an unwound zone at the beginning of the DNA double helix; (ii) the binding of RNA polymerase (*E*) at the beginning of the DNA molecule; (iii) the step-by-step advancement of RNA polymerase as the unwound zone advances on the DNA molecule; (iv) the step-by-step synthesis of the RNA chain as RNA polymerase advances; (v) the step-by-step detachment of the RNA from the DNA as the uncoiled zone advances and the region behind it is rewound.

The mechanism proposed here is described in more detail by the following chemical reactions:

$$\mathrm{DNA} + E \rightarrow \mathrm{DNA}\cdot E \qquad (0)$$

$$\mathrm{DNA}\cdot E + X_1\mathrm{TP} \rightleftarrows \mathrm{DNA}\cdot E\cdot X_1 \qquad (1a)$$

$$\mathrm{DNA}\cdot E + Y_{1K}\mathrm{TP} \rightleftarrows \mathrm{DNA}\cdot E\cdot Y_{1K} \qquad (1b)$$

$$\mathrm{DNA}\cdot E\cdot X_1 \rightarrow \mathrm{DNA}\cdot X_1\cdot E \qquad (1c)$$

$$\vdots$$

$$\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}E + X_i\mathrm{TP} \rightleftarrows \mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot X_i \qquad (2a)$$

$$\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E + Y_{iK}\mathrm{TP} \rightleftarrows \mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot Y_{iK} \qquad (2b)$$

$$\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot X_i \rightarrow \mathrm{DNA}\cdot X_1X_2\cdots X_i\cdot E \qquad (2c)$$

$$\vdots$$

$$\mathrm{DNA}\cdot X_1X_2\cdots X_n\cdot E \rightarrow \mathrm{DNA} + X_1X_2\cdots X_n + E \qquad (3)$$

In the growth reactions (2*a*, 2*b*) of the RNA strand it is assumed that the three non-complementary (false) ribonucleoside triphosphates (Y_KTP) compete with the complementary one (XTP) for that position on the DNA matrix which the latter ought to occupy, but that they are not included in the growing RNA chain. This assumption is based on a similar competitive inhibition (called 'low efficiency inhibition') observed in the synthesis of polyadelinic acid on single-stranded DNA in the presence of large concentrations ($> 10^{-4}$ mol/l.) of a nonadenilic YTP[2].

If a steady state and an excess of RNA polymerase are assumed, the foregoing reactions can be described by the following equations:

$$\vdots$$

$$\frac{dA_i^*}{dt} = \zeta c A_{i-1}^+ - (\zeta' + \nu) A_i^* = 0 \qquad (4)$$

$$\frac{dA_{iK}^0}{dt} = \eta c A_{i-1}^+ - \eta' A_{iK}^0 = 0 \qquad (5)$$

$$\frac{dA_i^+}{dt} = \nu A_i^* - (\zeta + 3\eta) c A_i^+ + \zeta' A_{i+1}^* + \sum_{K=1}^{3} \eta' A_{i+1,K}^0 = 0 \qquad (6)$$

$$\vdots$$

$$\frac{dA_n^+}{dt} = \nu A_n^* - \lambda A_n^+ = 0 \qquad (7)$$

$$\varphi = n\lambda A_n^+ \qquad (8)$$

$$N = \sum_{i=1}^{n} (A_i^+ + A_i^* + \sum_{K=1}^{3} A_{iK}^0) \qquad (9)$$

In these equations c is the concentration of the four ribonucleoside triphosphates (which are considered equal), A_i^+ the concentrations of the $\mathrm{DNA}\cdot X_1X_2\cdots X_i\cdot E$ complexes, A_i^* that of the $\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot X_i$ complexes, A_{iK}^0 that of the $\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot Y_{iK}$ complexes, n the length (nucleotide number, $n \approx 10^4$) of the synthesizing DNA strand, φ the RNA synthesis rate, N the number of DNA molecules with RNA polymerase, and ζ, ζ', ν, η, η' and λ the rate constants for the different reactions.

For the RNA synthesis rate Φ, expressed as the number of nucleotides polymerized per second and per molecule of DNA with RNA polymerase, one obtains:

$$\Phi \equiv \frac{\varphi}{N} = \frac{\zeta\nu c}{(\zeta'+\nu)\left\{1 - \frac{1}{n} + \left[\left(1 + \frac{\nu}{n\lambda}\right)\frac{\zeta}{\zeta'+\nu} + 3\frac{\eta}{\eta'}\right]c\right\}} \qquad (10)$$

If it is assumed that: (i) the step-by-step advancement of the RNA polymerase, the uncoiling of the DNA, and the inclusion of the ribonucleotide (X_i) in the growing RNA chain processes (2*c*) are fast in comparison with those (2*a*) of the formation and dissociation of the $\mathrm{DNA}\cdot X_1X_2\cdots X_{i-1}\cdot E\cdot X_i$ complexes (that is, $\nu \gg \zeta\eta'/3\eta$ and $\nu \gg \zeta'$), and (ii) the time needed for the release of the synthesized RNA molecule and the RNA polymerase is small in comparison with the time needed for the RNA synthesis (that is, $1/\lambda \ll n/\nu$), equation (10) becomes:

$$\frac{\varphi}{N} = \frac{\zeta c}{1 + 3\frac{\eta}{\eta'}c} \qquad (11)$$

The rate constant ζ is probably determined by collisions of the XTP molecules from the solution with the deoxynucleotide which has reached the RNA polymerase, and it would thus be[4,5] about 6×10^8 l.mol^{-1}s^{-1}.

We evaluate η/η' using the experimental results for the 'low efficiency' inhibition of the polyadenilic acid synthesis by competition with a nonadelinic YTP; the synthesis rate is reduced to half the value valid for 'high efficiency' inhibition (that is, for very small $10^{-6} - 10^{-7}$ mol/l. YTP concentrations), at concentrations of about 5×10^{-4} mol/l. for the non-adelinic YTP[3]. If this synthesis is considered to be similar to that of the RNA, and only with a different 'slippage' rate constant (instead of the ν constant for the processes (2*c*)), a value $\eta/\eta' \approx 2 \times 10^3$ l.$^{-1}$ mol is obtained.

With $\zeta = 6 \times 10^8$ l.mol^{-1}s^{-1} and $\eta/\eta' = 2 \times 10^3$ l.mol^{-1}, equation (11) shows that φ/N tends to $1{\cdot}0 \times 10^5$ for sufficiently high value of c and that half this rate is attained for $c \approx 1{\cdot}7 \times 10^{-4}$ l.$^{-1}$ mol.

The rate of the ribosomal RNA synthesis in *Escherichia coli* (where it represents about 90 per cent of the total RNA) is about 10^5 nucleotides polymerized per second[5] by the, at most, 10 DNA molecules which synthesize it[6,7]; this would correspond to $\varphi/N \approx 10^4 \ldots 10^5$.

We notice that for *in vitro* synthesis of RNA the reaction rate is much smaller than that predicted by equation (11), but that half of the high concentration rate is attained also for $c \approx 1{\cdot}3 \times 10^{-4}$ l.$^{-1}$ mol (ref. 8). The small *in vitro* rates are probably due to the fact that only a few of the DNA molecules have RNA polymerase; these rates increase proportionally with the amount of the enzyme added[9].

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Lipid Metabolism in Hypervitaminosis A

Since the report of Moore and Wang[1] on hypervitaminosis A in rats, it is now recognized that administration of excessive doses of vitamin A influences the general metabolism of the host. Ray and Sadhu[2] reported that administration of large doses of vitamin A to rats caused an increase in liver fat. March and Biely[3], and Wood[4] reported that administration of vitamin A to young chickens lowered the elevated plasma cholesterol which resulted from the inclusion of 1 per cent cholesterol in their diet. Kinley and Krause[5] also reported the hypocholesteraemic action of vitamin A in atherosclerotic patients. Pallotatta and Krause[6] reported that administration of vitamin A elevated serum phospholipids. Van Bruggen and Straumfjord[7], on the other hand, observed that long-term administration of large doses of vitamin A to patients

raised their plasma cholesterol and phospholipid levels. Misra[9] observed that administration of excessive doses of vitamin A to rats lowered their plasma cholesterol and raised phospholipid-levels. This communication describes the results obtained on the tissue lipids of rats given excessive doses of vitamin A.

Male Wistar albino rats weighing 145–150 g were divided into two groups of six each and were kept on stock diet and water *ad libitum*. To one group 10^5 I.U. of retinol (Prepalin, Glaxo Ltd., India) were given intramuscularly daily for ten days. Rats without retinol were the control. A daily record of their food consumption and a weekly record of their body-weight were maintained. On the tenth day rats in both groups were intraperitoneally given a saline solution of $NaH_2{}^{32}PO_4$ (100 μc./100 g body-weight). After 6 h, rats were killed by decapitation, and liver, kidney, small intestine (about 30 cm), brain and heart tissues were quickly removed, cleaned, weighed and transferred to a volume of chloroform : methanol (2 : 1, v/v) for lipid extraction. Intestine was rinsed thrice with ice-cold saline before lipid extraction. Blood was drawn before killing the animals for vitamin A assay in plasma by the method of Bessey *et al.*[9]. Lipids from the tissues were extracted according to the method of Folch *et al.*[10], and dissolved in 5 ml. chloroform. Weights of the tissue lipids were determined by weighing an aliquot to a constant weight. Lipid extracts of the tissues were analysed for cholesterol[11] and phosphorus[12]. ^{32}P activity of lipid solutions was determined in a window-less gasflow counter.

No difference was observed in the daily food consumption and body-weights of the rats in both groups at the time of death. Vitamin A values in plasma were: control, 60–80 I.U.; experimental, 600–800 I.U. per 100 ml. plasma. Rats given excessive doses of vitamin A showed rawness of the skin, epilation, bleeding around the nose and bent and limping posture Effect of hypervitaminosis A on tissue lipids of rats is shown in Table 1. Total lipids of liver, intestine, and brain showed a significant increase and kidney lipids a marked decrease in rats given excessive doses of vitamin A than the control group. Administration of large doses of vitamin A also caused significant elevations in the cholesterol levels of liver, kidney, intestine, and brain tissues as compared with the control group. Hypervitaminosis A did not affect phospholipid phosphorus levels and their specific activities of the above tissues except kidney where a marked increase in its phospholipid specific activity is noted (Table 1). Heart tissue lipids, cholesterol, and phospholipid phosphorus were not significantly affected in hypervitaminosis A.

Table 1. Effect of Hypervitaminosis A on Tissue Lipids of Rat

Tissues	Lipids (mg/g wet wt.) Cont.	Lipids (mg/g wet wt.) Exp.	Cholesterol (mg/g wet wt.) Cont.	Cholesterol (mg/g wet wt.) Exp.	Phospholipid phosphorus (mg/g wet wt.) Cont.	Phospholipid phosphorus (mg/g wet wt.) Exp.	Sp. act. (counts/min/μg P) Cont.	Sp. act. (counts/min/μg P) Exp.
Liver	37·9 ± 0·47	46·7 ± 1·29	1·78 ± 0·04	3·81 ± 0 05	0·77 ± 0 01	0·80 ± 0 02	109 7 ± 3·8	113·7 ± 3·9
	$P < 0·01$		$P < 0 001$		$P > 0·20$		$P > 0 40$	
Kidney	54·7 ± 2·34	35·7 ± 1·51	2·46 ± 0·21	3·44 ± 0·43	0·60 ± 0·05	0·64 ± 0·03	63·5 ± 1·3	83·4 ± 2 4
	$P < 0 01$		$P < 0·05$		$P > 0·40$		$P < 0·01$	
Intestine	36·7 ± 2·34	47·7 ± 1·47	1·65 ± 0·10	3·74 ± 0 14	0·34 ± 0 02	0·32 ± 0·01	74·3 ± 3·6	69·7 ± 4·1
	$P < 0·01$		$P < 0·001$		$P > 0 50$		$P > 0·40$	
Brain	53·5 ± 0·70	66 3 ± 2·19	11·74 ± 0·76	14 10 ± 0 67	1·31 ± 0 10	1·43 ± 0·13	3·7 ± 0·5	2 5 ± 0 4
	$P < 0·01$		$P < 0 05$		$P > 0 40$		$P > 0 05$	
Heart	33·9 ± 1 45	36 1 ± 2·83	1 48 ± 0 04	1·46 ± 0·11	0 82 ± 0 01	0·77 ± 0 02	5·3 ± 0 3	5·0 ± 0·4
	$P > 0 05$		$P > 0·80$		$P > 0 10$		$P > 0 10$	

The values are mean ± standard error of six animals.
P values < 0 05 are significant.

Reported results are in agreement with those observed by Ray and Sadhu[2]. It is difficult to arrive at any conclusion regarding the mechanism by which administration of excessive doses of vitamin A brings about the observations made on rat tissue lipids. It is also not certain whether vitamin A influences the lipid metabolism directly, or these changes result due to an indirect and unspecific reaction of vitamin A or a substance or substances derived from it.

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Ethionine-induced Hypoglycaemia

Although considerable attention has been directed to the role of impaired release of liver triglyceride into plasma in the development of the fatty liver induced in rats by ethionine[1,2], it seems unlikely that such a mechanism, by itself, adequately accounts for the fatty liver. Lombardi and Recknagel[1] have presented evidence showing that ethionine impaired hepatic triglyceride release in both male and female rats; however, it is well known that the male is surprisingly resistant to the development of a fatty liver[3]. Observations that glucose administration either prevents[3,4] or decreases[5,6] hepatic triglyceride accumulation directed attention to the possibility that hypoglycaemia occurs after an injection of ethionine, thus serving as a stimulus for fat accumulation in the liver.

Single injections of *d,l*-ethionine dissolved in isotonic saline (25 mg/ml.) were given intraperitoneally in a final dose of 75 mg/100 g body-weight to female and male Sprague–Dawley rats weighing 180–230 g. Methionine in equimolar doses, either alone or mixed with ethionine, was also administered in single injections to some animals. Control rats received comparable volumes of saline. Neutralized aqueous solutions of adenosine triphosphate (Sigma Chemical Co.), with concentrations of 100 mg/ml., were given subcutaneously in 100 mg doses at 0, 2 and 6 h. Food was denied to groups of animals (designated 'fasted') 12–15 h prior to injection and thereafter; however, water was allowed *ad libitum*. Further groups of animals (designated 'fed') were permitted access both to food and water prior to, and after, ethionine administration. The rats were anaesthetized with ether at varying intervals, bled from the aorta, and blood sugar determined in triplicate by the method of Somogyi[7]. The results are given in Table 1.

Blood glucose concentration fell 4 h after administration of ethionine in female rats fed up to the time of injection, and allowed access to food thereafter. Decreased blood sugar concentrations persisted for at least 48 h. A more marked lowering of blood sugar was observed in female rats fasted before and after the injection of ethionine. A distinct difference in susceptibility to hypoglycaemia was observed in male rats. Blood sugar did not fall in 'fed' males, and the decrease noted in 'fasted' males occurred relatively late, and was much smaller than in females.

Methionine and ATP prevented hypoglycaemia in 'fed' females. ATP lessened the fall in blood sugar induced by ethionine in 'fasted' females 4 h after injection, and at 8 h blood glucose-levels had returned to the values found in saline controls.

The mechanism of hypoglycaemia has not been elucidated by the present studies and this is at present being investigated. The observation that blood sugar fell 4 h

Table 1. Effect of Ethionine, Methionine and ATP on Blood Glucose Concentration

	Hours after injection					
	2	4	8	24	48	72
	Blood glucose concentration (mg per cent)					
'Fed'						
Female						
Saline	117·6 (4)*	109 6 (3)	96·5 (4)	105·9 (5)	116 5 (3)	98 0 (3)
Ethionine	113·0 (4)	63·4 (6)	64·4 (5)	73·7 (6)	76·1 (3)	91·2 (3)
Methionine		99·4 (3)	114·4 (8)	96·3 (6)		
Ethionine and methionine		106·9 (3)	100 9 (9)	94·5 (7)		
Saline and ATP		113·2 (4)	117·3 (4)			
Ethionine and ATP		112·4 (3)	102·7 (4)			
Male						
Saline	104 0 (5)	109·0 (4)	105·6 (4)	98·7 (5)		
Ethionine	114·1 (5)	108·8 (4)	94·6 (4)	99 1 (5)		
'Fasted'						
Female						
Saline		92·8 (4)	85 9 (4)	79·4 (4)		
Ethionine		56 3 (4)	51·2 (4)	56 2 (4)		
Saline and ATP		99·6 (3)	98 3 (3)			
Ethionine and ATP		76·7 (6)	88·7 (4)			
Male						
Saline		87·9 (4)	89 0 (5)	80·9 (4)		
Ethionine		85·0 (5)	75 9 (5)	71·7 (4)		

* Numbers in parentheses indicate number of animals in each group. Blood glucose values represent the mean for the animals in each group.

after ethionine in female rats previously fed up to the time of injection suggests that interference with gluconeogenesis was not the major factor, at least not in the early stages. Since (1) ATP levels in liver[8], and possibly in other tissues as well, decrease markedly after ethionine injection, and (2) the fall in blood sugar was modified in 'fasted' animals by administration of ATP, it is tempting to speculate that ethionine led to a significant increase in glucose utilization. Activation of phosphofructokinase by a fall in ATP might be expected to stimulate glycolysis[9]. If of sufficient magnitude, such an alteration could account for significant hypoglycaemia, and account for the decrease in liver glycogen previously noted after ethionine[10]. It is not known at present whether the differing propensities for male and female rats to develop hypoglycaemia is related to differing degrees of ATP depletion, as few measurements have been reported of ATP-levels for male rats. In one communication in which such data are presented[8], hepatic levels of ATP had fallen less drastically in males than in females at 5 h after administration of ethionine.

Regardless of the mechanism by which hypoglycaemia is produced, the present data also provide evidence that hypoglycaemia may play an important part in the genesis of fatty liver induced by ethionine. In recent years, considerable emphasis has been directed to the phenomenon of impaired triglyceride removal from liver as the major factor accounting for triglyceride accumulation after ethionine administration[1,2]. Relatively little attention has been directed to the possibility of increased fat delivery to the liver although it has been demonstrated that the increased fatty acids found in liver triglyceride are most probably derived from adipose tissue[11,12].

Profound hypoglycaemia, as noted in the fasted females, might be expected to stimulate release of non-esterified fatty acids from adipose tissue, and, indeed, increased levels of these have been observed in the blood of rats receiving ethionine[13,14]. Augmented delivery of non-esterified fatty acids to the liver with subsequent incorporation into triglyceride may be important before increased hepatic triglyceride levels become manifest. Thus, glucose administration, which is known to prevent[3,4] or decrease[5,6] hepatic triglyceride accumulation, may do so indirectly by alleviating hypoglycaemia. The well-known difference in susceptibility of female and male rats to development of fatty liver, not adequately accounted for by impaired hepatic triglyceride release, may also be explained by the differing propensities for developing hypoglycaemia. Thus, increased resistance to ethionine-induced fatty liver in males[15] may be related to their resistance in developing a fall in blood sugar. Finally, although ATP administration has been shown to augment hepatic ATP levels and to prevent the development of fatty liver[16,17]—presumably by correcting the ethionine-induced impairment in hepatic lipoprotein synthesis—this need not be the only mechanism. As shown by the present investigations, interference with the development of profound hypoglycaemia by ATP may also play an important protective part.

These speculations do not detract from the probable importance of impaired lipoprotein synthesis as a contributing factor in the genesis of ethionine fatty liver. Rather, they also direct attention to additional important factors, presumably including increased fat mobilization to the liver, in this form of acute experimentally induced fatty liver. Furthermore, the present data indicate that hypoglycaemia is probably the important stimulus for such augmented fat accumulation in the liver.

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α-Hydroxybutyric Dehydrogenase Activity of Human Vascular Tissue

In recent years, several investigations have been reported on the α-hydroxybutyric dehydrogenase (HBD) activity in human serum and in various animal organs[1–5]. This enzyme catalyses the reversible reaction:

α-hydroxybutyric acid + DPN ⇄ α-ketobutyric acid + DPNH

Reliable measurement of the enzyme activity is possible by determining the rate of reduction of α-ketobutyric acid with reduced diphosphopyridine nucleotide (DPNH). No research has previously been conducted on the HBD activity exhibited by vascular tissue. The present investigation was designed to determine the activity of this enzyme in various types of human blood vessels. To evaluate the association between arteriosclerosis and HBD values, enzymatic measurements were made separately on normal and lipid-arteriosclerotic portions of tissue.

The vascular samples were obtained fresh at autopsy at the St. Louis City Morgue from individuals ranging in age from 3 months to 85 years. After rinsing the specimens with isotonic saline solution, the adventitia was removed and 5 per cent aqueous homogenates of intima-media layers were prepared at 4° C with a tissue grinder (Kontes-Duall type). The homogenates were afterwards centrifuged at 25,000 r.p.m. and 0·05 ml. portions of the supernatants immediately used for measurement of enzyme activity. The HBD determinations were performed as described by the Sigma Chemical Co.[6]—a method similar to that used by Elliott and Wilkinson[2] for the assay of rabbit liver, heart and kidney tissue. The tests were conducted at 25° C.

The 0·05 ml. 5 per cent homogenate supernatant was first incubated with DPNH in 2·8 ml. phosphate buffer, *p*H 7·4, for 30 min; after this pre-incubation period, unspecific oxidation of DPNH was no longer detectable. 0·1 ml. of α-ketobutyrate solution was then added to the sample and, after thorough mixing, the rate of DPNH oxidation was determined at 340 mμ in a Beckman *DU* spectrophotometer provided with thermospacer equipment. The millimolar concentrations in the final reaction mixture were: α-ketobutyric acid, 4·25; DPNH, 0·1; phosphate buffer, 95·0. The optical density readings were made at 1-min intervals against a reference cuvette containing a dichromate solution of appropriate concentration. Linearity was observed between time of reaction and rate of DPNH oxidation during periods of 8–10 min; under the conditions used, the activity recorded was proportional to enzyme concentration. A blank in which the homogenate supernatant was replaced with water was run with each set of analyses. The observed HBD values are expressed as millimoles of substrate metabolized/g wet tissue/h. In addition, activities calculated on the basis of fresh tissue nitrogen content are reported.

Table 1. α-HYDROXYBUTYRIC DEHYDROGENASE ACTIVITIES OF HUMAN VASCULAR TISSUE

	No. of samples	mmoles of substrate metabolized/g wet tissue/h		mmoles of substrate metabolized/g tissue nitrogen/h	
		Mean	*S.D.*	Mean	*S.D.*
Thoracic descending aorta:					
Normal	54	0 329	0·131	8·51	3 37
Lipid-arteriosclerotic	31	0·252	0 098	7·53	2·62
Pulmonary artery	25	0·398	0·124	11 78	3 68
Coronary artery:					
Normal	15	0 458	0 196	13·96	5 92
Lipid-arteriosclerotic	5	0·200	0 099	6·48	2·82
Vena cava inferior	6	0·530	0·112	15·00	3 25

The mean HBD activities of vascular samples are listed in Table 1. Distinctly higher values were observed for the pulmonary artery, coronary artery, and inferior vena cava than for the aorta. Moderately lower enzymatic activities were displayed by arteriosclerotic than by normal tissue portions of the same aortic specimens (wet tissue = 81 per cent, t of difference = 3·95; tissue nitrogen = 89 per cent, t of difference = 1·85). A decrease in HBD activity with age was noted for both normal ($r = -0·27$, $t = 2·02$) and arteriosclerotic ($r = -0·56$, $t = 3·58$) aortic tissue.

Although the HBD activity observed for human aortic tissue is less than 0·5 per cent of that reported by Konttinen and Halonen[4] for liver tissue, a comparison of this dehydrogenase with other enzymes recorded for human arterial tissue[7] shows that α-hydroxybutyric dehydrogenase belongs to the group with highest vascular activities. This suggests that HBD may play a significant part in the intermediary metabolism of the vessel wall.

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Kynurenine in Rat Hair

THE pelage of certain murine rodents contains substantial amounts of fluorescent tryptophan metabolites[1]. In the laboratory rat *l*-kynurenine[2] is the principal compound present, with relatively small amounts of kynurenic acid, *N*α-acetyl-*l*-kynurenine and other fluorescent compounds. It is interesting to know what drain this constitutes on the total tryptophan pool in the rat[3,4]; and since tryptophan has been suggested as a possible precursor of red hair pigment, phenomelanin[5], how the amounts of kynurenine compare in the hair of rats with different coat colours.

H. Takahashi has determined the amount of kynurenine in the hair of albino rats and some strains of mice after strong hydrolysis in hydrochloric acid[6]. The method used was the ion-exchange chromatographic assay published by Brown and Price[7]. No appreciable amount of kynurenine was found in the hair investigated except albino rat hair. Albino rat hair, washed with very dilute detergent, gave an average value of 2 mg of kynurenine per 100 mg of dry hair weight. There is about 2 g of hair on a 125 g rat. This means that approximately 40 mg of kynurenine is 'excreted' with the growing hair during replacement of the post-natal pelage in rats between 4 and 7 weeks old, and corresponding amounts during subsequent periods of anagen in their hair cycle of the adult rat[8].

I have obtained slightly lower values by the method of Spăček[9], described here, and also found that the amount of kynurenine plus acetyl kynurenine (and kynurenic acid by chromatography[1]) is independent of coat colour, the content in the pelage being approximately the same in albino, black (melanin), and cinnamon (phenomelanin) rats. In the cinnamon rat there is consequently no evidence of a grossly altered kynurenine content in the hair due to production of phenomelanin. The possibility that kynurenine is taken up into rat hair directly from the circulating pool must be entertained, since this is true of iodide[10] and 9-phenyl-5 : 6-benzo-iso-alloxazine[11]. In the black rat fluorescence of the pelage is largely quenched by melanin *in situ*.

1 g of hair, weighed in room air, was placed in a 500-ml. boiling flask with 100-ml. distilled water, boiling granules and silicone antifoaming agent. The flask was placed on a pre-heated electric heater and 15 g sodium hydroxide added. The powerstat was adjusted to deliver the first 50 ml. of distillate in 20 min into a fractionating well immersed in an ice bath. After 50 ml. had collected, the condenser was washed with 10 ml. of water and this was added to the distillate. The distillate was transferred to a pre-cooled separatory funnel. The fractionating well was rinsed with 10 ml. of water and this was added to the contents of the separatory funnel. The separatory funnel and contents were cooled to 5° C and 50 ml. of cold diethyl ether was added and shaken. After 1 h separation at 5° C the ether layer was collected into a stoppered flask. The water layer was returned to the funnel and re-

Table 1. Mg of *l*-Kynurenine* in 1 g Rat Hair

Washed hair†	
Cinnamon	3·4
Cream dilute	3·0
Black	4·6
Albino	3·3
Unwashed hair	
Black	8·8
Albino ♂	9·4
♀	7·9
DL-kynurenine (2·5 mg)	2·1

* Including Nα-acetyl-*l*-kynurenine.
† Weighed after washing.

Table 2. Values obtained from Guinea-pig, Mouse and Human Hair (μg kynurenine per g of hair)

Guinea-pig (red)	25 μg/g*
(white)	4 μg/g
(black)	7 μg/g
Mouse (albino)	7 μg/g
Human (red)	27 μg/g*

* The slightly higher values obtained from red hair may have significance with respect to the synthesis of pheomelanin (ref. 5). This could also result from the presence of tryptophan *per se* with the method of determination used (ref. 14).

extracted with 25 ml. of cold ether. The water layer was discarded and the combined ether layers made up to 75 ml. and returned to the flash. Absorption at 360 mμ was determined in a Beckman *DU* spectrophotometer, the ether extract being kept in an ice bath during the procedure.

Clean, clipped rat hair was used in the investigation. A portion of this hair was washed through six 15-min changes of distilled water with constant stirring, drained dry in a Büchner funnel between changes, followed by a 15-min extraction with diethyl ether, after which the hair was thoroughly air dried. This is referred to as 'washed hair' in this communication. Healthy albino rats of the Holtzman strain and laboratory rats with cinnamon, black and cream dilute coat colour[12] were used in the investigation. The results of the assays are shown in Table 1. The same procedure was applied to samples of red human hair, guinea-pig hair and albino mouse hair, without obtaining significant values to indicate the presence of kynurenine in the hair (Table 2). A single determination of the amount of kynurenine lost in one 15-min washing with water accounted for 1·2 mg of a 1·7 mg difference between washed and unwashed hair. Paper chromatography of the fluorescent ether fraction with Mason and Berg's solvent system[13] revealed a single blue-white fluorescent spot, R_F 0·9 ±, which developed a salmon colour with Ehrlich's reagent. Determination of the ultra-violet absorption spectra of the presumptive

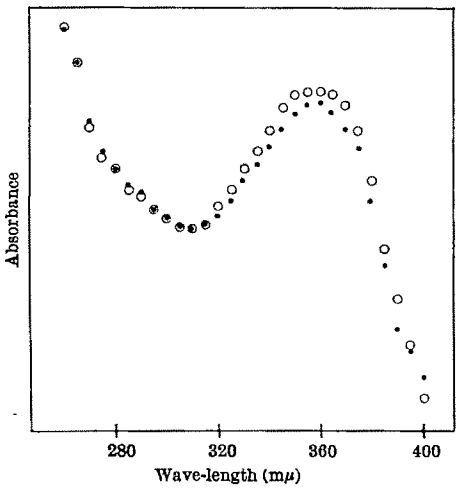


Fig. 1. Absorption spectrum of *o*-amino-acetophenone in diethyl ether. Solid dots plot distillate from rat hair, open circles plot distillate from known sample of D,L-kynurenine

o-amino-acetophenone obtained from rat hair and from D,L-kynurenine (Mann Biochemicals) revealed an absorption maximum at 358 mμ (Fig. 1).

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Inhibition of Choline Acetyltransferase by Excess Cysteine

The first demonstrations of an enzyme that would synthesize acetylcholine (ACh)—originally designated choline acetylase but now called choline acetyltransferase (acetyl-CoA: choline-O-acetyltransferase, *EC* 2.3.1.6.)—depended on the presence in the tissues of other endogenous enzymes which would synthesize acetyl-CoA. Even when the substrate role of acetyl-CoA had been appreciated, however, it was found that in order to realize the maximal activity of the enzyme, provision had to be made to couple it with another enzyme system which would produce acetyl-CoA continuously from suitable precursors. Compared with the production from such coupled systems the amount of ACh produced from added synthetic acetyl-CoA was as little as 20 per cent in the experiments of Reisberg[1] and about 50 per cent in those of Smallman[2] and of Morris and Tuček[3]. However, McCaman and Hunt[4] have obtained proportionately much higher yields of ACh in a buffer-substrate system containing synthetic acetyl-CoA, choline, eserine, magnesium sulphate, bovine plasma albumin and a high concentration of sodium chloride. Their incubations were conducted at *p*H 7·4. These authors considered that their higher yields of ACh depended on the replacement of potassium with a high concentration of sodium ions, but it appears that other factors may be more important.

We have now been able to demonstrate that rates of ACh synthesis either equal to, or greater than, those found by McCaman and Hunt[4] can be obtained from rabbit brain extracts when cysteine is omitted from the incubation medium. Our experiments have been performed on crude extracts of acetone-dried rabbit brain, on crude sucrose homogenates of the electric organ of *Torpedo marmorata* (see Morris, Bull and Hebb[5]) and on preparations of these enzymes partially purified by ammonium sulphate fractionation. The inhibitory effect of cysteine on the *Torpedo* enzyme when incubated with synthetic acetyl-CoA is shown in Fig. 1. In this experiment the incubation *p*H was 6·9, a value near the optimum for a

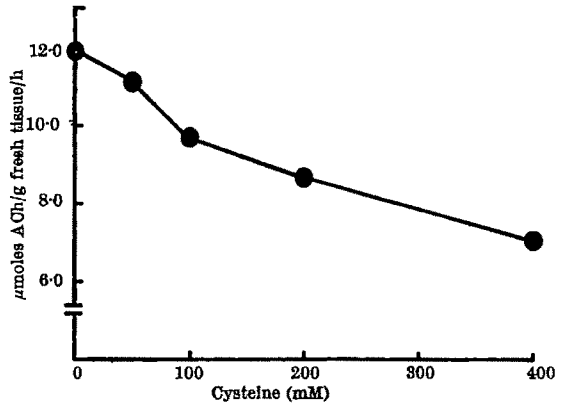


Fig. 1. Effect of cysteine on ACh synthesis by extracts of the electric organ of *Torpedo*. Incubation medium contained (μmoles/ml.): potassium chloride, 200; phosphate-citrate buffer, *p*H 6·9, 40; choline chloride, 24; eserine sulphate, 0·26; acetyl-CoA, 0·35; 20 μl. of partially purified *Torpedo* extract equivalent to 30 mg electric organ and various concentrations of cysteine. Temperature, 39°; *p*H 6·9; incubation time, 10 min. The yield of ACh from the coupled enzyme system at this *p*H and temperature was 14·6 μmoles ACh/g fresh tissue/h

coupled enzyme system containing phosphotransacetylase (acetyl-CoA : orthophosphate acetyltransferase, *EC* 2.3.1.8), acetylphosphate and CoA for the enzymatic production of acetyl-CoA. However, it appears that the *p*H optimum of choline acetyltransferase itself is nearer to 7·5, and at this *p*H the influence of cysteine on the yield of ACh from synthetic acetyl-CoA is correspondingly greater. The rate of synthesis of ACh from partially purified extracts of rabbit brain with synthetic acetyl-CoA at *p*H 7·5 in the absence of cysteine is greater than that obtained with the coupled system under optimal conditions (see Table 1). Cysteine is required in the coupled system for maximum activity.

Table 1. Effect of Cysteine, Sodium and Potassium on Acetylcholine Synthesis by Rabbit Brain Choline Acetyltransferase at *p*H 7·5

Enzyme	Incubation type	Cysteine (mM)	Sodium (mM)	Potassium (mM)	μMoles ACh per g fresh tissue per h
Crude	Acetyl-CoA	0	0	300	4·61
	Acetyl-CoA	0	300	0	5·41
	Coupled system	23	60	180	5·41
Partially purified	Acetyl-CoA	23	0	300	3·49
	Acetyl-CoA	0	0	300	8·61
	Acetyl-CoA	23	300	0	2·61
	Acetyl-CoA	0	300	0	6·30
	Coupled system	23	60	180	5·32

In both systems it is necessary to add monovalent cations, potassium or sodium, to obtain maximal yields of ACh. In some circumstances, we have found that potassium is better than sodium, but this is not invariably so. Sometimes sodium gives higher yields and this seems to depend on whether the enzyme is in the crude state and from what tissue it is obtained. It will be seen from Table 1 that 300 mM sodium supports a higher rate of ACh synthesis by crude rabbit brain than does an equivalent concentration of potassium, a result in qualitative agreement with McCaman and Hunt[4]. With partially purified enzymes, however, potassium is better than sodium.

In the experiments described here, the enzyme preparations were dialysed against 0·05 M-*tris*-thioglycollate buffer, *p*H 6·6, to reduce the endogenous sodium and potassium to a minimum. Subsequent measurements of these ions revealed that all but a trace had been removed. Enzymes containing no thioglycollate give much lower yields of ACh and crude rabbit brain extracts have the same specific activity (3·67 μmoles ACh/g fresh tissue/h) as reported by McCaman and Hunt[4]. It is thought that the low concentration (1 μmole/ml.) of thioglycollate present in most of our experiments may be necessary for the stability of the enzyme. In this connexion it is of interest that we have so far been unable to obtain identical rates of synthesis of ACh with the synthetic acetyl-CoA and coupled enzyme systems using partially purified placental choline acetyltransferase. The latter is considerably less stable than the enzyme from rabbit brain.

From experiments designed to determine the concentration of acetyl-CoA required to saturate our choline acetyltransferase preparations it is possible to obtain approximate K_m values for acetyl-CoA. At 39° C the K_m is $1{\cdot}67 \times 10^{-4}$ M for *Torpedo* and $4{\cdot}9 \times 10^{-5}$ M for rabbit brain enzyme. At this temperature the yield of ACh from *Torpedo* choline acetyltransferase with synthetic acetyl-CoA is 80–90 per cent of that obtained from the coupled enzyme system, but at 25° C, a temperature much closer to that which the enzyme in this species is normally exposed, the yields of ACh from the two incubation systems are equal.

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Brain Myelination after Neonatal Administration of Oestradiol

Oestradiol administered neonatally hastens functional brain maturation of the young rat as demonstrated by electroshock techniques[1]. The mechanism of this action is unknown, but it has been suggested that it is related to changes in brain metabolism and/or myelination[1]. Postnatal histological studies of the brain of the normal rat indicate that the rate of myelination in the hypothalamus is greatest between 10 and 20 days of age[2,3]. In the cortex this process begins on about day 12 and continues until about day 60 of age[4]. The present investigation was undertaken to learn whether or not early postnatal injection of oestradiol influences the rate of brain myelination in the developing rat, and whether it thereby hastens functional brain maturation.

Ten litters of six female rats each were kept with lactating mothers until they were killed at either 15 or 20 days of age. Food (a standard rat diet in pellets) and water were given *ad libitum* to the mothers throughout the experiment. The infants in each litter were divided equally into controls and rats to be injected with oestradiol. Oestradiol suspended in 'Mazola' oil was injected subcutaneously in a daily dose of 100 μg/100 g body-weight for 4 consecutive days beginning when the animals are 6 days old. Controls were injected with 'Mazola' oil alone. Half the animals were killed by decapitation on day 15 and the other half on day 20. These ages were selected because they correspond with critical changes in electroshock seizure responses[1].

After fixation in 10 per cent formalin for one week, the hypothalamus and sensory-motor cortex of each brain were separated, dehydrated, embedded in paraffin, sectioned at 6μ and stained with the modified Mann stain[5]. Fibres were selected at random and the smallest cross-sectional diameter of the myelin sheath and of the axis cylinder was measured with an ocular micrometer at a magnification of 1,000. The cross-sectional area of the myelin sheath was calculated by subtracting the area of the axis cylinder from the total fibre area. Ten measurements were taken for each animal. The significance of differences between groups was calculated by the *t*-test. The results obtained are summarized in Table 1.

In the hypothalamus of the controls, the cross-sectional area of myelin increased significantly between 15 and 20

Table 1. Mean Cross-sectional Areas of Myelin Sheaths in the Hypothalamus and Sensory-motor Cortex of Control and Oestradiol-treated Rats

Treatment	Age (days) 15	Age (days) 20	P‡ 15 versus 20 days
Hypothalamus (area in μ^2)			
Control	88 ± 3*	110 ± 6	< 0 01
Oestradiol, 100 μg/100 g body-weight injected from days 6–9	109 ± 4	117 ± 4	–
P† (controls versus oestradiol)	< 0 01	< 0 01	–
Sensory motor cortex (area in μ^2)			
Control	91 ± 3	86 ± 1	–
Oestradiol, 100 μg/100 g body-weight injected from days 6–9	119 ± 3	114 ± 2	–
P† (controls versus oestradiol)	< 0 01	< 0 01	–

* Mean ± *S.E.*

† Values based on *t*-test for significance of difference of means (controls versus oestradiol).

‡ Values based on *t*-test for significance of difference of means (15 versus 20 days).

days of age. In rats treated with oestradiol, myelin at 15 days of age was significantly greater than in the controls. Since myelin in the hypothalamus of treated rats does not significantly increase between 15 and 20 days of age, it would appear that in these rats, but not in the controls, the period of rapid myelination in the hypothalamus is almost complete at fifteen days. Thus the period of rapid myelination in the hypothalamus was accelerated by oestradiol.

In contrast to the hypothalamus, the sensory-motor cortex of control rats did not show a significant increase in myelin between 15 and 20 days of age. This is in agreement with other investigations[4] which report that myelination in these areas of the brain occurs slowly, over a relatively long period of time, and is complete at about 60 days of age. Similarly, the cortices of treated rats showed no increase in myelin between 15 and 20 days. At both ages, however, myelination was more complete after oestradiol.

The precocious laying down of the myelin sheath around nerve fibres in the rat brain, demonstrated here, may represent a mechanism by which early postnatal administration of oestradiol accelerates functional brain maturation. Although the nature of this mechanism is still unknown, it is suggested that oestradiol may affect some aspects of cholesterol metabolism which are critical for early myelination. Cholesterol is involved in myelination not only by its accumulation in the free form in the myelin sheath, but also by a possible role in the transport of fatty acids to and from the sites of triglyceride formation and breakdown[6]. Oestradiol has been shown to reduce serum cholesterol in the adult rat, but this has been demonstrated only through some actions of the liver[7]. Rats with diseases characterized by deficient myelination have brains greatly deficient in cholesterol as compared with controls[8]. It is suggested that the effects of oestradiol on myelination are, at least in part, a result of its action on cholesterol metabolism.

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Collagen Synthesis in Fibroblasts transformed by Oncogenic Viruses

The synthesis of collagen has been examined in cultures of various cell strains and established cell lines of fibroblastic origin[1–5]. All the strains and most of the established lines synthesized considerable quantities of collagen, but the rate of synthesis varied among the different strains and lines[4,6]. One line, 3*T*3, in contrast to others evolved from the same initial mouse embryo culture, was found to be severely restricted in its ability to synthesize collagen[2,7], while transformants of this line produced by polyoma virus or SV_{40} were able to synthesize collagen at higher rates[7]. It therefore appeared that collagen synthesis in cell line 3*T*3 was repressed and this repression was released by the viral transformation.

These experiments on 3*T*3 and its transformants were carried out on cultures grown in the Dulbecco-Vogt modification of Eagle's medium[8], which itself contained no ascorbic acid, but which was supplemented with 10 per cent calf serum. As fresh calf serum usually contains about 5 μg/ml. ascorbic acid[9], the final medium contained 0·5 μg/ml. or less. Since ascorbic acid is known to be important for collagen synthesis[10,11], the collagen synthesizing ability of cell strains[4,12] and lines has now been tested at higher concentrations of ascorbate. A concentration of 50 μg/ml. is more than sufficient to ensure a maximal rate of synthesis.

The rate of collagen synthesis may be estimated by determining the amount of hydroxyproline accumulated in the cell layer[2] or by exposing the cells to ^{14}C-labelled proline and measuring its incorporation into the non-dialysable hydroxyproline residues of collagen and into the proline residues of all the culture proteins. From the ratio of counts in HyPro to counts in Pro and knowing the abundance of HyPro in collagen and of Pro in the average cell protein, the ratio of collagen (C) synthesized to cell protein (P) synthesized $\left(\frac{\Delta C}{\Delta P}\right)$ during the period of exposure to the labelled precursor can be calculated[3]. This value is an index of differentiated function by the fibroblast.

Table 1 shows that in the absence of added ascorbate the mouse fibroblast line 3*T*6 gives a $\frac{\Delta C}{\Delta P}$ value of 3·5, while cell line 3*T*3 has a $\frac{\Delta C}{\Delta P}$ of only 0·55. Three viral transformants of 3*T*3—*SV*-3*T*3-5, *SV*-3*T*3-10 clone 1 and the doubly transformed *SV*-*PY*-3*T*3-11 clone 11 (ref. 13)—devote a larger fraction of their total protein synthesis to collagen formation than the parent line (up to nearly four-fold). This confirms the results obtained earlier by determining the quantity of collagen accumulated in the cell layer[7].

The relative ability of the cell lines to synthesize collagen is very different if 50 μg/ml. ascorbic acid is added to the medium prior to and during the 12-h period of exposure to the ^{14}C-proline. Line 3*T*6, previously considered to be the best of the lines for collagen synthesis, approximately doubles its rate of synthesis $\left(\frac{\Delta C}{\Delta P} = 6{\cdot}4\right)$, but 3*T*3 undergoes a relatively greater increase, the $\frac{\Delta C}{\Delta P}$ value rising from 0·55 to 7·2. It is therefore clear that in the presence of adequate levels of ascorbic acid, cell line 3*T*3 synthesized collagen as well as any other fibroblast line. In the viral transformants $\frac{\Delta C}{\Delta P}$ was less affected by ascorbate supplementation and was always lower than in the parent cell type (Table 1). The doubly transformed line, *SV*-*PY*-3*T*3-11 clone 11, did not respond to ascorbate supplementation at all; its $\frac{\Delta C}{\Delta P}$ values were comparable with

those found for *L*-929, an established fibroblast line with a long culture history[14] and some neoplastic potential[15]. In other experiments an SV_{40} transformed line derived from a human diploid fibroblast strain[16] was similarly found to be unresponsive to ascorbate supplementation and to have a lower rate of collagen synthesis than the parent strain.

Table 1. COLLAGEN SYNTHESIS BY FIBROBLAST LINES AND THEIR VIRAL TRANSFORMANTS

	HyPro/Pro (%)		$\Delta C/\Delta P$ (%)	
	low ascorbate	ascorbate supplemented	low ascorbate	ascorbate supplemented
3*T*6	9·47	15·4	3·5	6·4
3*T*3	1·60	17·2	0·55	7·2
SV-3*T*3-5	5·35	—	1·93	—
SV-3*T*3-10 clone 1	2·31	13·7	0·80	5·5
SV-3*T*3-5 clone 3	1·88	9·9	0·65	3·8
SV-*PY*-3*T*3-11 clone 11	4·65	4·24	1·66	1·51
L-929	4·56	4·65	1·60	1·66

Collagen synthesis in the transformed cell lines therefore differs from that in the parent cells in two respects: (1) When tested under conditions that allow the maximal rate of collagen formation (adequate ascorbate supplementation) the viral transformants produce less collagen per unit of protein synthesized than the parent line. A second differentiated function, hyaluronate synthesis, also is reduced in transformed 3*T*3 cells[17]. (2) Collagen synthesis in the transformed cells is less dependent on exogenously supplied ascorbate. The first result is consistent with the commonly observed loss of differentiated function occurring in neoplastic cells; the second with an increased metabolic autonomy.

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Incorporation of ^{35}S-methionine into Insulin

IN our investigations[1] of the biosynthesis of the *A* and *B* chains of insulin in rats by means of labelled amino-acids, we studied the incorporation of ^{35}S-methionine into that protein, the presence of which has not yet been experimentally demonstrated in insulin. There are some indications in the literature that the amino-acid composition of insulin in rats differs somewhat from that in animals of other species[2–4].

We carried out an investigation *in vivo* of the incorporation of ^{35}S-methionine into insulin of rats. The labelled amino-acid was injected intraperitoneally into the rat (1·2 μc./1·0 g body-weight; spec. act., 530 μc./mg). The animals were killed at various times after the injection. In some experiments insulin was isolated by Petting's method[5]. To purify the admixtures, the isolated insulin was reprecipitated many times at its isoelectric point. The radioactivity of the 'purified' insulin was determined, and results obtained are shown in Table 1.

Table 1. INCORPORATION OF ^{35}S-METHIONINE INTO RAT INSULIN AT VARIOUS TIMES AFTER INJECTION OF LABELLED AMINO-ACID (c.p.m./mg)

Time (min)	40	80	120
Radioactivity (c.)	240	540	680

Due to the possibility that the radioactivity found in insulin after injection of ^{35}S-methionine could be the result of a transformation of the labelled amino-acid into cysteine, followed by incorporation into a molecule of insulin, we determined the amino-acid residues of insulin to which activity belonged. For this purpose, insulin labelled *in vivo* with ^{35}S-methionine was oxidized by Sanger's method[6]; the chains were separated by high-voltage electrophoresis (1·0 mg oxidized insulin was transferred to the electrophoregram, and electrophoresis was carried out in phosphate buffer at *p*H 8·6 and 1,000 V). After eluting the insulin chains with dilute alkali (followed by evaporation under vacuum) the complete hydrolysate of each of the chains was radiochromatographed. The chromatograms of hydrolysates of the *A* and *B* chains of insulin showed radioactivity in the methionine and cysteine positions. In chain *A* 70 per cent of all activity was found in methionine and 30 per cent in cysteine, while in chain *B* the radioactivity of methionine and cysteine was the same (Table 2).

Table 2. DISTRIBUTION OF RADIOACTIVITY BETWEEN METHIONINE AND CYSTEINE RESIDUES IN INSULIN AND ITS *A* AND *B* CHAINS AFTER INJECTION OF LABELLED AMINO-ACID (c.p.m./mg)

Mode of isolation	Amino-acid residues	Insulin	Chain *A*	Chain *B*
Isoelectrical precipitation	^{35}S-methionine	540	350	509
	^{35}S-cysteine	498	150	417
Immunological	^{35}S-methionine	72	—	—
	^{35}S-cysteine	60	—	—

Thus, the data obtained show that ^{35}S-methionine is incorporated into both chains as such, and in addition that its radioactive sulphur resides in the cysteine residues of insulin.

The incorporation of ^{35}S-methionine into insulin in the process of biosynthesis of this protein was also confirmed by isolating insulin immunologically[7]. Complete acid hydrolysis and radiochromatography of the antigen–antibody complex revealed that the radioactivity resided in the methionine and cysteine positions (Table 2). It should be noted that in this experiment 55 per cent of the radioactivity of the original methionine was found incorporated in the cysteine residues of the insulin chains. This finding is in close agreement with the data obtained when insulin was isolated isoelectrically.

The present data indicate that ^{35}S-methionine is incorporated into rat insulin and that it is, in fact, a constituent amino-acid of this protein.

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PHYSIOLOGY

Influence of Umbilical Blood Fructose on the Production of Fructose by the Perfused Placenta of Sheep

Several investigations on the sheep placenta, perfused through the umbilical vessels, have shown that this tissue synthesizes fructose and that the initial precursor is glucose[1–4]. These investigations were performed in preparations in which the perfusion circuit was primed initially with maternal blood. Since the maternal blood contains little or no fructose, the admixture of this blood with foetal blood (contained in the placental vessels at the time of incorporation into the perfusion circuit) results in a perfusate which has a lower fructose concentration than was originally present in the foetal blood. Under these conditions, the fructose concentration of the blood recirculating through the placenta typically rises with time. The concentration rises rapidly at first, then more slowly, to reach within 1–2 h a plateau concentration in the range of 100–150 mg/100 ml.; values commonly found in foetal sheep blood. However, in one experiment reported by Alexander, Huggett, Nixon and Widdas[1] some fall was observed following the addition of fructose such that the concentration in the perfusate was greater than this range. Nixon[5] found in the isolated uterus of a pregnant sheep that the placental production of fructose was very much reduced when the fructose concentration in the umbilical arterial blood was similar to that found normally in foetal blood (the uterus was maintained by perfusion circuits through both the uterine and umbilical vessels). Both Alexander[6] and Nixon[5] have suggested that the production rate of 5–10 mg fructose/min previously observed[1] was due to the existence of a subnormal concentration of fructose in the umbilical perfusate. The purpose of the present work was to test this postulate by perfusing sheep placentae by the technique of Alexander, Huggett, Nixon and Widdas[1] with different fructose concentrations in the perfusing blood.

The animals used were Dorset Short-Horn sheep bearing foetuses of 100–120 days, anaesthetized by the spinal administration of procaine. The lower half of the ewe was placed in a saline bath at 38° C, the foetus was delivered by Caesarian section and then detached. Into the umbilical arteries were inserted cannulae fed from a reservoir by a variable speed rotary pump; cannulae inserted into the umbilical veins returned the blood to the reservoir. About 150 ml. of maternal blood was in the extracorporeal circuit. The perfusion pressure was about 40 mm mercury and the rates of blood flow were between 64–136 ml./min. Samples of blood were removed at intervals from the reservoir and the plasma deproteinized with 0·3 N sodium hydroxide and 5 per cent zinc sulphate. The concentration of fructose and glucose in the filtrates was determined by the resorcinol[7] and glucose oxidase[8] methods, respectively. After a period of recirculation the fructose concentration of the perfusate was altered by alternate flushing (venous outflow discarded) and recirculating with the new blood through the placental circulation; this procedure was repeated several times.

The results obtained in two experiments are shown in Fig. 1. In the first phase of experiment *A*, the concentration of fructose rose from 75 to 107 mg/100 ml over a period of 30 min. When, however, the perfusate was replaced by one containing fructose at about 180 mg/100 ml. no production of fructose was apparent. The cessation of fructose production was not due to a deterioration of the preparation because on returning the placenta to a perfusate with a low concentration of fructose, the concentration rose from 48 to 87 mg/100 ml. over 30 min.

In another experiment (not illustrated), which followed the same pattern, the initial 30 min of perfusion was accompanied by a rise in fructose concentration from 108 to 135 mg/100 ml. In the presence of a higher fructose concentration it rose only from 178 to 186 mg/100 ml. over the same period of time; however, on reverting back to a low concentration, the concentration rose from 27 to 100 mg fructose/100 ml. in 30 min.

In the experiment illustrated in *B*, the first phase of the experiment was conducted in the presence of a high fructose concentration followed by a phase at a low concentration and finally back to a high concentration. It will be seen that an increase in the fructose concentration only occurred when the concentration was low.

Using a 'through' perfusion technique, in which the pump drew on a large volume of blood and the outflow from the umbilical veins was collected at frequent intervals in separate containers, it was possible to compute the rate of fructose production in mg/min. In one such experiment where the concentration of fructose in the effluent blood was raised to 172 mg/100 ml. it was found, after a 10 min equilibration at this concentration, that the effluent blood collected in minute fractions had concentrations varying only from 164 to 176 mg fructose/100 ml. with blood flows between 105 and 109 ml./min. From knowledge of these arterio-venous concentration differences and of the rate of blood flow, the production or loss may be calculated. Over the 12 min of the observation period there was a net loss of 23·5 mg fructose which could be attributed to transplacental passage; this can occur at a rate of 0·4–2·0 mg/min in perfused preparations[5].

In all experiments the variations in fructose concentration were independent of the glucose concentration in the perfusate.

Hers[9], on the basis of *in vitro* studies, suggested that both the placenta and the foetal liver were involved in the synthesis of foetal blood fructose. It was suggested that in the placenta the conversion of glucose to sorbitol by aldose reductase (polyol: NADP oxidoreductase) occurred; the sorbitol then passed into the foetal circulation and was converted to fructose by sorbitol dehydrogenase (L-iditol: NAD oxidoreductase) in the foetal liver. While perfusion studies have confirmed that the foetal liver can convert sorbitol to fructose[10], the results of placental perfusion suggest that insufficient sorbitol is liberated from the placenta to substantiate the foregoing hypothesis. How-

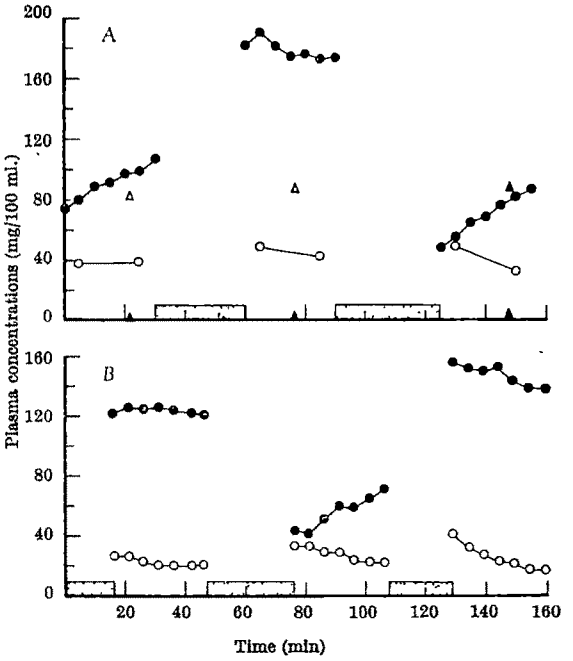


Fig. 1. Production of fructose by two perfused sheep placentae *A* and *B* in the presence of varying concentrations of fructose in the perfusate. The stippled area represents the equilibration period at the new concentration. ●, Umbilical plasma fructose; ○, umbilical plasma glucose, ▲, maternal plasma fructose; △, maternal plasma glucose

ever, it was suggested that a conversion of glucose→sorbitol→fructose occurred entirely within the placental cell and that the cell membrane was permeable to fructose but not to sorbitol[3,4]. The results presented here provide evidence that the foregoing reaction is suppressed by the phenomenon of product inhibition at high fructose concentrations. It is probable, therefore, that the rate of fructose production is governed by the rate at which it is lost from the foetal circulation through transplacental passage and utilization.

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Cyanide Excretion in the Rat

Much is now known about the body's metabolism of cyanide within a pattern of normal physiology. The conversion of cyanide to thiocyanate was first demonstrated in 1894 [1] and the enzymatic nature of this reaction was investigated in 1933 [2]). The enzyme system was named 'rhodanese' and it has been found to be specific for free cyanide without action on organically bound cyano groups. This property indicates that the excretion of thiocyanates after the administration of organic nitriles results not from a direct action on the nitrile itself, but from hydrogen cyanide which had first been removed from the nitrile molecule by another mechanism. Although this rhodanese/thiocyanate excretory pathway is considered to be the primary factor in the detoxification of cyanide there undoubtedly exist other pathways by which this may be mediated or assisted[3]. These implicate one-carbon compounds and also reveal that the radiocarbon of $Na^{14}CN$ appears in the methyl groups of choline and methionine and in the ureide carbons of allantoin. Some of it also finds its way into the cyano group of cyanocobalamin. It is known that hydroxocobalamin takes up cyanide avidly to form cyanocobalamin and can protect the experimental animal against lethal doses[4]. Latterly, a further pathway in the detoxification of cyanide has been implicated[5]. This shows that cyanide can combine with cystine *in vitro* to form 2-iminothiazolidine-4-carboxylic acid and that the formation of this acid also occurs *in vivo*. On injection of potassium cyanide subcutaneously into rats, the acid can be isolated from the urine in quantities corresponding to 15 per cent of the dose, although the main excretory product (80 per cent of the dose) is thiocyanate.

Cyanide has been extensively used by various routes as an experimental demyelinating agent[6–8]. Furthermore, it has now been shown that small doses given over a prolonged period of time may produce changes in the central nervous system of the rat without the production of manifest anoxia[9]. This elimination of the anoxic element produced by previous workers in the experimental demyelinating field lends support to the suggestion that a disorder of cyanide metabolism may be implicated in those diseases in which demyelination occurs within a picture of vitamin B_{12} deficiency[10,11].

The mechanism of cyanide demyelination is unknown. Although it has been stated that cyanide is not a cumulative poison[12] it was decided to investigate the excretion of cyanide/thiocyanate over a prolonged period of time. Adult albino Wistar rats were used in the experiment. They were maintained on a standard Oxoid modified diet 41*B*. A subcutaneous injection of 1 mg potassium cyanide was given once weekly for 20 weeks. A basal urine was collected prior to the initiation of the experiment and thereafter urines were collected at monthly intervals for the 24 h immediately following the injections. The estimation of urinary cyanide and thiocyanate was performed by a modification of the method of Aldridge[13]. This utilizes the conversion of cyanide and thiocyanate to cyanogen bromide followed by the determination of the cyanogen bromide by a colour reaction with a pyridine/benzidine reagent. 1·8 ml. N/10 hydrochloric acid had 0·2 ml. urine added and 0·1 ml. bromine water. 0·5 ml. of 2 per cent arsenicus acid was then added to the solution to remove excess bromine. It is important to shake the mixture well after the addition of each reagent. 3 ml. of the pyridine/benzidine reagent was then added and a colour change due to the unknown amount of cyanide and thiocyanate slowly develops and reaches a maximum in 25–30 min. This remains constant for about a further 30 min. It must be emphasized that the stability of the colour is dependent on temperature and allowance must be made for this fact if there is any great fluctuation from 20° C. With each determination, suitable blanks were also prepared by omitting the bromine water. Standard solutions of sodium thiocyanate (containing 3·4 μg/ml.) were made up using volumes of 0·2 ml., 0·5 ml. and 1·0 m. The graph obtained from these is virtually linear and allows the unknown to be calculated directly. If linearity is not established due to temperature distortion then a suitable curve may be drawn and the unknown read from this. The degree of colour change was estimated on a Hilger and Watts Spekker using an Ilford 4 spectral green filter. This reading minus the blank determination was then converted to μg thiocyanate/ml. urine. Urines were stored in the deep freeze after the initial assay and a duplicate performed 1 week later. The mean of these two readings was taken as representing the monthly level for the individual rat.

The results of this experiment show that seven of the eight rats subjected to the procedure gradually re-adapt their metabolic pathways to the injected cyanide so that it no longer appears in the urine as the quantities of thiocyanate found after the initial injection. The monthly output of these seven animals has been expressed as a mean excretion value of the individual mean values obtained (Table 1). One rat (Table 2) proved a notable exception to the general rule although at the conclusion of the experiment its capacity to excrete the cyanide as thiocyanate also appeared to be diminishing. Although many factors influence thiocyanate excretion (body mass, diet, renal clearance, etc.) and for obvious reasons a controlled 24-h sample was not obtainable from the rats used in this experiment, there can be little doubt that a definite trend has been established. The animal which showed a persistently raised thiocyanate excretion may provide a pointer to the fact that the individual rat's tolerance to the same cyanide stimulus shows a marked variation from the neuropathological point of view. Identical histologi-

Table 1. Mean Urinary Thiocyanate Levels expressed as μg/ml. in Seven Experimental Rats at Monthly Intervals

Basal	1	2	3	4	5
5 8	39 6	12 6	14 8	6 8	4 9

Table 2. Mean Urinary Thiocyanate Level expressed as μg/ml. in One Experimental Rat at Monthly Intervals

Basal	1	2	3	4	5
6 8	154·6	35·8	35·7	86·0	19·4

cal changes in the central nervous system are not reproducible in different animals otherwise similar in all respects[9].

The explanation for this disappearance of thiocyanate from the urine in response to the same cyanide stimulus is obviously of theoretical and possibly practical interest. It could be shown that delayed excretion was not the explanation because collecting urine for the second period of 24 h following the injections failed to demonstrate a rise in thiocyanate excretion compared with the figures for the first 24 h presented in this communication. It appeared possible that the cyanide/cystine pathway may have superseded the rhodanese/thiocyanate mechanism and it was obviously important to confirm or exclude this possibility. Cystine may be extremely important as a factor in central nervous system metabolism and it has been shown that this highly insoluble amino-acid can apparently pass through the blood brain barrier and become incorporated in neural proteins[14]. Chromatography of urine for 2-iminothiazolidine-4-carboxylic acid was accordingly performed (Dr. A. S. Azatoor) by the method of Wood and Cooley[5]. This substance could not be identified in the urine of a normal control rat, nor in the urine of a rat which had lost the capacity to excrete cyanide as thiocyanate, nor in the urine from the rat which had substantially retained this mechanism. Although it is claimed that 2-iminothiazolidine-4-carboxylic acid is an innocuous substance the evaluation of the possible effects of repeated injections does not appear to have been assessed in this context. However, our failure to demonstrate it in the urine in this experiment suggests that both the main excretory pathways for cyanide are affected by chronic unphysiological cyanide stimulation. We are left with the possibility that one-carbon compounds or the vitamin B_{12} cycle now play the major part in cyanide metabolism and it is known that the vitamin B_{12} activity of the liver is depleted by repeated injections of cyanide[9,15]. Vitamin B_{12} is stored in the liver as the coenzyme which is cyanide-free and on photolysis yields hydroxocobalamin. Finally, some other unknown degradation product of cyanide metabolism may injure the myelin sheath or cyanide *per se* interfere with some vital intramyelin metabolic pathway.

Although it has been stated that it is not the products of cyanide metabolism which are responsible for cyanide encephalopathy[16] this alteration in the excretory pathway raises all sorts of possibilities which may have important clinical connotations with respect to some disorders of the central nervous system in man.

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Interoceptive Conditional Response in Cortical Neurones

It has previously been reported[1] that, by presenting simultaneous visceral (splanchnic) and somatic (sciatic) afferent stimuli, evoked potentials can be conditioned in the sensorimotor cortex of the cat. As a continuation of these investigations, we examined cortical units during the same classical interoceptive conditioning procedure to demonstrate the possibility of this type of conditioning in the neurones of the sensorimotor cortex. There are data in the literature on response patterns of cortical units during exteroceptive conditioning in experiments with differing goals[2–4].

Twenty adult cats were anaesthetized with ether and tracheotomized. The left sciatic and splanchnic nerves were exposed, for sequential stimulation in a conditional paradigm. A section of the skull was removed over the sensorimotor area (posterior sigmoid and coronary gyri). In seven of the twenty animals chloralose (10 mg/kg) and local anaesthesia were then substituted for ether. In the other thirteen animals local anaesthesia was combined with immobilization by intravenous gallamine triethiodide and artificial respiration. Stainless-steel microelectrodes were inserted into the brain through a covering layer of agar, which maintained the pressure relations in cerebrospinal fluid and minimized arterial and respiratory pulsations.

The conditioning procedure was as follows: single sciatic shocks (10 V amplitude, 1 or 2 msec duration) were applied every 5 sec. Cells were sought which were either (*a*) silent except during sciatic stimulation (more numerous in chloralose anaesthesia) or (*b*) spontaneously firing, but with changing patterns on sciatic stimulation used as an unconditional stimulus.

When such cells were found responding to sciatic stimulation, the conditioning procedure began: single splanchnic shocks (5–10 V, 1–2 msec) as conditional stimuli were applied every 5 sec. Then splanchnic shocks were 'reinforced' with preceding sciatic stimulation, the conditional stimulus–unconditional stimulus interval being 200 msec. Usually a sequence of ten to forty pairings were presented with an interval of 5 sec between pairs. Finally, the conditional stimulus was presented alone. In each experiment from one to twelve units were 'trained' successively, and the firing pattern photographed.

The units examined were usually not initially responsive to the splanchnic stimulus (conditional stimulus), but fired to the sciatic shock (unconditional stimulus). Spontaneously firing units increased or decreased their firing rate. Altogether, 88 such units were found which changed their firing pattern to sciatic stimulation, and of these 88 units, 25 were capable of being conditioned. Fig. 1 is the record of a cell of a cat under chloralose anaesthesia. The cell, which was unresponsive to splanchnic stimulation before the pairing, gave a clear response after fifty reinforcements with the sciatic stimulus. In the seven animals narcotized with chloralose, 22 units responded on sciatic stimulation, and of these, five cells were conditionable, that is, they did not respond to a splanchnic stimulus unless this was repeatedly paired with sciatic shocks. These units then responded to splanchnic stimulation alone with a response that was strictly correlated with the time at which the sciatic response had appeared in the paired stimulus sequences. The criterion of conditioning was established on the basis of the temporal sequence of the unit firing pattern, and on the ability to 'extinguish' and 'retrain' the responsiveness to splanchnic stimulation.

Several units which fired spontaneously and irregularly before conditioning were not initially responsive to splanchnic stimuli, but became so after sciatic reinforcement. In the thirteen immobilized animals, 66 units were found which changed their firing rate in response to a sciatic stimulus, and twenty of these cells were condi-

tionable. In the majority of these units, or groups of units, the frequency of the firing increased following the sciatic shocks, but in some cases a diminution of the firing rate was observable. In one case this inhibition of the unit activity could be conditioned. In most cases we succeeded in holding the units in good condition for 20–60 min, and in many cells extinction and reconditioning were repeatedly performed. By conditioning the firing pattern of cortical units to splanchnic stimulation, we confirmed earlier data on conditioning of evoked potentials[1], as well as the data of other authors about alteration of unit activity by training[2–6].

Each neurone studied was responsive to sciatic stimulation but not to splanchnic stimulation, although the sensorimotor cortex has been described as the cortical projection area of both sciatic and the splanchnic afferents[7]. After training, however, using the splanchnic nerve stimulation as conditional, and the sciatic nerve stimulation as unconditional stimulus, some units also became responsive to a splanchnic stimulus. Thus, we obtained a relatively simply interoceptive conditioned response (that is, the conditional stimulus was a visceral afferent impulse).

These results may be interpreted as indicating that the neuronal basis of the process of learning might have its origin in a facilitatory state having some resemblance to post-tetanic potentiation[8,9], but clearly differing from it in the requirement for a specific time interval before the initiation of the conditional discharge. It is noteworthy that, after training, the conditional stimulus evokes a cell firing with the same delay as the unconditional stimulus previously applied. This precise timing in the delay of the conditional response may involve some as yet imperfectly understood excitation mechanism, perhaps related to such phenomena as long-lasting inhibitory postsynaptic potentials[10]. Our data do not indicate, however, whether these new neuronal responses arise in or are triggered from a particular cortico-subcortical system, or whether the generation of these delayed spikes is the property of all cortical or subcortical cells.

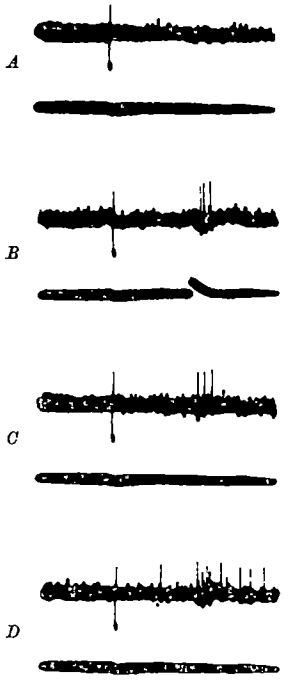


Fig. 1. Conditional responses of a group of cortical units. Conditional stimulus (to splanchnic nerve) appears as artefact in top trace in each case, and unconditional stimulus (to sciatic nerve) as notch in lower trace. Interstimulus interval 200 msec. *A*, Splanchnic stimulation alone initially produces no response. *B*, Paired stimulation of splanchnic and sciatic nerves, with group of 3 discharges to sciatic stimulus *C* and *D*, Induction of responses to splanchnic stimulation alone after paired reinforcements

Such a function in the mammalian brain may be similar to that of the 'pacemaker neurones' described recently in invertebrates[11].

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Aerosol Deposition in the Lungs of Space Travellers

The deposition of aerosols in the lungs of man during breathing constitutes an important mechanism for the entry of toxins or pathogenic organisms to the body. The respiratory tract is protected by an efficient ciliary mechanism for the rapid removal of particles deposited in the upper airways. Particles reaching the alveolar regions are removed more slowly. There are three mechanisms whereby inhaled particles are deposited on the walls of the airways—inertial impaction, sedimentation by gravity, and diffusion due to Brownian motion. The dimensions of the airways are such that large particles are deposited in the upper airway and have no opportunity of reaching the alveoli.

In the absence of gravity, as in an orbiting spacecraft, one may anticipate that particles normally deposited in the lungs by sedimentation (principally those particles of 1–8μ diameter) will be exhaled again with no deposition. Under the conditions of a reduced gravitational field as on the Moon an important distinction arises. While the deposition of particles in the range 1–8μ diameter will be less than normal, the particles of this size which are deposited will do so in a deeper region of the airways than normal. One effect of a reduced gravitational field may thus be to allow the access of large particles to the non-ciliated alveolar region of the lung. Particles of this size are important in the airborne spread of infection because they include the droplet nuclei carrying bacteria which are produced by the act of coughing or talking. The importance of this hazard to astronauts remains to be established.

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Motor Unit in Old Age

Decrease of muscular efficiency, fatigue and slowness of motor reactions are considered to be typical consequences of old age. No convincing evidence, however, has been brought forward as yet for the assumption that these motor deficiencies in old age are due to regressive changes, such as loss of nerve cells, disturbances of neuromuscular transmission or to degenerative atrophy of the muscle fibres themselves[1,2].

In many respects the muscles of an old animal resemble denervated muscles in their metabolic reactions. For example, the characteristic differences of metabolism between muscles with different function are lost[3] and proteolytic activity increases[4] in both denervated muscles and muscles of an old animal. On the other hand, the increased proteosynthesis in spinal motor neurones after nerve interruption which appears to be a characteristic reaction of a nerve cell to interruption of its cell process[5] is missing or very much delayed in the motor neurones of an old animal[6]. This corresponds to the delayed latent period (that is, the time before re-innervation of the peripheral nerve stump begins) during the regeneration process in an old animal[7] and there is also a considerable delay of recovery of muscle proteins of an old animal once re-innervation of muscle has started[7]. Thus, the atrophy of muscle and the metabolic disturbances observed could be due to central (regressive changes or loss of motor neurones) or peripheral (atrophy and/or degeneration of muscle fibres) changes. In both cases a decrease in the number of muscle fibres could be expected. Indeed, the total number of muscle fibres in the soleus muscle of adult rats has been found to decrease from 2,357 ± 78 (ref. 8) in adult (4-month-old) rats to 1,758 ± 39 in the soleus of old (2-year-old) rats.

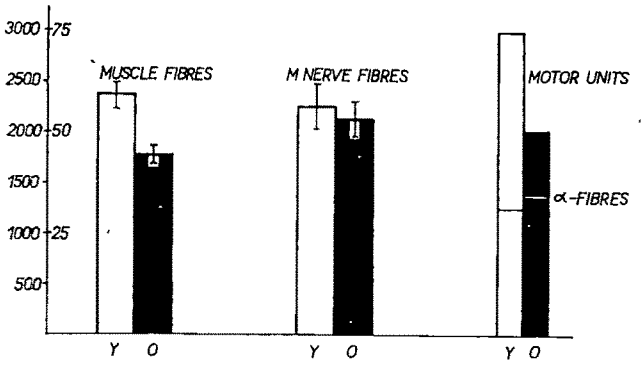


Fig. 1. Changes in total number of muscle fibres (left-hand side of ordinate) in soleus muscle of rats. Motor nerve fibres, α-nerve fibres, and motor units (right-hand side of ordinate) innervating the soleus muscle in young (*Y*) and old (*O*) rats

To determine the number of motor nerve fibres and the size of the motor unit innervating the soleus, dorsal roots L_3—S_2 were cut on one side in six animals and the motor nerves innervating the soleus muscle were removed 14–30 days later. Their number and diameters were determined in histological preparations stained using a modified Weigert method following fixation with 1 per cent osmic acid. The total number of motor nerve fibres innervating the soleus of old animals was 115 ± 2·31, compared with 120 ± 3·8 in adult animals. The number of α-motor fibres (more than 4μ) was 35 in the nerves of old, and 32 in those of adult animals. The histogram of the motor nerves of old animals showed a loss of bimodality, that is, only one maximum for fibres of 8·5–10μ instead of the two maxima (2·5–4μ and 8·5–10μ) found in the motor nerve fibres of adult animals, but no other changes in size distribution of the motor nerve fibres could be seen. Thus, it can be assumed that there is no loss of motor nerve cells in the spinal cord of an old animal. Due to loss of muscle fibres in muscles of an old animal, however, the size of the motor unit decreases by 33 per cent, that is, from 74 in muscles of adult rats to 50 in muscles of an old animal. It appears that this decrease in the size of the motor unit is due to peripheral changes and this assumption is substantiated by the changes in the sub-neural apparatus of the end plates as indicated by their cholinesterase activity. This shows diffusion, irregularity and granular fragmentation of the end plate structures. Beside random degeneration of the end plate, some terminal regeneration also occurs, which leads to irregularities in the pattern of innervation of the muscles of an old animal[9]. It remains to be seen how these peripheral effects are related to the previously mentioned disturbances of nerve-cell function[6,7].

The random degeneration and regeneration of end plates may explain the increased duration of the action potential in muscles of an old animal[10] and also the absence of 'denervation fibrillation' in muscles during old age.

We may conclude that, during old age, no loss of motor neurones occurs in the spinal cord of an old animal but that random degeneration of end plates leads to a loss of muscle fibres which results in a decrease in size of the motor unit.

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PHARMACOLOGY

Effects of Synthetic Angiotensin on Plasma Free Fatty Acid and Blood Glucose-levels

Recently, a number of investigators reported that the vasoactive peptides with smaller molecular weights, oxytocin, vasopressin and pitressin, decrease or increase plasma free fatty acid (FFA) levels in animals[1] and human subjects[2–4]. These peptides were also found to cause mobilization of FFA from the rat epididymal adipose tissues *in vitro*[5–8]. The present investigation was undertaken to examine the effects of a potent vasoactive peptide, angiotensin, on the plasma FFA and blood glucose-levels in dogs.

Six male dogs, fasted for 12 h, were anaesthetized with the intravenous administration of sodium pentobarbital (30 mg/kg). Mean systemic arterial pressure and heart rate were measured using techniques described previously[9]. To keep the activity of the sympathoadrenal system reasonably constant[10], 1 h was allowed to elapse from the time of the completion of the experimental preparation to the beginning of the measurements of the various parameters. Synthetic angiotensin ('Hypertensin') was infused intravenously at a rate of 0·1 μg/kg/min for 1 h. Blood samples were drawn from a femoral artery. The determination of blood sugar and plasma FFA levels were made in duplicate using the methods described by Nelson[11], Somogyi[12], Dole[13] and Trout *et al.*[14], respectively.

The results of the effects of angiotensin on plasma FFA and blood glucose levels are summarized in Table 1. Angiotensin always decreased heart rate and significantly increased mean systemic arterial pressure. Furthermore, angiotensin always significantly decreased both plasma FFA and blood glucose levels.

Table 1. Effects of the Continuous Intravenous Administration of Angiotensin (0·1 μg/kg/min) on Heart Rate, Mean Systemic Arterial Pressure, Blood Sugar-levels and Free Fatty Acid-levels

	Control	Angiotensin
Heart rate (beats/min)	143 ± 6	123 ± 4 ($P < 0.001$)
Mean systemic arterial pressure (mm mercury)	136 ± 6	167 ± 8 ($P < 0.025$)
Blood glucose levels (mg per cent)	128 ± 8	100 ± 5 ($P < 0.01$)
Plasma free fatty acid levels (μequiv./l.)	335 ± 21	156 ± 24 ($P < 0.005$)

Numerical values denote the mean ± standard error of the means of 6 dogs.

The effects of synthetic angiotensin on plasma FFA-levels and blood glucose-levels observed in the present investigation were essentially similar to those found previously with the other vasoactive peptides, pitressin and oxytocin[1-3]. Although the metabolic effects of pitressin and oxytocin have been referred to as 'insulin-like actions' by previous workers[1,5,6], Krahl[15] found that these vasoactive peptides do not promote protein synthesis. It is possible that angiotensin may also act in a similar fashion on protein synthesis. Recently, Feldberg and Lewis[16] observed that angiotensin stimulates directly catecholamines from the adrenal medulla in the cat. It is expected that the increased catecholamine secretion following the administration of angiotensin results in increases in plasma FFA and blood glucose-levels[13,17]. However, the results obtained from the present experiments do not coincide with this hypothesis in dogs. It is possible, however, that angiotensin may directly decrease plasma FFA- and blood glucose-levels, and these direct metabolic effects may overpower the indirect effects of catecholamines following the administration of angiotensin. Recently, Colville *et al.*[18] found that the cholinesterase inhibition with neostigmine lowered plasma FFA in dogs. Angiotensin increases arterial blood pressure and reflexly stimulates the parasympathetic nerve fibres. Hence, it is possible to suggest that angiotensin decreases plasma FFA by a similar manner. At present, however, the precise biochemical and physiological mechanisms responsible for the changes in plasma FFA- and blood glucose-levels are not clear. The *in vitro* investigation is indicated to elucidate this problem.

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Effect of Propranolol on the Peripheral Vascular Bed

A NEWLY synthesized compound, propranolol, was found to be a very potent, β-receptor adrenergic blocking agent[1]. Since the drug blocks the vasodilator (β-receptor stimulating) effects of catecholamines without affecting the vasoconstrictor (α-receptor stimulating) property, propranolol should be expected to cause the vasoconstriction of the peripheral vessels. However, Prichard and Gillam[2] recently found that propranolol lowered arterial blood pressure significantly in hypertensive patients. The present investigation was undertaken to examine the effect of propranolol on the peripheral vascular bed.

Dogs weighing between 20·5 and 25·0 kg were anaesthetized with sodium pentobarbital (30 mg/kg). In open chest dogs, a femoral artery was perfused with arterial blood at a constant and known rate by means of a Sigmamotor pump as described previously[3]. Systemic arterial pressure and femoral arterial perfusion pressure were measured continuously with Statham pressure transducers (*P23AA*). In this set-up, the effect of the drug on the peripheral vascular resistance was evaluated readily by the changes in the perfusion pressure. Heart rate and myocardial contractile force were measured continuously with an EFM tachometer and with a Walton–Brodie strain gauge arch[4,5] which was sutured on the right ventricular muscle. All parameters except heart rate were recorded continuously with an EFM recorder (*DR* 8).

As shown in the third part of Fig. 1, the effect of the intra-arterial injection of propranolol (25 μg/kg) on the femoral arterial perfusion was biphasic. The drug initially decreased the perfusion pressure markedly and rapidly but, within 1 min, the pressure began to increase and usually stabilized at a level slightly higher than control within 2 min. Since the femoral arterial blood flow was kept constant by means of a Sigmamotor pump and the systemic arterial pressure remained essentially unchanged throughout a period of the experiment, these biphasic changes in the perfusion pressure indicate that propranolol initially caused a transient vasodilatation and later a sustained vasoconstriction. As seen in the fourth part of Fig. 1, propranolol blocked completely the vasodilating effect of the intra-arterial administration of isoproterenol (0·01 μg/kg). However, the repeated administration of 25 μg/kg of propranolol caused an essentially similar degree of vasodilating effect as indicated by the decrease in the perfusion pressure. This effect was not modified by the intra-arterial injection of dichloroisoproterenol (0·8 mg/kg) or atropine (0·05 mg/kg). The magnitude of vasodilatation induced by propranolol was essentially proportional to the dose given, but that of the subsequent vasoconstriction became less and less striking with repeated administrations of the drug (25 μg/kg). However, much larger doses (more than 250 μg/kg) of propranolol, which significantly decreased systemic arterial pressure, markedly increased the femoral arterial perfusion pressure[6]. Qualitatively similar results were also obtained on the effects of propranolol on the peripheral vascular beds in the brachial artery.

From the present investigation, it seems evident that the intra-arterial administration of a small dose of propranolol dilates the regional arteries transiently before it constricts

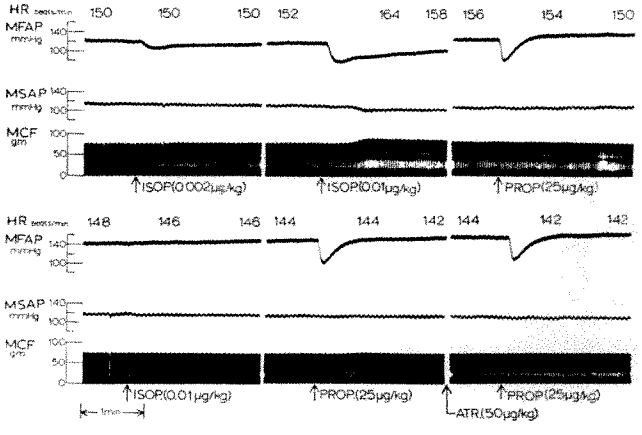


Fig. 1. Effect of propranolol on the peripheral circulation. *HR*, *MFAP*, *MSAP* and *MCF* denote, respectively, heart rate, mean femoral arterial perfusion pressure, mean systemic arterial pressure and myocardial contractile force of the right ventricle. *ISOP*, *PROP*, and *ATR* denote, respectively, isoproterenol, propranolol and atropine. These drugs were injected in the femoral artery. The femoral artery was perfused with arterial blood at a rate of 135 ml./min throughout a period of the experiment

the vessels by its β-receptor adrenergic blocking action. The initial vasodilatation is apparently due neither to stimulation of adrenergic β-receptor nor to that of cholinergic vasodilator fibres[7]. On the other hand, the administration of larger doses of propranolol constricts markedly the regional arteries mostly by the indirect stimulation of adrenergic α-receptor which is mediated through the increased reflex sympathoadrenal activities[6,7]. Obviously, the larger doses of propranolol always depress myocardial contractility and reduce stroke volume and cardiac output, thereby decreasing mean systemic and pulse pressures to elicit the cardiovascular reflexes[6,8,9]. Very recently, Prichard and Gillam[2] reported that propranolol may be useful for the management of essential hypertension. Although propranolol initially decreases the peripheral vascular resistance, the duration of the action is extremely short to be effective in the treatment of essential hypertension. Furthermore, we reported[6] that the doses of propranolol, which block effectively the adrenergic β-receptor, not only increase total peripheral resistance but also depress the ventricular function curve[10]. From the aforementioned reasons, it is rather difficult to comprehend the precise pharmacodynamic mechanism through which propranolol clinically alleviates essential hypertension.

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HISTOLOGY

Histochemical Demonstration of the Resorption of Xylose from Small Intestine

The high solubility of monosaccharides makes the topochemical demonstration of these substances in animal cells and tissues very difficult. In 1948, Okamoto, Kadota and Aoyama[1] described a method for the histochemical demonstration of sugars in tissue which is based on the insolubility of barium–glucose and barium–lactose in methyl alcohol. Gomori[2] showed that the specificity of this method was not sufficiently tested and the topochemical demonstration was unlikely to be very accurate. Müller[3] carried out tests with filter paper in order to discover whether it was possible to show the distribution of glucose by this method, and found a high specificity after fixation in a saturated solution of barium hydroxide in methyl alcohol. In experiments with animal tissues fixed at −10° C for 24 h he obtained a strong reaction in liver cells, but kidney cells were negative. Using freezing substitution with saturated barium hydroxide–methyl alcohol solution, Geyer[4] described the distribution of glucose in the kidney, and Stiller[5] was able to demonstrate the presence of glucose in the mature human placenta using this modification. Ehrenbrand[6] used cold microtome sections for the localization of glucose and probable galactose in the secreting mammary gland of hamsters.

Recently we succeeded in demonstrating solutions of monosaccharides, especially stereoisomeric sugars such as glucose, mannose and galactose, as arabinose and xylose or fructose. The solutions were dipped on filter paper and fixed with the saturated barium hydroxide–methyl alcohol solution at −22° C and were sharply localized up to concentrations of from 0·08 to 0·005 per cent. In contrast, sugar phosphates are difficult to demonstrate and the results of this experience suggest that disturbances of sugar phosphates by the topochemical demonstration of monosaccharides under physiological conditions are improbable.

Experiments were undertaken using white mice previously starved for 24 h and fed on 10 per cent xylose solution in order to study the resorption of sugars from the small intestine. The cryostat technique was used, in conjunction with fixation of the sections, in cold (−22° C) saturated barium hydroxide–methyl alcohol solution. It proved impossible to localize this sugar topochemically in the cells of intestine. After this, better results were obtained by modifying the fixation procedure and using a saturated solution of strontium hydroxide in methyl alcohol. First, tests were carried out on filter paper. Fig. 1 demonstrates the difference between the behaviour of xylose after fixation in saturated barium hydroxide–methyl alcohol solution at −22° C (Fig. 1*a*) and after fixation in saturated strontium hydroxide–methyl alcohol under the same conditions. These experiments showed a sharp localization and a deep blackness after fixation in strontium hydroxide–methanol and treatment with an alcoholic silver nitrate solution (Fig. 1*b*). Application of this modification to tissue samples from mice fed xylose resulted in the topochemical demonstration of xylose resorption.

Various stages of the resorption can be shown. Within the epithelial cells of the mucosa, granules in the supranuclear zone first become visible. Later these granules can be shown throughout the whole of the cytoplasm of the cells (Fig. 2). At the bases of the crypts the reaction is stronger. This is the main pattern, but occasionally the intercellular spaces reacted more strongly than did the cytoplasm of the cells. Afterwards, a diffuse positive reaction was observed in the stroma of the villi and the endothelial cells of the capillaries also reacted positively.

The results make it clear that xylose can be shown topochemically by this method during the process of resorption (the controls, which were not fed xylose, were negative). The developments reported here also show that xylose may enter mucosa cells in various stages. Furthermore, it is clear that the majority of the resorption of xylose from the intestine is achieved in a percellular way, and that less than half is achieved in an intercellular one.

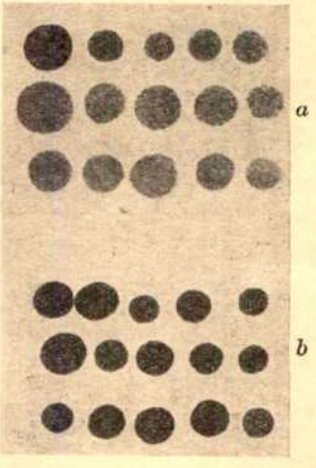


Fig. 1. Comparison of fixation of xylose on filter-paper at −22° C. (*a*) Fixation in saturated barium hydroxide–methyl alcohol; (*b*) fixation in saturated strontium hydroxide–methyl alcohol. Concentrations of xylose range from 10 per cent to 0·0006 per cent, beginning in the upper left-hand corner of each part. The concentration of xylose in each spot is half that in the previous spot



Fig. 2. Demonstration of xylose at a later stage of the process of resorption

It is possible that a more complete study including early stages of sugar transfer might show further differences in the process of resorption, especially in various parts of the intestine.

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IMMUNOLOGY

Effect of Large Quantities of Homologous Globulin on Cells of the Plasma Series in Lymphatic Nodes

THE homoeostatic mechanisms acting in the lymphoid cell system are still obscure. The hypothesis of mutual feed-back humoral interrelationships between the various mesenchymal cell derivatives is being tested in this laboratory[1,2]. Thymus suppresses the proliferation of plasma cells and directly or indirectly stimulates the maturation of these cells. This is the cause of the delayed and prolonged antibody formation in animals thymectomized at birth[3]. This communication describes experiments which we undertook to discover whether excess globulin suppresses the multiplication of plasma cells.

Non-inbred albino rats were used. Total globulins, as well as albumin fraction, were sedimented from the same lot of pooled rat sera by 50 per cent saturation with ammonium sulphate, dissolved in distilled water, and dialysed against physiological saline at *p*H 7. The globulin and albumin were filtered through the Chamberland candle L_3. The protein nitrogen was estimated in the solutions of globulin and albumin. The protein content was equalized in the samples of both solutions before administration to the animals.

The general schedule of experiments was as follows. The rats were divided into three groups. The first received globulin, the second albumin, the third saline. In all groups, the rats were injected subcutaneously in the footpad of the right hind leg with 0·1 Freund adjuvant mixed with an equal quantity of saline. In experiments Nos. 3 and 5 the rats received 0·1–0·5 ml. globulin solution (or albumin solution or saline) in the right footpad, and 2·4–4·5 ml. intraperitoneally, before injection of the Freund adjuvant, and three times daily from 3 days thereafter. The amount of protein injected into each rat was 1,012 mg in experiment No. 3 and 884 mg in experiment No. 5. In experiment No. 4, globulin (and albumin and saline respectively) were injected into the hind right leg on the fifth day after the administration of Freund adjuvant. 1 ml. was administered six times over a period of 2 days (total, 277 mg). The rats were killed, and prints of the cut surfaces of various lymph nodes were made on coverslides, fixed with methanol and stained with azureosin. In each preparation, a differential cell count was made in thirty fields of vision (130–150 cells per field), as described by Pokrovskaya *et al.*[4]. In all experiments there was some suppression of plasma cells in popliteal and lumbar (retroperitoneal) lymph nodes in the groups of rats injected with globulin as compared with the rats injected with albumin.

A statistically significant reproducible effect was noted in the lymph nodes lying behind the thymus ('parathymic') (Table 1). There was a marked suppression of cells of the plasma series in these lymph nodes, even in experiment No. 4, in which proteins were injected in the hind leg only.

These experiments show that globulin and albumin act on cells of the plasma series in different ways. The suppressive action of excess globulin in regulating the multiplication of cells of the plasma series is, perhaps, a basic homeostatic mechanism. It is still debatable whether this action of blood proteins on plasma cells is direct, or whether it is mediated by various systems. There is, as yet, no satisfactory explanation as to why the parathymic lymph nodes are more susceptible to homologous blood proteins than other lymph nodes. It is interesting to note that, in the course of accidental involution of lymphatic organs after injection of cortisol, the parathymic lymph nodes behaved in a different manner from the other lymph nodes (unpublished data).

Experiments using paper electrophoresis showed that daily injection of 14 ml. blood globulin for 4 days did not lead to hyperglobulinaemia: 12 h after the last injection the level of plasma globulin was quite normal. This is an indication of the existence of a strong homoeostatic mechanism for the elimination or destruction of excess of globulin.

Table 1. EFFECT OF HOMOLOGOUS SERUM GLOBULIN AND ALBUMIN ON PLASMA CELLS IN THORACIC PARATHYMIC LYMPH NODES*

Cells	Experiment No. 3					Experiment No. 5					Experiment No. 4				
	Groups of rats injected with					Groups of rats injected with					Groups of rats injected with				
	Globulin 1	Albumin 2	P_{1-2}†	Saline 3	P_{1-3}‡	Globulin 1	Albumin 2	P_{1-2}	Saline 3	P_{1-3}	Globulin 1	Albumin 2	P_{1-2}	Saline 3	P_{1-3}
Reticular	243	275	—	266	—	410	289	999	431	—	225	247	—	246	—
Blasts	89	106	983	36	—	59	87	789	22	930	21	40	930	39	816
Plasma blasts	18·4	30·6	716	16·5	—	16	40	860	9	430	3·6	11	984	6·5	644
Plasma cells	216	458	962	264	730	165	420	995	278	920	117	407	999	247	878

* The table shows the absolute quantity of the respective kind of cells.
† P_{1-2}, Significance between quantity of cells of group 1 (globulin injected) and group 2 (albumin injected).
‡ P_{1-3}, Significance between quantity of cells of group 1 (globulin injected) and group 3 (saline injected).

No doubt, future experiments will elucidate the relationship of this phenomenon to the suppression of antibody formation in the primary response by means of passive administration of the specific homologous antibody[5,6].

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Sensitization of Mice with Human IgA

It is well known that an increased vascular permeability produced by various agents can be visualized by the leakage of intravenously administered dye through the affected vessels. The vascularity and high histamine and serotonin content of mouse ears[1] suggested that these organs might be useful in the visualization of *in vivo* antigen–antibody reactions. While investigating the ability of intravenously administered pollen extracts to induce blueing of the ears of mice[2], Kind *et al.* also found that mice sensitized with bovine serum albumin (BSA) developed blueing of the ears when later challenged intravenously with BSA and Evans blue dye. When blueing of the ears was produced in normal mice by the intravenous injection of BSA, rabbit anti-BSA and dye, no latent period was found to be necessary for the 'fixation' of antigen or antibody[3]. Indeed, in mice, maximal incidence of anaphylaxis with rabbit antibody can be obtained by injecting antibody and antigen intravenously in rapid succession[4]. The apparent absence of a requirement for tissue fixation in mice suggested that blueing of the ears in anaphylactic mice might be useful for detecting such proteins as normal human IgA or myeloma IgA, which will not sensitize guinea-pig skin for reverse passive cutaneous anaphylaxis probably because of the lack of fixation sites on such molecules[5].

Swiss–Webster female mice weighing 17–20 g were inoculated intravenously with 0·05 ml. of various dilutions of normal human serum (NHS). One hour later the animals were again injected intravenously with 0·1 ml. of goat anti-human IgA (Hyland Laboratories, Los Angeles, California) and 0·2 ml. of 0·5 per cent Evans blue dye. The anti-IgA serum was found by immunoelectrophoresis to develop a single, strong precipitin line with whole human serum. The data in Table 1 indicate that as little as 0·05 ml. of a 1 : 20 dilution of NHS was able to sensitize mice. The concentration of IgA in normal human serum is 150 to 400 μg/ml. (ref. 6). Since no pure IgA was available and since an anti-serum to IgA, although absorbed to make it specific, might contain small amounts of antibodies to other serum components, it was necessary to demonstrate by additional experiments that the observed blueing of the ears was actually due to IgA–anti-IgA interaction. The data in Table 1 indicate that an IgA myeloma serum which contained about ten times more IgA than did NHS could sensitize mice for blueing of the ears at a dilution of 1 : 200 in contrast to NHS, which could not be diluted more than 1 : 20 and still retain its ability to sensitize. In gel-diffusion investigations this IgA myeloma serum produced a single precipitin band with anti-IgA and this band fused (reaction of identity) with the band formed by NHS and anti-IgA. The data in Table 2 indicate that cord

Table 1. Sensitization of Mice with a Normal Human Serum and with an IgA Myeloma Serum

Mice sensitized intravenously with: 0·05 ml. normal human serum (NHS)	Mice challenged intravenously 60 min later with: 0·1 ml. goat anti-IgA plus 0·2 ml. of 0·5 per cent Evans blue dye	No. of mice reacting (blue ears) Total No. challenged 10/16
NHS 1 : 10	,,	7/11
NHS 1 : 20	,,	2/6
NHS 1 : 50	,,	0/5
Saline	,,	1/20
0·05 ml. IgA myeloma serum (diluted 1 : 200)	,,	8/14
0·05 ml. IgA myeloma serum (diluted 1 : 500)	,,	0/5
0·5 ml. IgA myeloma serum (diluted 1 : 200)	Dye plus saline	0/5
0·05 ml. NHS	,,	0/5

Table 2. Sensitization of Mice with Cord and Maternal Sera

Mice sensitized with:	No. of mice reacting (BE)* / No. of mice tested	IgA present in serum (by immunoelectrophoresis)
maternal serum †	4/8	+
cord serum	0/6	−
maternal serum	2/3	+
cord serum	0/5	−
maternal serum	4/5	+
cord serum	1/5	−
maternal serum	3/4	+
cord serum	1/5	−

* Mice injected with 0·05 ml. of cord or maternal serum were challenged 60 min later with 0·1 ml. of goat anti-IgA and 0·2 ml. of 0·5 per cent Evans blue dye. Control mice injected with antiserum plus dye or antigen plus dye produced no blueing of the ears (BE).
† Four different pairs of cord and maternal sera were tested.

sera which contain no IgA detectable by immunoelectrophoresis (as previously demonstrated by Allansmith *et al.*[7]) rarely sensitized mice for blueing of the ears with goat anti-IgA, whereas maternal sera shown to contain IgA by immunoelectrophoresis were effective sensitizers. Mice failing to give blueing of the ears after injection of cord serum, goat anti-IgA, and dye were later challenged intraperitoneally with goat anti-IgG. Blueing of the ears occurred in all animals tested[8]. Although no pure IgA was available for these investigations, the results obtained with an IgA myeloma serum (greatly increased sensitization) and with cord sera (greatly reduced sensitization) strongly suggest that human IgA can sensitize mice. Since it has been shown that human IgM, like human IgA, will not sensitize guinea-pig tissues[8], the method described here may also be useful for quick detection of human IgM proteins or their fragments.

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Electrophoretic Behaviour of *H* and *L* Chains of Human Serum and Colostrum Gamma-globulin

Dissociation of gamma-globulin molecules by reduction in urea with such thiol reagents as mercapto-ethanol and thioglycolate[1,2], or in neutral aqueous solution[3], or by *S*-sulphonation[4,5], allows the identification of two types of polypeptide chain, called *H* (*A*) and *L* (*B*), which may be separated by gel filtration.

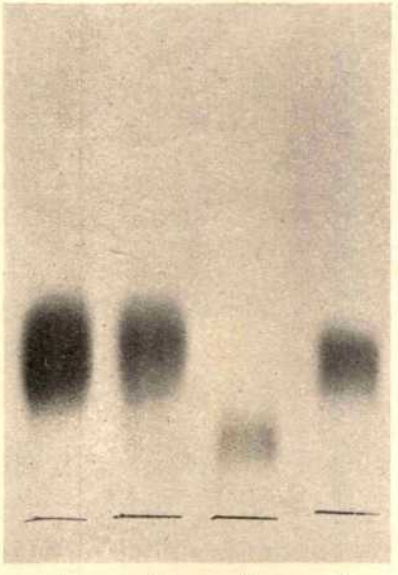
The *L* chain is the carrier of antigenic and allotypic determinants common to all types of immunoglobulins; its high degree of heterogeneity is apparent on starch-gel electrophoresis in formic acid and urea[6] and especially in glycine and urea. This mode of electrophoretic separation allows separation of *L* chains of all investigated animal species into approximately ten components, which, according to Cohen and Porter[7], probably differ in amino-acid composition.

In contrast to this, *H* chains are the carriers of the properties characteristic for each class of immunoglobulins. The heterogeneity of this chain is apparent from these characteristic properties and from the fact that it contains two N-terminal amino-acids[8]. However, electrophoretic separation of this chain into more components has not yet been achieved, although its extensive diffusion zone, which has the same mobility as the initial gamma-globulin, confirms its heterogeneity.

The experiments reported here were designed to investigate the differences in electrophoretic behaviour of polypeptide chains of gamma-*G* globulins in human blood serum and of the immunoglobulin of human colostrum, which was found to correspond in electrophoretic properties to serum gamma-*A* globulin, but which carries antigenic determinants specific both for gamma-*A* and gamma-*G* serum globulins[9,10].

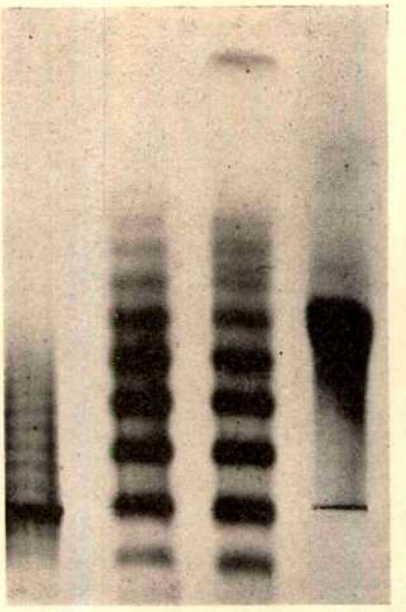
H- and *L*-type chains were prepared from immuno-electrophoretically homogeneous preparations of gamma-globulin[11] by *S*-sulphonation for 20 h (ref. 5) in 0·5 M ammonium buffer, *p*H 8·6, containing cupric ions. Isolation of chains was performed on 'Sephadex *G*-100' columns in 6 M urea and 0·05 M formic acid. Solutions of the chains were neutralized, transferred to 0·05 M ammonium carbonate on 'Sephadex *G*-25' and lyophilized. Units prepared in this way were found to be quite soluble in the buffers normally used.

Fig. 1 shows the analysis of *H* and *L* chains of human serum and colostrum gamma-globulin by electrophoresis in starch gel containing formic acid and urea. It is clear that the *L* chain from serum and colostrum forms zones quite similar in shape and mobility, while the migration of colostrum *H* chain is apparently slower than the migration of *H* chain from serum gamma-globulin. These findings indicate that the *H* chains of human colostrum immunoglobulins behave very similarly to the *H* chains of serum gamma-*A* globulin. However, striking differences



A *B* *C* *D*

Fig. 1. Starch-gel electrophoresis in 0·05 M formic acid and 6 M urea. *A*, *L* chain of serum γ-globulin; *B*, *L* chain of colostrum γ-globulin; *C*, *H* chain of colostrum γ-globulin; *D*, *H* chain of serum γ-globulin



A *B* *C* *D*

Fig. 2. Starch-gel electrophoresis in 0·035 M glycine and 8 M urea. *A*, *H* chain of serum γ-globulin; *B*, *L* chain of serum γ-globulin; *C*, *L* chain of colostrum γ-globulin; *D*, *H* chain of colostrum γ-globulin

were found in the analysis of the same material by starch-gel electrophoresis, using 0·035 M glycine and 8 M urea. Fig. 2 shows that this procedure separated *L* chains into the usual number of zones, two of which migrate cathodically, while one remains at the start and seven migrate to the anode. Comparing the spectra of both chains tested, they were found to be very similar, with the exception of the fifth and sixth zone, which doubled in the direction of the anode in the case of colostrum chain, and moreover another intensive, very fast-moving zone was found in this colostrum chain, which is absent in serum material.

As with the *L* chain, we succeeded in separating the *H* chains of serum gamma-globulin into a number of zones, the character and mobility of which are quite different. These zones are narrow, with relatively small differences in electrophoretic mobility, showing that these components are closely related. In contrast to this, the same chain, isolated from colostrum immunoglobulin, formed one wide zone with greater mobility than the serum *H* chain, and moreover showed only a trace of one faster and one slower zone.

Separation of the *H* chain of human serum gamma-globulin into individual fractions by glycinated starch-gel electrophoresis has, until now, been unsuccessful[7]. The successful separation of the *H* chain into ten fractions in our experiments may be imputed to the aforementioned relatively gentle method of preparing the chains. The possibility of separating the *H* chain into fractions is not limited to the human material; similar results were obtained with pig material, and this is considered to be further evidence for the high heterogeneity of this chain. The experiments of Poulik and Shuster[12], who succeeded in fractionating papain *F* fragment (part of the *H* chain) into ten to thirteen components, support the supposition that this heterogeneity is not an artefact caused by the method of preparation of the chains. In contrast to this, *H* chains of colostrum immunoglobulin seem to be less heterogeneic, as they only form one wide zone. This is in agreement with the assumption that the mammary gland selects only a part of heterogeneic mixture of blood serum immunoglobulins[13].

Differences between *L* chains of serum and colostrum gamma-globulin, expressed by the presence of a fast-

moving zone only in the colostrum material, confirm that even the *L* chains of the preparations compared are not quite the same.

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PATHOLOGY

Phosphorylase Activity in Megakaryocytes

In 1956, Scavo and Andres[1], and later others[2–4], reported that glycogen in megakaryocytes was decreased in chronic idiopathic thrombocytopenic purpura (ITP) with megakaryocytic hyperplasia before splenectomy. Also, some of these investigators[1,2] showed that megakaryocytic glycogen increased after splenectomy and that the increase coincided with the appearance of budding megakaryocytes and numerous platelet masses in bone marrow. Jamra and Lorenzi[5], however, following Daniell's[6] system for semi-quantitating megakaryocytic glycogen, noted that in presplenectomy patients with ITP there was an increase in large coarse PAS positive glycogen granules and concluded that this histochemical finding might prove to be pathognomonic of ITP with megakaryocytic hyperplasia. Because of the importance of phosphorylase, required for the initiation of glycogenolysis, it was decided to determine if the large masses of glycogen in megakaryocytes in this condition might be cumulative secondary to a relative phosphorylase deficiency, that is, an acquired megakaryocytic phosphorylase deficiency glycogenosis.

Quaglino and Hayhoe[7] described a method for the histochemical detection of haemic phosphorylase based on a modification of the technique originally developed by Takeuchi and Kinoshita[8]. As a degradative enzyme, phosphorylase is specific for the phosphorylytic cleavage of the -1-4 linkages of glycogen to yield glucose-1-PO_4. The reaction is apparently irreversible *in vivo*. However, *in vitro*, in the presence of large amounts of glucose-1-PO_4 and a glycogen primer, the reaction can be reversed, and the newly synthesized polysaccharide can be demonstrated by iodine staining; the density of the staining reaction is thought possibly to be related to the chain length[9].

Fresh coverslip smears of bone marrow were air-dried at 4° C for 2–3 h. After the smears had been fixed in 70 per cent acetone for 30 sec, they were incubated in the substrate solution prepared according to Quaglino and Hayhoe[7]. Positive controls were obtained by using previously tested specimens which had been stored at −10° C for not longer than 36 h; negative controls included unincubated smears. Occasionally a few megakaryocytes in unincubated smears showed a light-brown cytoplasmic staining reaction consistent with areas of pre-formed glycogen, but no dark blue or blue-black staining reactions were noted in any negative controls.

Staining reaction and distribution in megakaryocytes (Figs. 1–3). In each case a minimum of 50 consecutive magakaryocytes were evaluated for intensity of staining reaction. Enzymatic activity was semi-quantitated according to the following scheme:

Value	Staining activity
0	Absent or faint.
1	Distinct diffuse or distinct fine granularity.
2	Distinct fine granularity plus a few coarse granules.
3	Many coarse granules.

Bone marrow from five patients who were evaluated for non-neoplastic and non-thrombocytopenic haematologic dyscrasias had an average megakaryocytic phosphorylase score (MPS) of 85 with a range of 74–99. Three patients with chronic ITP before splenectomy had an average score of 141 with a range of 104–173. One patient with a reactive thrombocytosis following traumatic fracture and haemorrhage had an MPS of 204.

The intracellular site of phosphorylase activity, as determined by periodic acid–Schiff reaction after diastase digestion, closely parallels that of pre-formed glycogen. Quaglino and Hayhoe[7] suggest the similar intracellular distribution of pre-formed glycogen and phosphorylase activity may be related to the presence of pre-formed glycogen acting as a built-in primer. The higher average phosphorylase scores for patients with ITP, therefore, correspond to the increased number of coarse PAS-

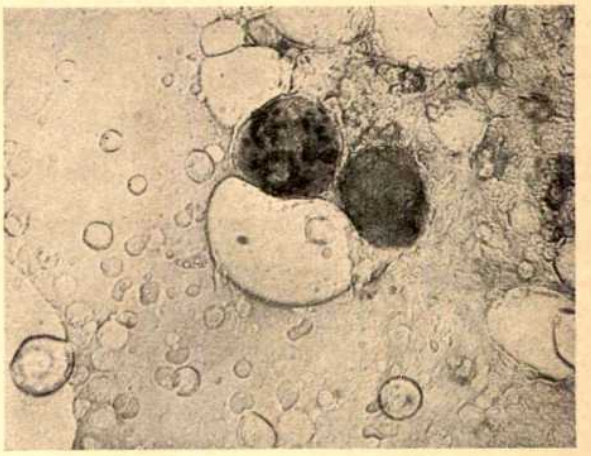


Fig. 1. Phosphorylase activity in megakaryocytes. Two megakaryocytes from a patient with chronic ITP before splenectomy. The more intensely staining cell is scored as 3; the less intensely staining cell is scored as 1

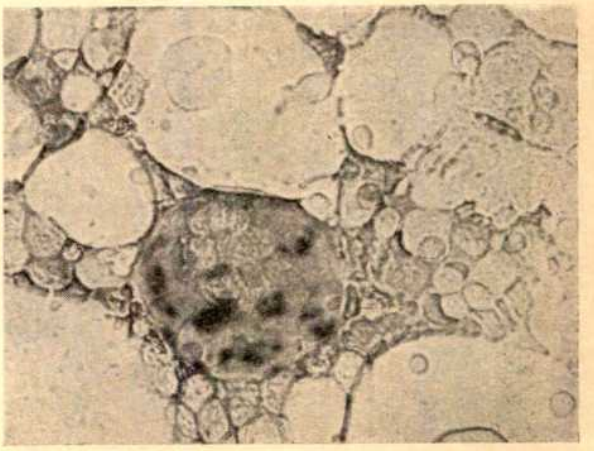


Fig. 2. Pre-splenectomy. Phosphorylase activity in a megakaryocyte. A few coarse granules give this cell an MPS of 2

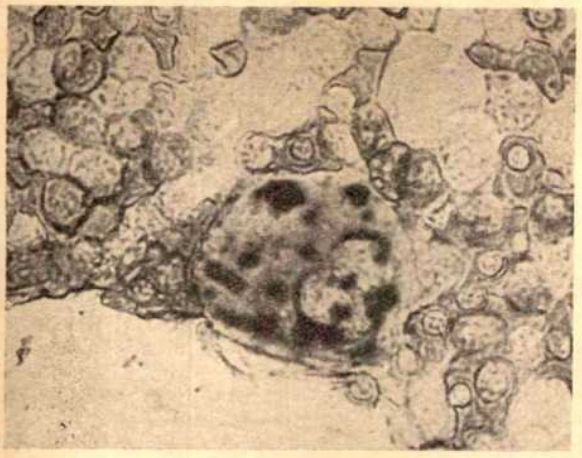


Fig. 3. Pre-splenectomy. Phosphorylase activity in a megakaryocyte. Many coarse granules rate an MPS of 3

positive granules. The high MPS in the patient with reactive thrombocytosis after traumatic haemorrhage is consistent with increased megakaryocytic glycogen as noted by Daniell[6] in humans after acute blood loss and as found by Etcheverry and Grignaschi[3] in experimental animals.

These preliminary investigations suggest there may be considerable variation in megakaryocytic phosphorylase activity and also that the enzymatic activity roughly parallels the amount of intracellular glycogen. There is, however, no indication by this *in vitro* technique that there is a phosphorylase deficiency in chronic ITP before splenectomy. The possibility of the absence of or diminished concentration of a primer or initiating factor necessary for phosphorylytic cleavage *in vivo* remains to be examined.

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Action of Riley's Enzyme-elevating Virus on Tumour-bearing Mice

RILEY virus (RV) is a passenger agent occurring in most mouse tumours which are subjected to repeated transplanting passages[1–6]. The influence of this virus on tumour-bearing hosts has not yet been clarified, though its stimulating and accelerating effects on tumour-growth have been discussed[3,5]. There is a significant elevation of LDH-values after virus-infection of tumour-bearing mice as compared with tumour-free hosts[1,3,5] (termed 'tumour-virus synergism' by Riley[5]). This phenomenon is of special interest, because the natural occurrence of RV is almost restricted to tumour-bearing mice. Enhanced values of plasma-LDH in tumour hosts[7] might be induced mainly by RV. We have investigated the effects of RV on three different tumours of mice previously free of the agent.

First, mice with spontaneous mammary tumours (Swiss- and *C3H/He J*/Han-lines), collected from older breeding females, were inoculated with about 3,000–4,000 *I.D.* of RV by intraperitoneal injections. Values of plasma-LDH were followed by enzyme determination at 2- or 3-day intervals up to more than 4 weeks. Levels of plasma-LDH increased almost two-fold as compared with tumour-free Swiss mice infected with the same amount of virus. Table 1 shows the development of enzyme elevation, giving the values at six selected days only. Values are representative means for many animals from several repeated experiments.

The second species of tumour-host developed polyoma-induced carcinomata following infection of new-born Swiss-*NMRI*/Han mice with *SE*-polyoma virus. These tumour-animals showed a similar reaction of plasma LDH after primary infection with RV to that of mice with spontaneous mammary tumours. We have an impression that tumour weight might be a substantial factor influencing LDH-increase in some animals; but it was not possible to determine a relation between enzyme elevation and tumour mass.

The average values of plasma LDH in both kinds of tumour cover both very low and extremely high responses of plasma enzyme to virus inoculation. The latter enhancement was comparable with the previously reported synergistic action[3,5], but in our experiments the single animals with high, short peaks of LDH died rapidly.

Table 1. PLASMA LDH IN THREE TUMOUR SPECIES AFTER INFECTION WITH RILEY'S LDH-ELEVATING VIRUS

Days after RV infection	0	4	7	10	14	21	28
Spontaneous mammary tumours	58	155	263	233	185	202	145
Polyoma virus-induced tumours	89	217	239	233	222	233	171
Transplanted tumours (carcinoma 284)	39	224	208	261	325	364	396
Tumour-free controls	31	148	150	149	154	148	150

Numbers give enzyme activities in Buecher units per ml. plasma. Values are means from forty animals of at least six repeated experiments in each of the three species of tumours and controls.

After these findings of a moderate virus-tumour synergism in hosts with primary tumours, a transplanted tumour was used to investigate the action of RV. The tumour was developed in this laboratory by transplantation of a spontaneous mammary carcinoma of an inbred *C3H/HeJ* Han mouse to inbred *C3H*-hosts. The tumour is now in its fourteenth passage of transplantation and is referred to as carcinoma 284; animals of the sixth to eighth passages were used in these experiments.

About 14 days after RV-infection of the transplanted carcinoma 284 the results differed as follows: an almost tenfold increase of plasma-LDH resulted 14–28 days after virus inoculation, whereas mice with primary tumours showed an approximately six- to seven-fold increase, compared with a five-fold increase in the controls, during this period of observation. Therefore, the synergistic action of RV with tumour-bearing hosts was demonstrated most clearly in animals bearing the transplanted carcinoma 284. This result seems to be of interest, because host mice with the various transplantation tumours, usually infected with RV, show very enhanced plasma-LDH as compared to virus-infected tumour-free controls.

Titres of RV in transplanted carcinoma 284 were not elevated as compared to spontaneous or polyoma-induced tumours, though we found that the titre was 100–1,000 times higher in the tumours than in the organs of the same host. The synergistic effect in tumour animals cannot, therefore, be explained by an enhanced virus propagation. In accordance with the impaired clearance of LDH after infections with RV (refs. 4, 8), it might be considered that enzyme-discharging cells of the reticulo-endothelial system are blocked by influences of the tumour tissue before virus infection.

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Amyloidosis in Mice with a Transplantable Plasma Cell Tumour

Transplantable plasma cell tumours of mice resemble human myelomatosis in the following features: morphology of the tumour cells[1], production of globulins[2], Bence Jones type of proteinuria[3], osteolytic bone lesions[4,5], and myeloma kidney[3,6]. All these features, however, are not present simultaneously in any one strain of neoplasm. Since systemic amyloidosis has been reported in 14 per cent of cases of human myelomatosis[7], the presence of amyloid in mouse myelomatosis was investigated.

The tumour chosen for study was the *ADJ/PC5* which arose in a *BALB/c* mouse following an intraperitoneal injection of incomplete Freund's adjuvant and heat-killed staphylococci[8]. The 61st generation of this tumour was transplanted subcutaneously by the trochar technique into the right flank of ten 8-week-old female *BALB/c* mice using 2-mm fragments of a 14-day donor tumour. A further series of ten mice served as controls. The animals were maintained on a diet of rat cake, supplemented with biscuits, wheat and maize. They were killed by cervical fracture on the 24th experimental day.

The serum of the tumour-bearing animals was characterized on paper electrophoresis by a slow-moving narrow band of γ-globulin, which on immunoelectrophoresis was a γ G globulin, and on ultracentrifugal analysis had a sedimentation coefficient of 6·4*S* ($S_{20}W$). Animals with large tumours showed proteinuria, but Bence Jones protein was not detected.

Representative pieces of tissue were taken from the tumour, liver, spleen, kidney, heart, tongue and femur of each mouse. They were fixed in 4 per cent formaldehyde saline, and the bones were decalcified in buffered formic acid. The tissues were processed routinely and stained with haematoxylin and eosin, methyl violet[9], congo red[10], thioflavine-*T* (ref. 11), periodic acid–Schiff (PAS)[12], van Gieson, silver[13] and methyl green-pyronin[14].

The tumour was a well-defined lobulated fleshy mass confined to the subcutaneous tissue. Macroscopically no metastases were found at post-mortem. Of the visceral organs, only the spleen showed definite enlargement. Microscopical examination of the tumour showed a variable appearance (Fig. 1). There were sheets of well-differentiated plasma cells at some sites, with frankly necrotic areas at others. A large part of the tumour, however, consisted of lobulated masses of pleomorphic cells. These cells were large, round or oval in shape with basophilic cytoplasm, and showed intense pyroninophilia. The nuclei were large and irregular, and there were numerous bizarre mitotic figures. Collagen fibres were sparse, but a well-defined reticulin network outlined the lobulated structure of the neoplasm. There was no histological evidence of tumour metastases, and the bone marrow was free of tumour.

Table 1. Distribution of Amyloid in *BALB/c* Mice carrying the *ADJ/PC5* Tumour

Tumour mouse No.	Amyloid present in the following organs								
	Tumour	Liver	Glomerulus	Tubules	Myeloma casts	Spleen	Heart	Tongue	Bone
(1)	+	+	+	+	+	+	–	–	–
(2)	+	+	–	+	–	–	–	–	–
(3)	+	+	+	+	–	–	–	–	–
(4)	+	+	+	–	+	–	+	–	–
(5)	–	–	–	–	–	–	–	–	–
(6)	–	–	–	–	–	–	–	–	–
(7)	+	+	+	–	+	–	–	–	–
(8)	+	+	+	–	–	+	–	–	–
(9)	+	+	+	–	+	–	–	–	–
(10)	+	+	+	+	+	–			–

Microscopic foci of amyloid were found in eight of the ten tumour-bearing animals, but in none of the control mice. Amyloid was identified by the intense pale green fluorescence with thioflavine-*T*, a strong PAS-positive reaction, and a khaki colour imparted by the van Gieson stain. Congo red binding was weak; birefringence, dichroism and methyl violet metachromasia were absent. Amyloid fibrils were demonstrated with the electron microscope[15]. The thioflavine-*T* reaction was used to find the distribution of amyloid (Table 1); false positive reactions of certain structures were strictly excluded[16], and the lack of autofluorescence was a further requirement[17].

The tumour and liver were involved in eight mice. In the tumour, amyloid was usually found in the vascular sinusoids (Fig. 2). In the liver amyloid was present in the sinusoids and the portal and centrilobular veins (Fig. 3). Renal involvement was noted in the glomeruli of seven mice, and in the tubules of four (Fig. 4), while myeloma casts were found in six mice. Myeloma casts showed identical reactions to those of amyloid (Fig. 5)

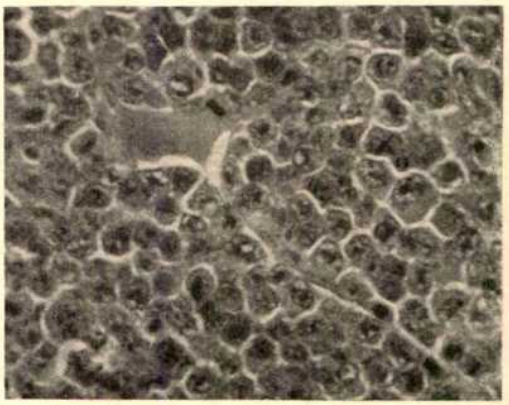


Fig. 1. Plasma cell neoplasm *ADJ/PC5*, showing cellular pleomorphism, and a small focus of amyloid (stained with haematoxylin eosin, × *c.* 365)

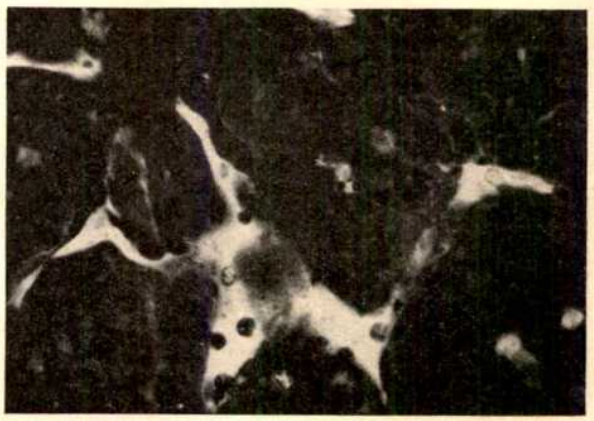


Fig. 2. Fluorescent microphotograph showing amyloid among cells of the plasma cell neoplasm (stained with thioflavine-*T*, × *c.* 365)

and they are therefore comparable with myeloma casts in man[18,19]. Small perifollicular foci of amyloid were observed in the spleens of two mice and the heart was involved in one animal.

Atypically staining amyloid occurs in a proportion of cases of human myelomatosis and was also found in mice bearing the *ADJ/PC5* tumour. The distribution was not that of the classical 'primary' amyloidosis usually found in human myeloma. However, as Symmers[20] has shown, there is considerable overlap in the distribution of amyloid between the primary and secondary varieties.

It appears that the plasma cell tumour was responsible for amyloid formation and the observations made are consistent with the cellular theory of Teilum[21]. Osserman *et al.*[22] have recently presented evidence to show that plasma cell neoplasia or dyscrasia may be found in all cases of human amyloidosis. The presence of amyloid in mice bearing the plasma cell tumour lends support to these views.



Fig. 3. Amyloid in the sinusoids and centrilobular vein of the liver (stained with thioflavine-*T*, × *c.* 365)

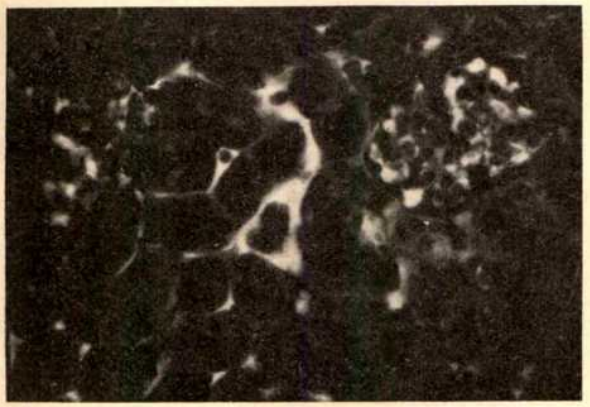


Fig. 4. Amyloid in the glomerular capillaries and renal tubules (stained with thioflavine-*T*, × *c.* 365)

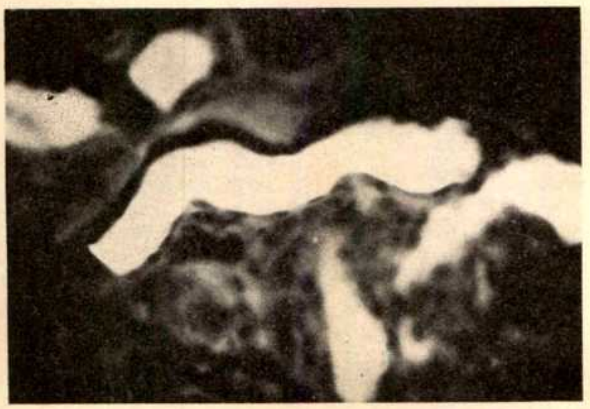


Fig. 5. Myeloma casts in the renal tubules (stained with thioflavine-*T*, × *c.* 365)

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Micro-architecture of Skin and its Behaviour under Stress

IT has recently been shown that the mechanical characteristics of human skin are peculiar both *in vivo*[1] and *in vitro*[2] and do not correspond exactly with those of any known isotropic material. It was found that the increase in the length of portions of skin under increasing load was relatively large at first, but a point was reached when further loading produced little increase in length up to the point at which the specimen failed completely. Moreover, it was suggested that the behaviour of skin under mechanical stress is related to the anatomical arrangement of the collagen and elastic fibres in the dermis which our preliminary histological investigations tend to confirm. We have found, in addition, certain unexpected changes particularly in the dermal collagen after stress[3].

The collagen within the dermis can be divided into three layers according to its histological architecture; 1, a narrow sub-epidermal zone of loosely arranged fine fibres; 2, a wider mid zone of more compact coarse fibres; 3, a deep zone of variable thickness which consists of coarse collagen fibres more loosely arranged than in the mid zone. In all three layers the collagen fibres are normally laid down in a three-dimensional interlacing network.

For our histological investigations we used mainly human skin from the abdominal wall obtained at necropsy, because of its greater availability, and a limited amount of work has been done on skin taken from various sites in live patients during plastic operations. Normally two portions of skin were obtained, one taken horizontally across the abdomen and the other vertically. Specimens were investigated following varying degrees of stress up to and including failure, and after having been stretched over varying periods of time. Some specimens were fixed while still in the stretched condition and others were allowed to relax before fixation.

When skin is stretched the collagen fibres of the dermis lose their random three-dimensional arrangement and become orientated along the line of stress, that is, parallel with the surface. At low loads only a few fibres are re-orientated, but with increasing strain progressively more fibres become altered until all the collagen fibres in the mid zone lie parallel to the surface. Eventually transverse fracture lines appear in the fibres and these increase until complete disruption of the skin occurs. This orientation of the collagen fibres in the line of stress is obvious no matter in which direction the force is applied. The sub-epidermal and deep zones of the dermis follow the same pattern as the mid zone but lag somewhat behind. It is surprising to find that elastic and reticulin fibres seem to be largely unaffected by stretching; they are merely displaced by and compressed between the orientated bundles of collagen.

Certain unexpected staining differences were observed after stretching. With Mallory's trichrome stain[4] the normal collagen stains blue. In contrast, stressed collagen fibres stain red (fuchsinophilia) and thus resemble smooth muscle. This alteration is completely reproducible when stressed and control specimens are compared in successive sections from a single block of stressed skin, and whether the staining technique is manual or entirely mechanical in an automatic staining machine. We have obtained the same results with modifications of Mallory's method, for example Masson[5], picromallory[6], Foot[7] and the Falg method[8]. Lendrum's Martius-Scarlet-Blue[6] method demonstrates the change particularly well.

The alterations in orientation and staining appear to be closely linked, but usually the fibres become orientated before the change in staining properties occurs. The greater the stress the greater the number of collagen fibres which become fuchsinophil.

If the skin is stretched and allowed to relax before fixation it is noticeable that after small degrees of stretching the collagen fibres return to their three-dimensional interlacing pattern but retain their affinity for acid fuchsin. At greater stress levels the fibres remain orientated in the line of stress, but show a wavy, concertina pattern after relaxation in addition to retaining fuchsinophilia. Fracture lines only occur when stretching is great enough to produce changes which are irreversible on relaxation.

It is not yet clear what alteration is responsible for the change in staining reaction, but it is probably connected with the unique micro-structure of collagen. The collagen fibril consists of long-chain protein molecules arranged in regular parallel straight rows within a matrix of acid mucopolysaccharides (Curran and Clark[9]). It may be that in the normal collagen fibril only the mucopolysaccharide is stained and that this produces the blue reaction by any of the trichrome methods. On the other hand, in the stressed fibril the mucopolysaccharide is disrupted allowing access of the dye to the protocollagen which is fuchsinophil. In support of this possibility some of our material shows that these fuchsinophil fibres are surrounded by a faintly discernible blue envelope. This, however, fails to explain why the blue reaction of the mucopolysaccharide should largely disappear. Further, special stains for acid mucopolysaccharides, for example, thionin, toluidine blue and methylene blue extinction, do not disclose any difference between normal and stressed collagen.

Whatever the cause, we find that the tinctorial changes in stressed collagen closely resemble those described as 'fibrinoid degeneration of collagen' in some of the collagen diseases, for instance, scleroderma and the rheumatoid nodule. It is entirely different from the fibrinoid exudate seen in the walls of blood vessels or the renal glomeruli in cases of malignant hypertension, in polyarteritis nodosa and diabetic vasculosis. We believe that the so-called fibrinoid necrosis of collagen, whatever the cause, has no relation to fibrin, and we suggest that it would be more rationally termed 'stressed collagen' using the term stress in the wide sense of damage caused by any agency whether physical, chemical or biological.

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Is Hodgkin's Disease a Syndrome of Hypothymism?

There has been much discussion recently, based on both X-ray and autopsy evidence, about the role of the thymus in the development of Hodgkin's disease[1,2]. Quite recently, Hoffbrand[3] failed to show auto-immune phenomena or thymus involvement in Hodgkin's disease.

On the other hand, the immunology of Hodgkin's disease strongly indicates a profound impairment of the lymphoid system. In recent years, the presence of dissociation of antibody formation and of immunological reactions of the delayed type has been clearly established in Hodgkin's disease.

Even in the early stages of Hodgkin's disease there is a marked suppression of immunological responses of the delayed type to different antigens, tuberculin, mumps virus, *Candida albicans*, *Trichophyton gypseum*, diphtheria toxoid, streptokinase-streptodornase and dinitrochlorobenzene. The response to homografts of skin and bone marrow is similarly affected. At the same time, antibody formation to a large number of antigens remains unimpaired (for a review of this topic see refs. 4–6). Similarities between malignant lymphomas and graft-versus-host reactions were noted by Kaplan and Smithers[7], while Green *et al.*[8] questioned whether patients with Hodgkin's disease were lymphoid chimeras. Aisenberg[5] claims that, in contrast to the immunological status in Hodgkin's disease, both immunological responses of the delayed type and antibody formation are absent in graft-versus-host reactions. Aisenberg made the same observation when comparing the status of neonatally thymectomized animals and the status in Hodgkin's disease: "Thus it seems that a number of clinical features of Hodgkin's disease including the immunological defect cannot be adequately explained by incriminating a loss of thymic function. However, in the terminal stage of disease many features are similar to the post-thymectomy state, and it appears possible that the two could represent the same end stage of different etiologic processes".

New data on the histopathology and immunology of the post-neonatal thymectomy state raise the question whether Hodgkin's disease can be considered as a syndrome of hypothymism. Earlier, it was established that, in neonatally thymectomized animals, both antibody production and immunological responses of the delayed type were suppressed[9,10]. This was in accord with the well-known depletion of small lymphocytes in lymphoid organs[9–11] combined with the proliferation of reticular and plasma cells[11,12]. In contrast to these initial data, Svet-Moldavsky *et al.*[11] showed that neonatally thymectomized mice retain the capacity to produce antibody. For some antigens (*Vi*-typhoid antigen, live influenza virus) antibodies are produced immediately after the primary immunization, for others (sheep erythrocytes, *O*-typhoid

antigen) only after re-immunization[11,12]. This is very similar to the production of antibodies to tetanus toxoid in patients with Hodgkin's disease only after re-immunization which was noted by Barr and Fairley[13].

In neonatally thymectomized mice in which immunological reactivity had been recovered by re-immunization, some lymph nodes and the spleen recover the normal histological pattern, while other lymph nodes retain scantiness of lymphocytes, absence of germinal centres, and hyperplasia of reticular and plasma cells[11,12]. Quite recently, it was demonstrated in this laboratory[12] that neonatally thymectomized mice can also produce antibody to sheep erythrocytes after the primary immunization. In this case, antibody production begins later but persists longer than in normal animals. Antibody can reach even higher titres than normally. Such antibody production is explained by an enhanced proliferation of immature cells of the plasma series and a slowing down of their maturation.

All the foregoing evidence indicates that, in neonatally thymectomized animals, there occurs not a general loss of the immune response, but its clear dissociation, which is identical in detail with the immunological defect in Hodgkin's disease. The histopathological pattern in neonatally thymectomized mice subjected to the effects of antigens, with sharp variations in the structure of different lymph nodes in one and the same animal, is strikingly similar to that seen in Hodgkin's disease.

We suggest that the pathogenesis of Hodgkin's disease is based on a definite hypothymic-hypolymphocytic syndrome characterized by depletion (and possibly a quantitative insufficiency) of the lymphocytes of lymphoid organs, lymphopenia in the peripheral blood, enhanced proliferation of reticular and immature plasma cells and loss of immunological responses of the delayed type with retained capacity for antibody production. Proliferation of cells of the plasma series may result in hypergammaglobulinaemia which cannot, so far, be explained.

Of course, individual features of the syndrome of hypothymism may be manifested to a greater or lesser extent. The pattern of the syndrome may change considerably, due, for example, to secondary chronic infections or to the involvement of other systems. Prolonged antigenic stimulation in the syndrome of hypothymism may result in the formation of cells of the Sternberg–Berezovsky type.

This concept of the syndrome of hypothymism is not equivalent to hypofunction or lack of function of the thymus. This syndrome may depend on hypofunction of the thymus while at the same time developing from a blocking of the transmission of hormonal or other thymus effects to the effector systems of different levels. Indeed, the syndrome does not necessarily involve a morphological involvement of the thymus in the process.

Of special importance in this syndrome is the impairment of the reciprocal equilibrium of the system of lymphocytes and cells of the plasma series. I believe that antagonistic interaction of these two cellular systems constitutes one of the fundamental homoeostatic mechanisms of the lymphoid system. Selective impairment of lymphocytes or depletion of lymphocytes for any reason (as a result of X-irradiation, neonatal thymectomy, etc.) results in an enhanced proliferation of reticular cells and cells of the plasma series. This proliferation is a very important element of the hypothymic-hypolymphocytic syndrome and underlies the transformation of Hodgkin's disease into reticulosarcomatosis and the existence of many intermediate forms between these diseases.

All the foregoing strongly suggests that transplantation of the thymus tissue should be undertaken for therapeutic purposes in patients with Hodgkin's disease. Even if such grafting will not completely cure the disease, it should result in a marked improvement of all phenomena associated with the state of hypothymism. The absence of immunological responses of the delayed type in patients with Hodgkin's disease would ensure the durability of the thymus homograft.

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BIOLOGY

Photographic Method of Recording the Activity of Small Animals using Repetitive Electronic Flash

REPETITIVE or strobed electronic flash has been commonly used in techniques for the analysis of animal locomotion. Some of the methods involved several flashes on to a single photograph, notably in connexion with work on the flight of birds and bats. Dryl[1,2] has described a 'photomacrographic' method for recording the activity of mobile protozoans. This utilized a time exposure to a continuous source of light illuminating the animals against a dark background. His method was developed from similar 'photomicrographic' techniques for activity recording used by other workers[3–6].

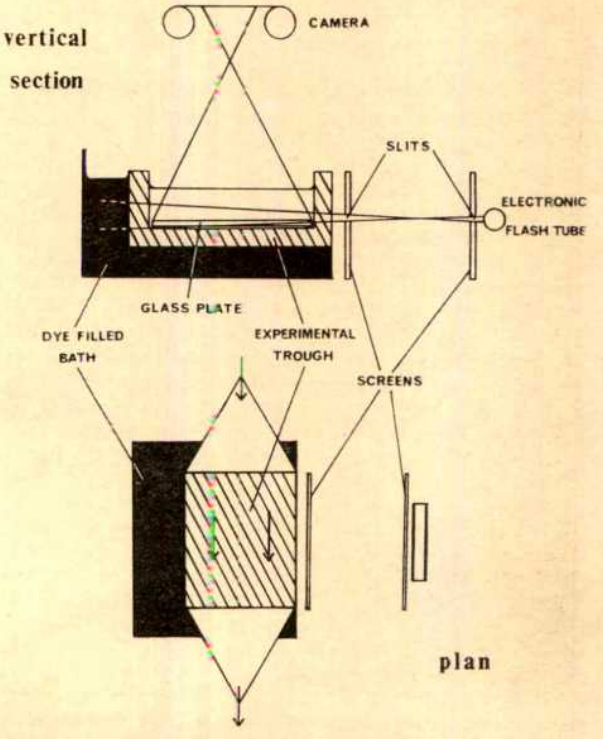


Fig. 1. Diagram of the activity recorder. The arrows in the plan of the apparatus denote the direction of water flow through the trough, and the hatched area the recording field

The apparatus described here (Fig. 1) was designed for recording the activity of small aquatic animals in a shallow trough of water. This measured 8·1 × 10·7 cm and was 2 cm deep. Trail records of the animals appeared as series of discrete images or as a continuous track. This depended on the flashing rate of an electronic flash system, and on the speed of movement of the animals. They were allowed to move freely on glass plates lying on the bottom of the trough in a horizontally uniform flow of water. The brief flashes (about 1 msec) illuminated the animal so that it appeared as a linear series of images of reflected light against a dark background. This was achieved by directing the flashes through two narrow horizontal slits. The light emerged as nearly parallel flat beams across the bottom of the trough. It was important to minimize stray scattered light which reduced the efficiency of the dark background. On long exposures, this tended to fog the single frame of photographic film on which the record was made. The film was exposed in a conventional 35 mm camera with the aperture set at *f*4 and the shutter left open. It was mounted on a light-proof box over the trough, and focused on the animal. Micro-copying film (Kodak 'Microfile'), which is very slow (A.S.A. 2), fine grained and contrasty, was found to be most suitable. The trough was immersed in a bath of red dye which served to increase the efficiency of the dark background by absorbing some of the stray scattered light. Transparent 'Perspex' sheet was used to construct the trough and parts of the rest of the apparatus. The light source and the screens were shielded in so that the light only passed through the narrow slits. Holes were provided for ventilation to prevent overheating on long runs of flashing.

Under these conditions, records of the relatively slow-moving activity of certain small lamellibranchs (Fig. 2) were made. These records were made over several hours, usually at a flashing rate of once every 10 sec, in the course of an investigation[7] of the behaviour involved in the maintenance of their 'commensal' associations. An ordinary portable single flash unit (Mecablitz 108) was used and a Palmer mechanical time switch was included in the flash tube triggering circuit so that it would fire repetitively. This allowed a range of flashing rates (limited by the re-cycling period of the flash unit) from once every 5 sec.

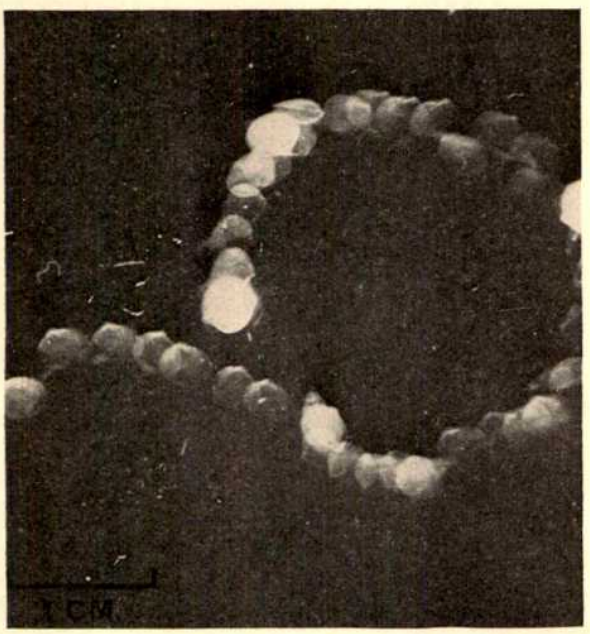


Fig. 2. Part of the photographic record made by a single specimen of the bivalve *Montacuta substriata* walking across the bottom of the trough. The orientation of the animal can be discerned from the individual image components of the track. The more saturated images indicate where the animal was stationary for a period

A more reliable electronic method for triggering a flash tube has since been used. This was constructed by the Benthos Company of North Falmouth, Massachusetts, U.S.A. It incorporated a silicon-controlled rectifier as an electronic switch for the discharge of a capacitor. The latter triggered the flash tube (G.E. *FT*-30) which yielded about 10 w sec/flash. This was found to be quite adequate for my recording conditions. Adjustment of a resistor-capacitor system allowed the flashing rate to be varied over a wide range.

The size of the recording field and the size and the nature of the activity of the experimental animal would determine the exact nature of the components of the electronic flash system in other applications. It should be as simple to construct and use this recording method for terrestrial as with aquatic organisms.

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Intraretinal Discrimination of Horizontal and Vertical Planes of Polarized Light by Octopus

Since the early investigations of von Frisch[1,2], it has been known that some arthropods have an ability to discriminate different planes of polarized light. Recent investigations, using intracellular microelectrodes[3–5], have disclosed that the receptor cells themselves are the analyser for polarized light, although there still remains disagreement as to the existence of the intraocular analyser[6].

In a behavioural study, Moody and Parriss[7] found that the octopus could differentiate planes of polarized light at right angles. Based on this observation and also on the structural similarity in the rhabdomeres between the insect and cephalopod, these authors have proposed a hypothesis that the mechanism for analysing the plane of polarization lies in the octopus retina. In the present investigation of the octopus electroretinogram (ERG), it was found that the octopus retina can indeed discriminate between the horizontal and vertical planes of polarization, and the horizontal–vertical arrangement of the rhabdomere tubules was considered to be the structure responsible for the discrimination.

The animals used were *Octopus vulgaris*. The cornea, lens and vitreous fluid were removed, and the retina was flattened, sclera downwards, on a large plate of silver–silver chloride which served as the indifferent electrode. An *in situ* orientation of the retina was maintained so that the horizontal line of the retina (which corresponds to the direction of the slit pupil) was parallel to the front edge of the experimental table. The light source was a glow modulator tube (Sylvania *R*1131*C*) which was activated by an electronic pulse generator. The light source, condenser lens and 'Polaroid' filter were arranged so that a parallel beam of polarized light was projected perpendicularly to the retina. By this arrangement of the optical system and retina, all possible factors that might cause intensity variation with rotation of 'Polaroid', such as reflexion, refraction, scattering and absorption in the optical pathway, could be minimized. The relative angle between a plane of polarization and the horizontal line of the retina was varied by rotating the 'Polaroid' which was interposed between the retina and lens.

On illumination of the retina by polarized light, a sustained negative response (ERG) was recorded from glass

capillary microelectrodes placed on the retinal surface. The ERG had the characteristics already described elsewhere[8]. In a number of cases the ERG was found to fluctuate in amplitude periodically as the 'Polaroid' was rotated around the optical axis, having two maxima and two minima in one complete turn. But the fluctuation of the ERG must be an experimental error, because it could be minimized by complete flattening of the retinal surface.

The technique adopted was selective adaptation. The retina was first illuminated intermittently by a flash of light (100 msec in duration, about once every 5 sec) while the 'Polaroid' was rotated during a dark period (control run). When fluctuation of the ERG was seen in the control run, the recording site was moved to another retinal area and the control run was repeated until no variation of ERG was found. But this procedure was rarely needed, whenever the retinal surface was held flat. Then a steady light of 20–60 sec in duration was delivered while the 'Polaroid' was fixed at a certain position (selective adaptation). Following light adaptation, the same procedure was repeated as in the course of control run (test run). The result is shown in Fig. 1*A*. For adapting light at 0°, with the plane of polarization perpendicular to the horizontal axis of the retina, the minimal response to test lights appeared at 0° and 180°, and the maximum at 90° and 270° (Fig. 1*A*, top). When the plane of polarization of adapting light was at 90°, the minimum appeared at 90° and 270°, and the maximum at 0° and 180° in the test run (Fig. 1*A*, middle). No such effect of selective adaptation was observed when the e-vector of adapting light was at 45° (Fig. 1*A*, bottom) or at 135°.

The similar effect of selective adaptation could also be demonstrated by a different method. The retina was illuminated repetitively by a flash of polarized light at 0°, and, when the ERG was reduced in amplitude due to light adaptation, the 'Polaroid' was rotated from 0° to 90°. Then the same number of flashes were given at 90°, and the 'Polaroid' was again rotated back to 0°. This procedure was repeated several times. Enhancement of ERG was readily seen immediately after the plane of polarization was rotated either from 0° to 90° or from 90° to 0° (Fig. 1*B*, top and bottom). However, when the e-vector was rotated from 45° to 135° or vice versa, no such temporary increment of response was seen (Fig. 1*B*, middle). From these observations, it is obvious that the octopus retina differentiates the horizontal and vertical planes of polarization, but not oblique planes of 45° and 135°.

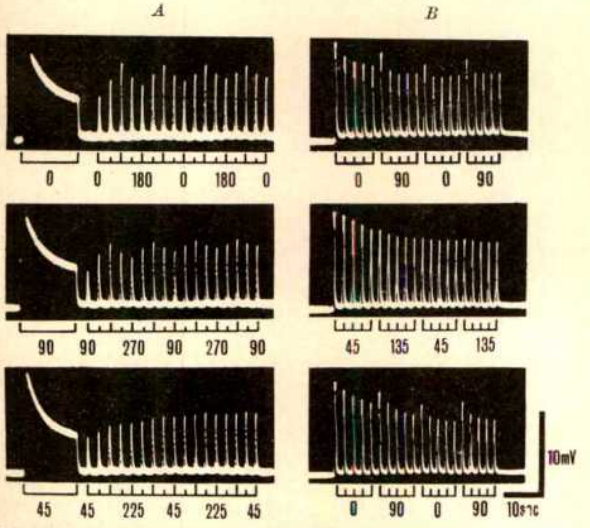


Fig. 1. Oscilloscope tracing of octopus ERG. DC recording. *A*, Retina was first light-adapted at 0° (vertical) for top, 90° (horizontal) for middle, and 45° (oblique) for bottom record. The 'Polaroid' was then rotated at 45° interval. *B*, Five flashes in same plane of polarization, then the 'Polaroid' was rotated by 90°, and the same procedure was repeated. Experiment was carried out from top to bottom in both *A* and *B*. Numerals under each record indicate position of 'Polaroid' in degree

If the e-vector of adapting light is deviated from the standard direction (horizontal line of the retina) by $\delta(<45°)$, then the question arises whether the minimum responses would appear at δ (and maximum at $\delta + 90°$), or the minimum would appear still at 0° (and maximum at 90°). The next experiment answered this question. The retina was first light-adapted at 30° or 330° (that is, ±30° deviated from 0°), then the test run followed. The result was the same as in the case of selective adaptation at 0°, the minimum appearing at 0° and maximum at 90° for adapting light at 0° ± 30°. For adapting light at 90° ± 30°, the minimum appeared at 90° and maximum at 0°. Thus, the position at which the minimum and maximum appeared in the test run was independent of the direction of the e-vector of adapting light, when the e-vector was deviated from the standard direction of the retina by less than 45°. If the e-vector of adapting light was deviated equally from the two standard directions (±45° from horizontal line), no minimum and maximum appeared in the test run.

The same experiment was repeated at various regions in the retina. The results were the same at all retinal areas, showing no regional differences.

These results suggest that in the octopus retina there are two analysers, one lying horizontally and the other vertically, and that they are selectively stimulated and consequently light-adapted by either horizontal or vertical components of incident light. It follows that the octopus retina can discriminate the horizontal and vertical planes of polarization, but not the oblique planes of 45° and 135°. Since all factors which cause intensity variation by reflexion or scattering with rotation of the 'Polaroid' had been eliminated in the stimulating system and dioptric apparatus of the eye, the analysing mechanism must lie in the retina. In the octopus retina the rhabdomes are arranged in a rectangular array, lying in the horizontal and vertical directions[9], and in these directions closely packed fine tubules, rhabdomeres, leave the receptor cell surface perpendicularly[7]. Thus it may be concluded that the horizontal–vertical arrangement of rhabdomeres could provide the morphological basis for the discrimination of plane of polarization in the octopus.

Additional details of these and related experiments will be published separately[10].

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Circadian Rhythms in the Feeding Behaviour of *CBA* Mice

MANY vital processes have marked diurnal rhythms in natural circumstances; and in the laboratory, even under the most constant conditions possible, they still show cycles with a period approximating 24 h ('circadian rhythms'[1]). From his observations on rats, however, Siegel[2] concludes that the diurnal rhythm in the feeding behaviour of this species disappears in 6–10 days in animals

kept under continuous light. (The same conclusion was previously reached by Baker[3], but in our opinion on the basis of an inadequate analysis of the feeding behaviour of his animals.) This conclusion was justly criticized by Aschoff[4]; Siegel's pooling of data for a group of subjects may have obscured the existence of non-synchronous cycles in the different individuals. Therefore, as a preliminary to other investigations on the physiological mechanisms of feeding behaviour in mammals, we have re-investigated this point.

Our subjects were *CBA* mice, kept for two generations under continuous light and constant temperature (24° C). The data reported here refer to 20 males, 5 months old at the start of the experiment, kept individually in 22 × 22 × 15 cm cages, where they could hear but not see one another. Water and dry laboratory food were given *ad libitum*. Automatic records[5] were taken of the time patterns of gnawing at this food in each individual over 24-h periods.

Typically, the mouse gnaws until it has loosened a small piece of food. Then it withdraws from the food to ingest this 'bite'. In all cases there are marked fluctuations in the frequency of bites in the course of the 24-h observation. To analyse these fluctuations, we divided each observation into six 4-h periods, and counted the number of bites in each of these. If we term the period with the greatest number of bites the 'peak period', a record can obtain 0–5 pre-peak periods and 5–0 post-peak periods, depending on whether the peak fell early or late in the observation. For each period of a record the number of bites in that period was expressed as a percentage of that in the peak period of that record. Fig. 1 gives the median of the percentage for each period (total of seventy-five observations on twenty individuals). The distribution-free confidence intervals for each median are shown with confidence coefficients as close to 0·950 as possible. The actual coefficients are shown at the top of Fig. 1 (see Kendall and Stuart[6]). Fig. 1 strongly suggests that a rhythm is present. Although the precise periodicity underlying the original data cannot be derived from this figure, the form of the curve suggests a rhythm of about 24 h, that is, a circadian rhythm.

The statistical significance of this rhythm was tested as follows. The null hypothesis, that is, that our observations could be considered as a purely random series, was tested against the alternative hypothesis that there is an underlying periodicity of approximately 24 h by calculating the total number of ways in which six numbers can be so arranged as to give a result in agreement with such an alternative hypothesis. This number, when divided by the total number of different ways in which six numbers can be arranged (6! = 720), yields the probability under the null hypothesis that a random batch of six numbers in random order agrees with the alternative hypothesis. The number of times one can have agreement in N such

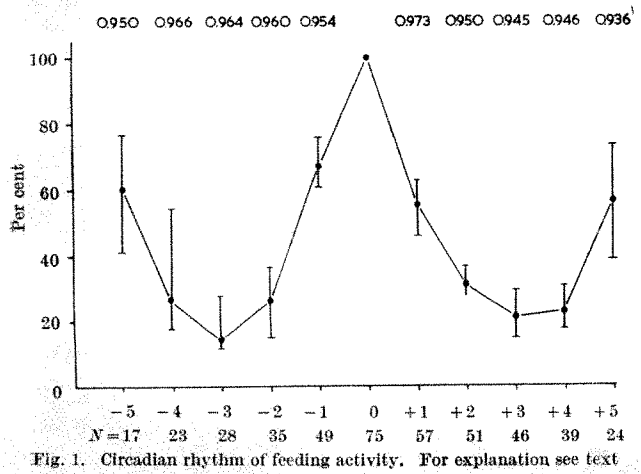


Fig. 1. Circadian rhythm of feeding activity. For explanation see text

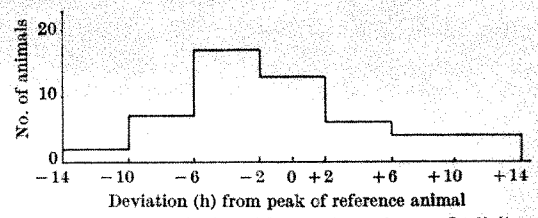


Fig. 2. Frequency distribution of degree of asynchrony of activity peaks (in relation to an arbitrarily chosen reference, individual); compilation of data for the entire period of observations

batches is, then, a binomial variable. The test consists of rejecting the null hypothesis if the number of times agreement has been found is greater than x, where the probability of obtaining x agreements or more under the null hypothesis is less than or equal to the level of significance chosen. In our data, agreement was present in 48 out of 75 cases ($P \ll 0{\cdot}01$).

We conclude, in contrast to Siegel, that at least in mice kept under continuous light, feeding behaviour retains a marked circadian rhythm. It may be added that our data on rats kept under continuous light for two generations also indicate such a rhythm[7].

We have analysed our data in the way just described, for two reasons. First, although there was some indication of inter-individual synchronization, the peak periods of different individuals on the same day often lay far apart (Fig. 2). Secondly, we have not observed any individual over a prolonged uninterrupted series of days; however, in a number of cases the same individual was observed twice on consecutive days or with an interval of only 1, 2 or 3 days. The average period length, estimated from these data, is 25·3 h (average over 97 cycles in ten individuals).

Some preliminary results are also available on the feeding patterns of *CBA* mice injected with aurothioglucose (ATG). During the 'dynamic phase', resulting from this treatment, the animals are strongly hyperphagic. Bite frequency is much increased in all periods, but there is still some indication of a peak period in each record. With the rise in body-weight consequent on the hyperphagia, food intake gradually returns to about normal values, and the animals enter the 'static obese phase'. During the latter, the circadian rhythms of the ATG-treated mice appear identical to those of intact controls. These data suggest that the brain lesions induced by ATG do not affect the mechanism underlying the circadian rhythm in our mice.

Finally, the following example illustrates that these persistent circadian rhythms are to be reckoned with in the interpretation of experiments on feeding behaviour. We have measured feeding activity in mice after food deprivation up to 24 h. Our data strongly suggest that bite frequency after a given deprivation depends on whether feeding is resumed in the active or in the inactive phase of the circadian cycle. Since it appears virtually impossible to eradicate these circadian rhythms from feeding behaviour, we now find it preferable to keep our subjects under conditions where at least the cycles of the different individuals are synchronized, that is, where there is a 'natural' alternation of darkness and light.

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Aurothioglucose Sensitivity of *CBA* Mice injected at Two Different Times of Day

Some years ago Brecher and Waxler[1] described for the first time obesity in mice resulting from aurothioglucose (ATG) injection. Since then it has been found that ATG can cause lesions in different areas of the brain[2–4]. Among these, the lesions in the ventromedian hypothalamic nuclei are held responsible for the observed obesity. However, it was also found that the most effective ATG doses caused a high mortality[5].

As the susceptibility of animals to certain drugs, toxic substances, etc., is well known to be strongly dependent on their diurnal rhythm[6], we have carried out experiments to see to what extent this is true for ATG. *CBA* mice (males and females) were kept under a day–night rhythm of 12 h light (0700–1900 h) and 12 h darkness (1900–0700 h). The ages of these animals varied from 4 to 6 months, that is, they were all full-grown. Two different ATG doses were used (0·4 mg/g and 0·5 mg/g bodyweight) of a 10 per cent ATG suspension in oil ('Solganal *B* Oleosum', Schering). The mice were divided into two equal groups of comparable composition with respect to age and sex. One group was injected during their inactive phase (1200 h), the other group during their active phase (2000 h). All the animals were deprived of food for 3 h immediately before injection. The animals were weighed daily at 0830 h.

The results are summarized in Table 1.

Table 1

Group	I		II	
Time of injection:	Day	Night	Day	Night
ATG dose	0·4 mg		0·5 mg	
No. of animals	19	19	31	31
Weight *a**	21·9	22·2	27·3	25·1
Weight *b*†	32·7	31·6	36·2	33·9
Died	1	0	22	5

* Mean body-weight (g) before injection.
† Mean body-weight (g) 1 month after injection.

These data show that the ATG injection strongly promotes weight increase, considering the fact that the weight of normal mice of the same age increases by only 1–3 g per month. There is also a striking difference in mortality between the 'day' and 'night' animals of group II. Apparently the animals are much more sensitive to the toxic qualities of ATG at 1200 h than at 2000 h. To a much lesser degree this can be found in group I. One of the 'day' animals died, some were sick for some days after the injection, whereas of the 'night' animals in this group none showed any sign of illness. I never observed illness or mortality in animals injected with a dose of 0·3 mg/g mouse (23 animals). In this latter case weight increase is only slightly greater than normal.

From these data it can be concluded that the susceptibility of mice to the toxic effect of ATG clearly depends on their metabolic state. It may be that this decreased susceptibility is comparable with that described after food deprivation[7]. There are no indications that there is a difference in the resulting obesity of the comparable day and night groups.

It is noteworthy that twelve of the twenty-seven animals that died in group II went through a phase (starting about 24 h after the injection and lasting for some hours) of frantic biting. During these hours the tongue is seriously injured and becomes a swollen bloody mass protruding from the month. These animals then stop eating and drinking and die after some days. Whether this frantic biting is an aggressive or an exaggerated feeding activity cannot yet be decided.

What the precise cause of death of all these animals may be is unknown to me, but I now know that ATG mortality can be decreased considerably by injecting the animals at the beginning of their active phase.

Because ATG plays a part in the treatment of, for example, rheumatism and asthma, the findings reported here may also have a medical significance. It would seem important to examine more systematically the effect of drugs, medicines, radiation treatment, etc., in relation to the natural day–night rhythm of the organism treated.

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Haplospora globosa Kjellm. and *Scaphospora speciosa* Kjellm. in Culture

The reproduction and life-cycle of the order Tilopteridales have attracted the interest of phycologists for a long time. Reinke[1] was the first to investigate these problems and, although he was unable to provide any definite evidence, he concluded that the algae in question represent alternating generations of one species, a sporophyte producing non-motile quadrinucleate monospores and an oogamous gametophyte. Reinke's view was adopted by such authorities as Kylin[2,3], Oltmanns[4] and Smith[5], but rejected by Sauvageau[6] and Christensen[7].

Reinke[1], Nienburg[8] and Dammann[9] tried to culture *Haplospora globosa* Kjellm. and *Tilopteris mertensii* (Turn. in Sm.) Kütz. but without great success. In the former species, only germlings from monospores were obtained.

Haplospora globosa is widely distributed on North Atlantic coasts. In the Oslofjord, the *Haplospora* stage as well as the *Scaphospora* stage, originally described as *Scaphospora speciosa* Kjellm., are rather common in spring (Sundene[10]). Specimens of the *Haplospora* stage were collected in May 1964 near the Biological station, Drøbak, in the middle of the fjord, and cultures were established in Erdschreiber at 5° C with daylight. The specimens were fertile, with stalked monosporangia. Monospores were released and some were isolated before settling, while most of the cultures were started from sporelings attached to the bottom of the mother-dishes. The majority of these sub-cultures were contaminated by diatoms, but some

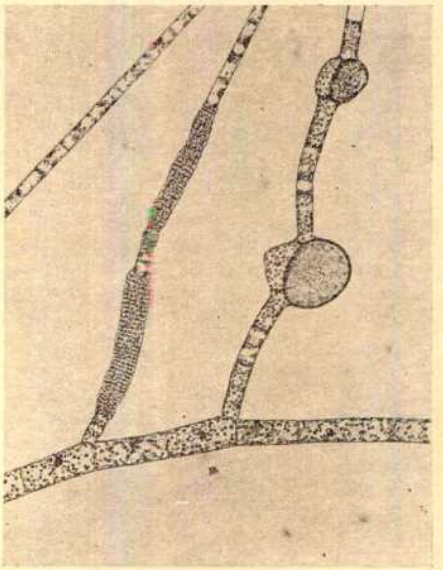


Fig. 1. Branch of the *Scaphospora* stage of *Haplospora globosa* with two antheridia and two oogonia. (× 95)

proved to be unialgal. At first the sporelings grew very slowly and developed during the summer to small, procumbent and zigzag-bent filaments. In the autumn upright shoots sprouted from these filaments. They grew very rapidly, forming tufts 5–10 cm high during the following winter and, early in March, they became fertile, carrying large sessile unilocular as well as plurilocular reproductive organs. The structure of the vegetative thallus and of the reproductive organs accorded with the description of *Scaphospora speciosa* (Fig. 1).

The unilocular reproductive organs of the *Scaphospora* stage contained one large non-motile cell which, after release, settled on the bottom of the dish. From the plurilocular organs a great number of zoids were liberated and a great many of them were observed congregating around the non-motile cells, an indication that they were attracted by the latter.

These observations corroborate Reinke's view that the *Scaphospora* stage represents the gametophyte in the life cycle of *Haplospora globosa* and that the non-motile cells are eggs and the zoids spermatozoids.

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Powdery Mildew Fungus on Cucurbits in the Transvaal Province of South Africa

IN her work on South African *Erysiphaceae*, Doidge[1] has provisionally identified the powdery mildew fungus that causes a troublesome disease on Cucurbitaceae throughout the country as *Erysiphe cichoracearum* DC. After two different mildew fungi were found to attack cucurbits in Europe[2], Doidge[3] realized that this identification should be regarded as tentative, but because the perithecial stage of the fungus had not been found in South Africa, more precise identification was considered impossible. Since then, a number of publications have appeared which have pointed out that it is possible to distinguish between the two fungi in question, namely *Erysiphe cichoracearum* DC. ex Merat emend. Salmon and *Sphaerotheca fuliginea* (Schlecht. ex Fries) Poll., on the basis of their conidial characteristics[4–7]. The conidia of the *Sphaerotheca* species contain conspicuous discoid or rod-shaped fibrosin bodies which were first observed and described by Bouwens[8]. They can be clearly seen if the spores are microscopically observed in a 3 per cent aqueous potassium hydroxide solution as recommended by Kable and Ballantyne[7]. In the conidia of *E. cichoracearum*, however, these bodies are lacking. Though both species produce conidia in long chains, the way in which the conidia germinate also differs. The germ tubes of *E. cichoracearum* are all simple and straight, but among those of *S. fuliginea* a number occur that are forked.

Samples of powdery mildew from cucurbits, collected during the past growing season in various parts of the Transvaal Province, all showed long chains of conidia with well-developed fibrosin bodies present in every specimen. Wherever germination of the conidia was found possible, forked germ tubes, like those illustrated by Boerema and Van Kesteren[5], were also observed. The variation of conidial dimensions, 25·2–37·8 × 14·7–23·1μ, in spores of the cucurbit mildew from the Transvaal falls within the range of dimensions given for *S. fuliginea* by Clare[6] (25·0–46·5 × 14·0–25·0μ).

It is therefore concluded that powdery mildew on cucurbits in Transvaal is caused by the fungus *Sphaerotheca fuliginea* (Schlecht. ex Fries) Poll. The disease was observed on cultivars of the following Cucurbitaceae: *Cucumis melo* L., *C. sativus* L., *Cucurbita pepo* L., *C. maxima* Duch. and *C. moschata* Duch. The mildew generally caused a more severe disease on species of *Cucurbita* than on those of *Cucumis*. Considering that even in tobacco-growing areas where *E. cichoracearum* was prevalent, cucurbit mildew samples only showed the presence of *S. fuliginea*, the indications are that in South Africa, like in Australia[4,6], *S. fuliginea* is the only or most prevalent cucurbit powdery mildew.

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The Dugong

WHEREAS the manatee of the tropical Atlantic has been the subject of widespread interest in recent years, the related dugong of the Indo-Pacific region has received scant attention. An increasing scarcity over most of the range of the animal has been assumed on the evidence available; for example, few remain in the Red Sea, around Ceylon or Malaysia.

We have recently completed a preliminary survey of the dugongs on the coast of Queensland, in Torres Strait, and elsewhere in the Australasian region. Personal travel and the questioning of innumerable individuals with local knowledge, together with much and continuing correspondence with coastal missions and native reserves, lead on integration to certain encouraging conclusions. Additional correspondence with those having local and personal knowledge will be welcome.

Our conclusion is that in parts of the Australian north-east the dugong is not to be regarded as a rare animal. In places its numbers may even be increasing now, following excessive hunting in the nineteenth century and in the early decades of this century. The world's stock of dugongs is certainly much greater than that of manatees.

On the Australian coasts the hunting of dugongs nowadays is wisely confined to native peoples, whose traditional diet includes these animals. Because of the dugong's acute hearing the process of hunting is not easy: the use of engines in their vicinity is fatal to success, in a way which does not apply in turtle-hunting which is the usual alternative. Because of its difficulty, and because of the progressive movement of the native peoples concerned on to a wage basis and the use of purchased foodstuffs, the hunting pressure on the dugongs would seem to be diminishing in many places.

We believe that under present pressures the dugong stocks on the Australian coasts are secure, although there may be a few places of local excessive killing as certain native groups increase in population. Apart from native hunting, a small number of dugongs are killed inadvertently in fish traps, in official shark-netting programmes for the benefit of bathers, and a few by 'sportsmen'.

We have been able to accumulate, besides extensive personal testimonies, a collection of fresh skeletal and other material, older skulls from kitchen middens and, in

particular, numerous tusks from which it is hoped to determine the relative ages of the animals being killed for food. Further quantities of fresh material and information, it is hoped, will continue to reach us from Queensland and the Northern Territory of Australia.

Our own future investigations will consist of detailed analysis of the evidence and material collected. The opportunity now exists for others to take up the investigation of the dugong's detailed breeding biology by intensive work on freshly killed animals. There are just one or two places, now known to us, where dugongs are taken regularly for food in sufficient numbers and where local help and facilities would be adequate.

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MICROBIOLOGY

Leptospires isolated from Frog Kidneys

In the summer of 1964, cases of leptospirosis developed in man as a result of swimming in a farm creek. During epidemiological studies, six leopard frogs (*Rana pipiens*) were collected from these waters. Blood samples were obtained from the frogs. Two pools of kidneys (*A* and *B*) were made from three frogs each. Leptospires were not observed on dark-field microscopy of the kidney suspensions. These were diluted with Stuart's liquid media (1 : 10–1 : 100,000) and 2–3 drops from the dilutions inoculated[1] into semi-solid media: bovine-albumin 'Tween 80' (ref. 2) containing 5-fluorouracil[3] (96 μg/ml.) (Hoffman-LaRoche, Inc., Nutley, New Jersey) and Fletcher's semi-solid media[1]. The inoculated media were incubated at 28°–30° C for 90 days and examined periodically by dark-field microscopy. Leptospires were isolated from pool *B* in albumin 'Tween 80' medium on the twentieth day after inoculation. Observations of the frog isolate by electron microscopy indicated that the organisms were morphologically characteristic of the genus *Leptospira*[4].

The positive culture was transferred to bovine albumin 'Tween 80' with 5-fluorouracil, Fletcher's semi-solid and Stuart's liquid media. These media were adequate for growth and the isolate could be continuously subcultured.

Standard rabbit antisera against *L. ballum*, *L. canicola*, *L. icterohaemorrhagiae*, *L. bataviae*, *L. grippotyphosa*, *L. pyrogenes*, *L. autumnalis*, *L. pomona*, *L. sejroe*, *L. australis*, *L. hyos*, *L. mini georgia*, *L. biflexa*, and *L. kremastos* were used for preliminary identification of the isolate. The isolate was propagated in Stuart's medium for use in the microscopic agglutination test and the subsequent antigen standardized by nephelometry, to a nephelometer reading of 25–30 (ref. 5). The isolate reacted against *L. ballum* antiserum in a dilution of 1 : 25 with the microscopic agglutination test. Rabbit antiserum against the isolate was prepared from a liquid culture standardized to the same bacterial density as that used in the microscopic agglutination test. The antiserum produced reacted with its homologous antigen in a 1 : 12,800 dilution. The rabbit antiserum was tested against antigens of twelve leptospiral serotypes previously listed (excluding *L. biflexa* and *L. kremastos*). The rabbit antiserum reacted against *L. ballum* to an end titre of 1 : 25. The microscopic agglutination test was performed with serum from frogs 1 and 2 (*A*) and frogs 4, 5 and 6 (*B*) against the homologous isolate, *L. pomona*, *L. biflexa*, and *L. ballum*. The microscopic agglutination test was negative in dilutions of serum as low as 1 : 10. In addition, the same serum pools were tested using the rapid macroscopic slide agglutination test[1], with negative results.

Investigations have been conducted to determine the pathogenic characteristics of the isolate in guinea-pigs, hamsters, gerbils, and mice. Actively growing cultures of the frog isolate were inoculated intraperitoneally into the species. No fever or weight loss was observed in the animals following inoculation. The frog isolate has not been recovered from blood culture or from the kidneys of the experimental animals. Additional laboratory studies are in progress to determine the role of the isolate as a pathogen. The isolate has been sent to the World Health Organization/Food and Agriculture Organization and National Leptospirosis Reference Laboratories for definitive identification.

A review of the literature indicates that the role of amphibians and reptiles in the epidemiology of leptospirosis is not as well defined as in man, domestic animals, and wildlife.

In studies of frogs (*Rana* sp.), Van Thiel[6] found a high percentage of sera reacted against many pathogenic and apathogenic strains of leptospires. He states that this may be due to natural immunity which can be broken when frogs are experimentally inoculated with large doses of virulent *L. icterohaemorrhagiae*. Leptospires were recovered from the kidneys and livers up to 7 days after experimental inoculation. Van Thiel concluded that frogs under natural conditions did not shed leptospires in the urine or play a part in the epidemiology of Weil's disease. Babudieri[7] was unable to isolate pathogenic leptospires from some species of amphibians caught in the rice fields of Italy. He reports that others have had similar negative results; Uhlenhuth and Fromme found leptospires in the intestine of the frog, but Babudieri states that these were probably not pathogenic.

Combiesco *et al.*[8] and Van der Hoeden *et al.*[9] reported finding leptospiral agglutinins in the serum of reptiles. Abdulla and Karstad[10] experimentally inoculated snakes with *L. pomona*. Uninoculated snakes kept as cage mates became infected. Both groups were carriers of leptospires in liver and kidney tissues and also produced agglutinins against *L. pomona*. Two species of turtles were similarly infected by inoculation with *L. pomona*. Neither transmission of the infection to cage mates or sustained infection was observed in the turtles. In Florida, White[11] found leptospiral agglutinins in snakes, but leptospires were not isolated from the liver or kidney tissues. Ferris *et al.*[12] isolated *L. ballum* from the kidney tissue of the hog-nosed snake (*Heterodon platyrhinis*) in Illinois, but agglutinins against *L. ballum* were not detected. In Slovakia, Plesko *et al.*[13] isolated *L. sejroe* from one of thirty-seven lizards; leptospiral agglutinins were found in ten of thirty-seven tested.

Although the isolate discussed in this communication was cultured from the kidney of the frog, the role of the unidentified leptospire has not been defined as either a pathogen or a saprophyte. The significance of the 1 : 25 microscopic agglutination titre against *L. ballum* is at present unknown.

Four major questions become apparent: (*a*) What part do the poikilothermic amphibians and reptiles or other lower forms of animal life play in the maintenance and continuity of leptospires in the environment? (*b*) Lower forms of life serve as food for other animals, but are some amphibians the culprits—carriers and shedders of leptospires—or victims or both? (*c*) When pathogenic leptospires are discharged into water, do they remain pathogenic for animals and man, do they transform into saprophytic or free-living forms for survival, or do they gradually disintegrate and die? (*d*) Do lower forms of animal life become intermediate hosts and provide a missing link in the chain of transmission which results from transformation of leptospires from saprophytes into pathogens that infect other animals and man?

Further investigations of amphibians, reptiles, and lower forms of life and their environmental interrelationships may help to define the role of pathogenic or saprophytic leptospires in the epidemiology of leptospirosis.

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Effect of Cellular Lipid on the Sensitivity of some Gram-positive Bacteria to Penicillins

It is possible to increase the lipid content of Gram-positive bacteria by sub-culture in nutrient broth containing glycerol and to deplete cellular lipid by treatment of the cells with pancreatic lipase.

Using benzylpenicillin, cloxacillin, methicillin and quinacillin[1] and *Bacillus subtilis*, N.C.T.C. 3601, *Streptococcus faecalis*, N.C.I.B. 8553 and *Staphylococcus aureus* (three strains) Oxford, N.C.T.C. 8452 and 100110 isolated at Queen Mary's Hospital for Children, Carshalton, we find that lipid enhancement invariably increases the resistance, as measured by the minimum inhibitory concentration for a constant inoculum of 10^3 cells/ml. and depletion invariably produces an increase in sensitivity. Results are summarized in Table 1.

Total lipid was determined by extracting dried cells with chloroform–methanol, 2 : 1, and by electrophoresis in the presence of 10^{-4} M sodium dodecylsulphate[2].

Preliminary experiments, using thin-layer chromatography and silicic acid-impregnated paper chromatography, indicated that this change in resistance could not be attributed to any particular component of the bacterial lipid as obtained by solvent extraction.

It is clear that changes in lipid content of cells markedly effect their susceptibility to penicillins and it is not impossible that such changes may occur *in vivo*, subsequent to infection.

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AGRICULTURE

Paternal Effect on Grain Size in Wheat

Varieties of wheat can differ in yield of grain even when unfavourable factors such as disease, unsuitable soil nutrient status and lodging are eliminated as completely as possible. Methods of breeding for high yield could be made more objective if the physiological processes responsible for the yield differences were understood. There are two possibilities. First the assimilative capacity of the plant after anthesis may be the principal limiting factor, or secondly, the grain may be a limited sink for photosynthates, either as a result of its rate of growth or its ultimate size.

The investigation reported here was carried out to obtain information on the potential capacity of the grain as a sink for photosynthates. Tests were made for genetically determined differences in grain size. The effects of variation in grain number, and of the assimilative capacity of the plant, on the supply of photosynthates to each grain were eliminated by making comparisons using common maternal parents. Genetic differences in the grain were obtained by artificial cross-pollination.

Table 1. Naturally Selfed Parents

	Mean weight per grain (mg)
'Holdfast'	41·6
'Cappelle-Desprez'	58·4
F_1 'Holdfast' × 'Cappelle Desprez'	53·5
Least significant difference $P < 0.05$	3 3
$P < 0.01$	4·7

Two varieties of winter wheat were used: 'Cappelle–Desprez' which has large grain and 'Holdfast' which has relatively small grain (Table 1). In 1963 the varieties were crossed reciprocally and also selfed by hand, a separate ear being used for each treatment. The experimental variation was too great for a paternal effect to be detected. This effect was likely to be small, because the male parent contributes only one-third of the endosperm genotype.

Table 1. Effect of Lipid on the Sensitivity of Gram-positive Bacteria to Penicillins

Organism	Treatment	Lipid content (% w/w of whole dried cells)	Mobility (μsec^{-1} V^{-1} cm^{-1}) in the presence of 10^{-4} M sodium dodecyl-sulphate	Minimum inhibitory concentration (μg/ml.)			
				Quinacillin	Benzyl-penicillin	Methicillin	Cloxacillin
Staph. aureus (Oxford)	Control	6·39	−2·84	0 3	0·03	8·0	0·35
	Grown with glycerol	18·3	−4·56	20 0	2·0	20 0	8·0
	Lipase treated	6·4	−2·40	0 1	0·01	6·0	0·25
Staph. aureus N.C.T.C. 8452	Control	6·5	−3·21	0·7	Destroyed	1·0	0·5
	Grown with glycerol	7·0	−3·68	2 0	Destroyed	2·0	0 8
Staph aureus 100110	Control	—	−3·69	5·0	100	26	1·0
	Grown with glycerol	—	−3·68	5 0	100	26	1·0
	Lipase treated	—	−3·0	3·5	100	24	0·8
Strep. faecalis	Control	5·3	−2·42	110	3·2	45	40
	Grown with glycerol	5·9	−3·01	350	10·0	100	200
B. subtilis	Control	8·6	−4·56	8·0	0·6	0 4	0·01
	Grown with glycerol	19·0	−5·48	40	5·0	1 0	0·03
	Lipase treated	8·3	−3·45	5·0	0·3	0·2	0·008

In 1964, genetic differences in the grain were obtained by backcrossing the reciprocal F_1 hybrids, as female parents, with each variety. Therefore, owing to genetic segregation in the F_1, the genetic variation in the endosperm was greater than in the previous experiment. To facilitate crossing, the small basal and apical spikelets, and also all florets in excess of two per spikelet, were removed from the F_1 ears. To improve the accuracy, and to ensure that photosynthates were available similarly to grain in the two treatments, each maternal ear received both backcrosses, one rank of spikelets being crossed by 'Cappelle-Desprez' and the other by 'Holdfast'. The two reciprocal F_1 hybrids behaved similarly, and the results for all the 29 treated ears have been combined (Table 2).

Table 2. BACKCROSSES OF F_1 HYBRIDS BY THE TWO PARENTS

	Mean No. of grains set	Percentage of grains set	Mean grain weight (mg)
Backcross by 'Holdfast'	9·7	86 6	52 0
Backcross by 'Cappelle-Desprez'	9·4	88·1	54 0
Variance ratio	0·58 (Not significant)	0·39 (Not significant)	12·7 ($P < 0·01$)

It is apparent, from the significant paternal effect in the backcrosses, that the genotype of the grain was a determinant of grain size. Because photosynthates were available similarly for each treatment, this effect also implies that yield was not solely dependent on the assimilative capacity of the plant.

By taking other observations into account, it is possible to consider how the inter-relations of photosynthate supply and the characteristics of the developing grain determine yield. Experimental reduction of the photosynthetic area also indicates that the assimilative capacity is not always fully exploited. For example, Thorne[1] found that compensation occurred in shading experiments with barley; and Miller *et al.*[2] found, for eight varieties of wheat, a mean reduction in yield of only 10·5 per cent for complete defoliation at the ligule one week after anthesis, and 4·8 per cent when half of every leaf was removed. Yield is not, however, entirely dependent on the characteristics of the developing grain. When few grains develop on each ear, as in partially sterile interspecific hybrids and in ears which have been emasculated and only a few florets pollinated, the grains are considerably larger than normal.

Consequently, we may conclude (Fig. 1) that yield of grain depends on the assimilative capacity of the plant, the genotype of the grain and on the number of grains produced. The interactions of these characters and their effects on yield are shown by the arrows. There is not, however, sufficient information to determine their relative importance.

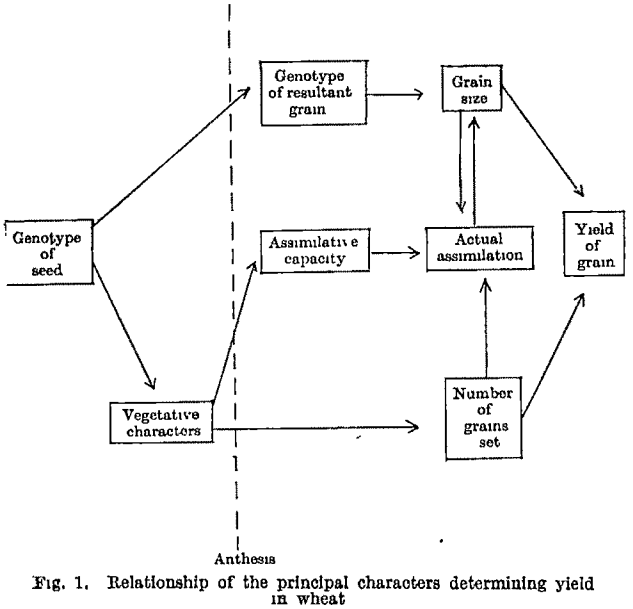


Fig. 1. Relationship of the principal characters determining yield in wheat

Because yield of grain is partially dependent on the genetic characteristics of the grain, increase in yield could be obtained by treatments which increased the number of grains set. As the number of florets per spikelet in wheat is not delimited until the later stages of ear development, the treatments, which might be of a hormonal nature, need not produce any major change in vegetative characters.

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SOIL SCIENCE

Removal of Plutonium-239, Tungsten-185 and Lead-210 from Soils

THE increasing use of plutonium and the possibility of the activation of tungsten and lead by intense neutron flux produced by nuclear devices suggested a need for some investigations of the plant–soil interrelationship of these elements. Lead-210, ^{185}W and ^{239}Pu were the nuclides used.

Previous experiments in this laboratory[1] and findings of others[2] indicated that only small amounts of ^{239}Pu were translocated from the soil to the plant foliage (leaf–soil ratios $\sim 0·0001$). Data were generally variable because of the low counting rates. The results were technically improved in these experiments by the increased concentration of ^{239}Pu in the soil. Keaton[3] reported small amounts of lead accumulated in plant tissues, and Chow *et al.*[4] discussed lead isotopes in petrol and aerosols as a possible soil contaminant in areas with large numbers of cars. Limited information is available on the uptake of tungsten[5] from soils. This communication contains results showing the amounts of ^{239}Pu, ^{185}W and ^{210}Pb that were removed from different soils by plants, and the effectiveness of several extracting solutions in removing ^{239}Pu and ^{185}W from contaminated soils.

Barley (*Hordeum vulgare*, var. Arivat L.) was grown for 18 days in three different soils using a modified Neubauer technique[6]. The isotopes were mixed with 100 g oven-dry soil, then placed in a container. After planting, the soil was brought to field capacity by daily watering. The soils were: Ephrata fine sandy loam, a slightly alkaline soil; Milville silt loam, a calcareous soil; and Cinebar silt loam, a medium acid forest soil. The isotopes were added as plutonium nitrate, potassium tungstate (specific activity, 2·7 mc./mg) and lead chloride (specific activity, 81 mc./g) dry soil for ^{185}W and ^{210}Pb and 10 μc./g for the ^{239}Pu. The activity in the extraction experiments for both soils was 0·1 μc./g dry soil. The standard soil extraction procedure was used[7].

The amount of ^{239}Pu and ^{210}Pb accumulated in tissues was very limited, but ^{185}W was taken up readily (Table 1). The uptakes of these radioisotopes by barley were soil dependent, particularly that of ^{185}W. Concentration of ^{185}W in plants grown in Ephrata soil was fifty-five times higher than for those grown in Cinebar soil. The lower uptake from acid (Cinebar) soil suggests that tungsten was taken up as an anion, probably WO_4^{--}. The uptakes of ^{210}Pb and ^{239}Pu were greatest from the acid soil, which is true for most cations. No toxic symptoms were observed, and yields (Table 1) for each soil type were nearly the same with all the nuclides. Yields in controls, not shown, were not significantly different from treatment yields.

The removal of ^{239}Pu and ^{185}W from the different soils into suspending solutions is shown in Table 2. The

Table 1. Uptake of ^{239}Pu, ^{185}W, and ^{210}Pb by Barley from Three Soils using a Neubauer Technique

Isotope	Soil type	Soil activity (μc./g)	Plant yield (g)	Concentration in tissue (nc./g)	Removal by plant (per cent)
^{239}Pu	Ephrata	10	1·5 ± 0·02	1·3 ± 0·13	0 00019
^{239}Pu	Milville	10	1·5 ± 0 02	0·4 ± 0 08	0·00006
^{239}Pu	Cinebar	10	1·0 ± 0·02	2·2 ± 0·37	0·00022
^{185}W	Ephrata	0·1	1 2 ± 0·1	380 ± 5	4·5
^{185}W	Milville	0·1	1·4 ± 0·1	80 ± 5	1·1
^{185}W	Cinebar	0·1	0 8 ± 0·1	7 ± 1	0·06
^{210}Pb	Ephrata	0 1	1·6 ± 0·02	0·2 ± 0·02	0·003
^{210}Pb	Milville	0 1	1·8 ± 0 03	0·2 ± 0 05	0·003
^{210}Pb	Cinebar	0·1	1·0 ± 0·03	0·5 ± 0 05	0 005

± = One standard deviation.

Table 2. Removal of ^{239}Pu and ^{185}W from Soils using Different Extractants

Isotope	Soil	Per cent removed by the following extractants: 1·0 N NH_4 AC	0 1 N HNO_3	1 0 N HNO_3	0·2 mM DTPA*
^{239}Pu	Ephrata	0 06 ± 0 02	0·14 ± 0·01	0·25 ± 0 02	0·1 ± 0·03
^{239}Pu	Cinebar	0 05 ± 0·01	0·64 ± 0·08	0·32 ± 0·08	0 03 ± 0 004
^{239}Pu	Milville	0 06 ± 0 004	0·01 ± 0·004		0·43 ± 0·005
^{185}W	Ephrata	12 0 ± 1·0			
^{185}W	Cinebar	1·4 ± 0·2			
^{185}W	Milville	4 8 ± 0·8			

* Calcium-trisodium salt of diethylenetriaminepentaacetic acid (supplied through the courtesy of the Geigy Chemical Corporation).
± = One standard deviation

greatest amount removed was the 12 per cent of ^{185}W extracted from Ephrata soil with ammonium acetate. The removal of ^{185}W by ammonium acetate extraction, and by barley, follows the same pattern in all three soils, although the amount removed by the ammonium acetate was much greater than that removed by barley. The highest amount of ^{239}Pu removed was 0·64 per cent from the Cinebar soil by 0·1 N nitric acid. Again the extracting solutions removed much more ^{239}Pu from soils than did the plants.

Barley grown in the different soils accumulated only very small amounts of ^{210}Pb, less ^{239}Pu, whereas ^{185}W was accumulated in biologically significant quantities. These results show that the soil extracting procedures in common use are not reliable estimates of the quantities of these radioisotopes that can be removed by plants.

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PSYCHOLOGY

Müller-Lyer Haptic Illusion and a Confusion Theory Explanation

Many geometric visual illusions have haptic analogues[1]. The most studied is the Müller–Lyer figure. Gregory[2] has recently given strong evidence in support of a perspective theory of the visual variant. Day[3] has argued in favour of a common explanation of both—a suggestion which would rule out the perspective theory, as the haptic variant has been demonstrated on congenitally blind subjects[4]. It seems that the simplest explanation of the Müller–Lyer haptic illusion has been ignored. This is the confusion theory[5]. Under this the illusion results from the mere confusion of the arrow-heads' apices with their fins.

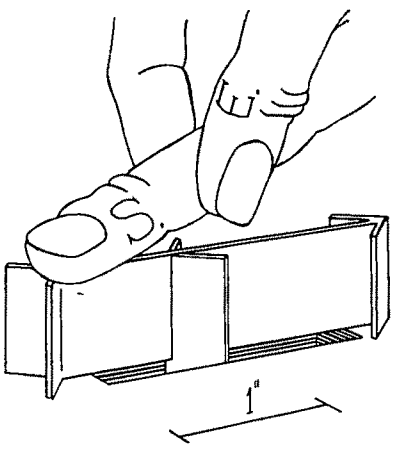


Fig. 1

In the present experiment Revesz's passive technique[1] was used. E. drew S.'s index finger from a position of rest on one end of the figure longitudinally over the rest without stopping at the other end (Fig. 1). S. then said which segment of the centre line he thought was bigger. To avoid confusion the figure was originally described to S. demonstratively by touch using the terms 'point' and 'line' only, and he was instructed to ignore the shorter lines as much as possible in making his judgments. The method of limits was used—the position of the centre arrow-head was varied along the centre line for each judgment. Two variables were investigated—the direction of finger movement and the direction in which the arrow of the figure pointed. Both could be either towards or away from S. Seven students were tested.

From the confusion explanation one would expect the three points where the centre line is intersected to be always confused with the fins except when the finger is initially placed at the pointed end. A clear impression of this point only should then be received because S.'s finger is initially at rest on this same point. Consequently the illusion should be greater when the finger is begun at the tail end of the figure. An analysis of variance confirmed this ($P < 0·001$). Contrary to expectation, further analysis showed that when S.'s finger was initially placed at the pointed end the illusion was not significant ($P < 0·15$ on a one-tail t-test).

We chose the passive technique as it appeared to give us more control over the stimulus. However, we found it a very difficult and inelegant method; S.'s hands were often too limp or too stiff or they perspired. Despite these criticisms the results seem positive enough to give some support to the confusion theory.

With this explanation, repeated inspection trials would be expected to lead to an improved perception of the figure with less confusion and a consequent decrement in the size of the illusion. This has been found by Rudel and Teuber[6] although they used a different technique and a Müller–Lyer figure five times as large as our own. They also found a transfer of decrement from touch to vision and vision to touch. This remains unexplained by our results, which endorse the view that the two illusions require different explanations.

Ann Watson
C. French

Department of Psychology,
University of Aberdeen.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 28

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W C.2), at 5 30 p m.—Dr. P. R Thornton "Scanning Electron Microscopy—Its Use in the Evaluation of Semiconductor Devices and Materials".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C 2), at 5 30 p m —Mr D J. Rhodes and Dr A. Wright "Induced Voltages in the Sheaths of Cross-bonded A C. Cables".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C 1), at 5 30 p m —Prof. C. J. F. Bottcher (Leiden): "Pathology and Chemistry".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W C.1), at 6 p m —Prof J. W. Tukey (Princeton) "Numerical Spectrum Analysis· I. Introduction".*

Tuesday, March 1

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W C.1), at 1.15 p m.—Dr. J. B Hasted "Radio Waves in Water".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 2 p.m —Discussion Meeting on "Gamma and Infra-red Camera Techniques".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W C 2), at 5.30 p m —Mr A J White. "Analysis and Design of Model Reference Adaptive Control Systems".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. R A. Coombs "The Cell Surface as Revealed by Immunological Studies". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m —Discussion Meeting on "Diesel Injection Equipment for Larger Engines".

Wednesday, March 2

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C 2), at 9 30 a.m and 2.15 p m —Colloquium on "Microphones".

BRITISH POSTGRADUATE MEDICAL FEDERATION, UNIVERSITY OF LONDON (at the Institute of Dental Surgery, Eastman Dental Hospital, Gray's Inn Road, London, W C.1), at 5.30 p.m.—Dr. J. W. S. Harris. "Experimental Studies on Closure and Cleft Formation in the Secondary Palate". (Second of three lectures on "The Scientific Basis of Dentistry".)*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W C.2), at 5 30 p m.—Discussion Meeting on "Performance Requirements of Pilot-wire Differential Protection Systems", opened by Mr. J. Rushton, Mr D. W. Lewis, and Dr W. D Humpage.

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W 1), at 5 30 p m.—Dr. C. M. H. Pedler. "The Retina as a Computer—An Electron-Microscopical Study".*

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W 1), at 6 p.m —Mr P. N Nikiforuk and Mr. D. R. Westlund: "The Large Signal Response of a Loaded High-Pressure Hydraulic Servomechanism"

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 6 p m.—Prof J. W Tukey (Princeton) "Numerical Spectrum Analysis: II. Some Geophysical Applications" *

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Northern Polytechnic Chemical Society, at the Northern Polytechnic, Holloway Road, London, N 7), at 7 p.m.—Prof. C. W. Rees : "Recent Advances in Benzyne Chemistry".

Thursday, March 3

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p m.—Mr. B. T Kelly, Mr. W. H. Martin and Mr P. T. Nettley: "Dimensional Changes in Pyrolytic Graphite Under Fast-Neutron Irradiation"; "Dimensional Changes in Polycrystalline Graphites Under Fast-Neutron Irradiation".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W C 1), at 5 p.m.—Prof. S. V. Perry. "Biochemistry of Muscle".*

INSTITUTE OF REFRIGERATION (at the National College of Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S E.1), at 5.30 p m.—Mr. A. Enemark: "Winding Temperatures and Insulating Materials in Hermetic Compressors".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5 30 p m.—Mr. Howard A McKinley. "LNG Movements by Sea—Present Developments and Future Prospects".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W C.1), at 5.30 p.m.—Dr E. J. Moran Campbell: "Exercise Tolerance". (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF CHEMICAL ENGINEERS, GRADUATES AND STUDENTS SECTION (at University College, Gower Street, London, W.C 1), at 7 30 p.m.—Annual Centre Meeting. Mr. Teichmann: "Handling Explosives".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C 2), at 7.30 p.m —Lord Todd, F.R.S.. "The Changing Face of Organic Chemistry" (Second Annual Medal Lecture).

Friday, March 4

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 10.55 a.m.—Symposium on "The Experimental Taxonomy of Flowering Plants".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W 12), at 4 p.m —Dr. G. Fearnley: "Fibrinolytic Drugs".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C 1), at 6 p m.—Prof J. W. Tukey (Princeton) "Numerical Spectrum Analysis· III, Modernizing the Technical Apparatus" *

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1). at 9 p m.—Prof B. H Flowers, F.R.S.· "Physics with High Energy Electrons"

Saturday, March 5

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S E.23), at 3.30 p m.—Mr. Nigel Heppel "A Naturalist in Nigeria".*

Monday, March 7

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. P. J. N. Norris. "An Introduction to Silicon Controlled Rectifiers".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6 30 p.m —Dr. H M. Finniston "Experimenting in Experiments".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS IN BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (March 4).

JUNIOR RESEARCH OFFICER (graduate) for supervisory duties in the laboratories of the DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University College of Wales, Aberystwyth (March 5).

PROGRAMMING ASSISTANTS (2) (graduates, preferably with some knowledge of computing) IN THE COMPUTING DEPARTMENT, for work which will involve programming and the supervision of programmers—The Secretary of the University Court, The University, Glasgow (March 5).

LECTURER or ASSISTANT LECTURER (with a special interest in bacteriology or microbial genetics) IN BOTANY—The Secretary, Birkbeck College (University of London), Malet Street, London, W C.1 (March 7).

UNIVERSITY DEMONSTRATOR (with an appropriate honours degree and preferably research and/or teaching experience) IN ANIMAL PHYSIOLOGY—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (March 9).

LECTURER IN NUMERICAL ANALYSIS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Leicester (March 10).

ASSISTANT LECTURER (botanist or plant biochemist) IN BOTANY—The Secretary, The Queen's University, Belfast, Northern Ireland (March 11).

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Manchester, 13, quoting Ref. 31/66 (March 12).

ASSISTANT LECTURER or LECTURER in the field of inorganic chemistry—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (March 12).

LECTURER or ASSISTANT LECTURER IN SOCIOLOGY—The Secretary, The University, Edinburgh (March 12).

CHAIR OF NATURAL HISTORY IN THE DEPARTMENT OF ZOOLOGY—Charles H. Stewart, Secretary to the Curators of Patronage, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (March 14).

LECTURER or ASSISTANT LECTURER IN INORGANIC CHEMISTRY (in one of these appointments preference will be given to candidates with an interest in organo-metallic or transition element chemistry)—The Deputy Secretary, The University, Southampton (March 14).

LECTURER or ASSISTANT LECTURER IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Leeds, 2 (March 14).

LECTURER or ASSISTANT LECTURER (one or more, preferably with research experience in either catalysis or absorption, or in techniques used in these fields) IN PHYSICAL CHEMISTRY—The Secretary, The University, Edinburgh (March 14).

ASSISTANT LECTURER (with some experience in structural petrology) IN THE DEPARTMENT OF GEOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (March 15).

LECTURER IN RADIATION BIOLOGY AND PHYSICS—The Secretary, University of St. Andrews, Queen's College, Dundee (March 15).

LECTURER (with special interests in plant biochemistry or microbial genetics or morphogenesis) IN BOTANY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 15).

ASSISTANT LECTURER (with interests in sedimentology or palaeoecology) IN THE DEPARTMENT OF GEOLOGY—The Secretary, The University, Edinburgh (March 16).

LECTURER and ASSISTANT LECTURERS (2) IN PHYSICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 19).

LECTURER or ASSISTANT LECTURER (medically qualified) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Leeds, 2 (March 21).

RESEARCH ASSISTANT or SENIOR (Post-doctoral) RESEARCH ASSISTANT IN ORGANIC CHEMISTRY for investigations related to fluorinated polymers—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 21).

TEMPORARY ASSISTANT LECTURER (with a good honours degree and a special interest in social geography) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds, 2 (March 21).

PRINCIPAL LECTURER IN SCIENCE AND HEAD OF THE SCIENCE DEPARTMENT—The Principal, Bede College, Durham (March 26).

SCIENTIFIC OFFICER (with a good honours degree, and preferably training in genetics and statistics) to engage in research on grass breeding techniques, including the evaluation of selection criteria—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (March 26).

ASSISTANT LECTURER (with a veterinary degree) IN VETERINARY PATHOLOGY—The Registrar and Secretary, The University, Bristol (March 31)

CHAIR OF STATISTICS AT BIRKBECK COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C 1 (March 31).

ENTOMOLOGIST or BOTANIST IN THE PLANT PATHOLOGY DEPARTMENT to work on insect transmission of viruses to plants, including cereals and grasses—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (March 31).

FELLOW (with a Ph.D. or equivalent degree in chemical engineering or one of the physical sciences) IN HIGH INTENSITY COMBUSTION OF FINE PARTICLES, University of Canterbury, Christchurch, New Zealand, to study and develop small burners capable of burning pulverized coal—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (March 31).

SECOND CHAIR IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Deputy Secretary, The University, Southampton (April 1).

SIR WILLIAM DUNN READER IN BIOCHEMISTRY—T. D. Kellaway, Department of Pathology, The University, Tennis Court Road, Cambridge (April 18).

PROFESSORS (3) OF ENGINEERING—The Registrar, University Registry, The Old Schools, Cambridge (April 19).

ASSISTANT PHYSICIST (Basic Grade) TO WORK IN THE RADIOTHERAPY DEPARTMENT AND THE CLINICAL ISOTOPE LABORATORY—The Group Secretary, Portsmouth Group Hospital Management Committee, Saint Mary's Hospital, Milton Road, Portsmouth.

CHAIR OF BIOCHEMISTRY—The Registrar and Secretary, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential".

EXPERIMENTAL OFFICER (with a degree in some branch of biology) IN THE DEPARTMENT OF PATHOLOGY to work as a member of a team investigating the behaviour of children's tumours in tissue culture—Dr. J. K. Steward, Tissue Culture Laboratory, Department of Pathology, University of Manchester, Manchester, 13.

HEAD (with high academic qualifications and considerable research experience in laboratory and field experimentation with crop plants, not necessarily vegetables) OF THE CHEMISTRY SECTION—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick.

LECTURER IN SOCIOLOGY IN THE DEPARTMENT OF ARTS, SCIENCE AND EDUCATION—The Registrar, Goldsmiths' College (University of London), London, S.E.14.

LECTURER or ASSISTANT LECTURER (preferably with knowledge of one or more of the following fields: process control and dynamics, mass and heat transfer, particle mechanics, thermodynamics, or chemical reaction engineering) IN CHEMICAL ENGINEERING—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 59/2/G.

LECTURER or SENIOR LECTURER (honours graduate with a special interest in mycology and plant microbiology) IN BOTANY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

LECTURER (well qualified in physics and preferably experienced in some branch of advanced electronics) IN THE DEPARTMENT OF PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURERS or ASSISTANT LECTURERS (or exceptionally Senior Lecturers or Readers) IN NUMERICAL ANALYSIS—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

POSTDOCTORAL FELLOW IN THE CHEMICAL LABORATORY for work in carbohydrate chemistry with Dr. R. D. Guthrie—The Assistant Registrar (Establishmert), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

RESEARCH ASSISTANT (with a first degree in a biological science) IN THE DEPARTMENT OF ZOOLOGY to work on problems of cell behaviour in tissue culture—The Secretary, University College, Gower Street, London, W.C.1.

RESEARCH ASSISTANT (honours graduate or graduating this summer) IN BOTANY—Head of the Department of Botany and Zoology, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH PATHOLOGIST or HISTOLOGIST for work on atherosclerosis—Dr. E. B. Smith, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.1.

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STATISTICIAN or AGRONOMIST (with good statistical experience) to assist research workers in developing countries overseas in the design, conduct, and interpretation of experiments on perennial plantation crops—The Secretary, East Malling Research Station, East Malling, Maidstone, Kent.

TECHNICIAN, and JUNIOR TECHNICIANS (2) IN THE DEPARTMENT OF PHYSICS—Mr D. R. Ringer, Department of Physics, The University, Nottingham.

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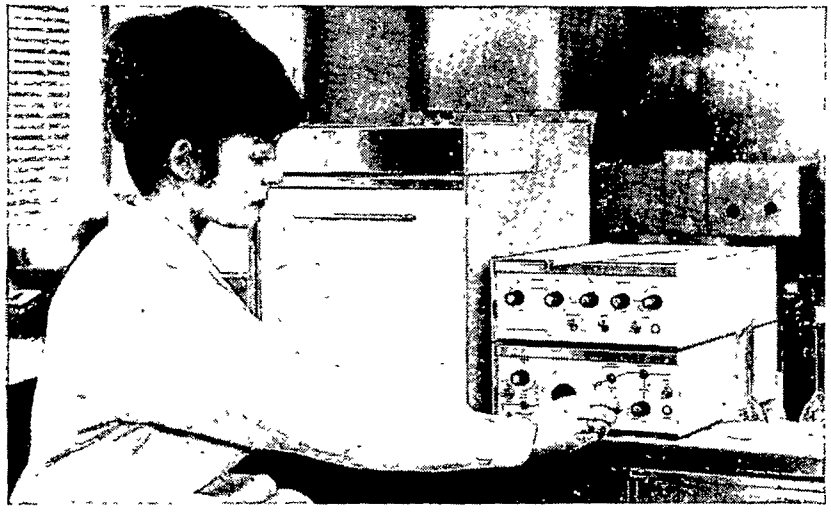
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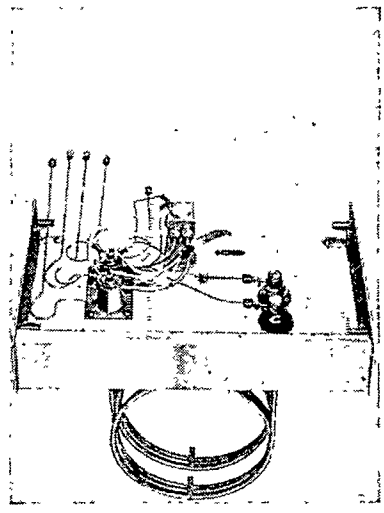
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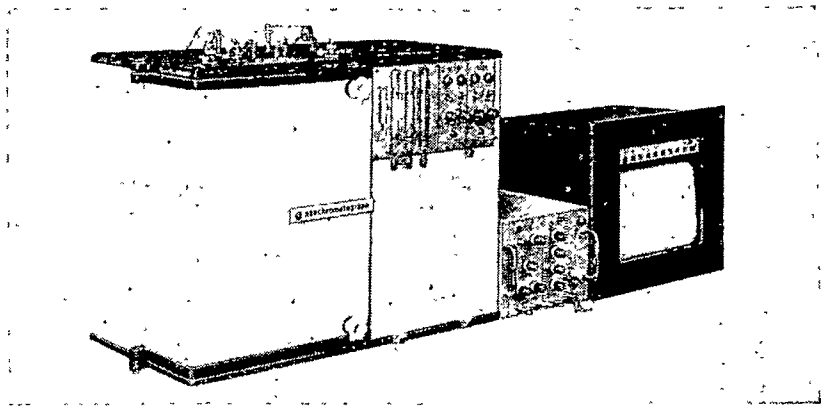
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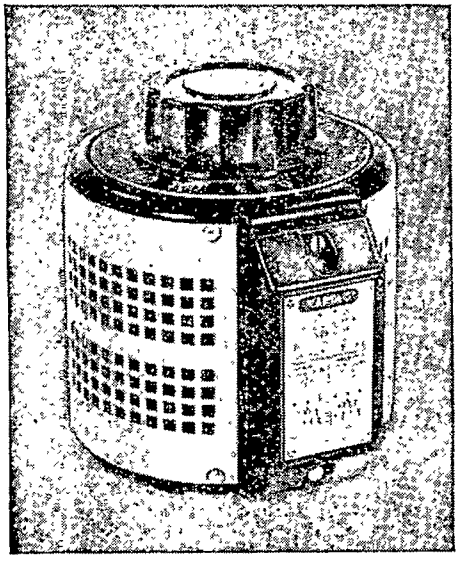
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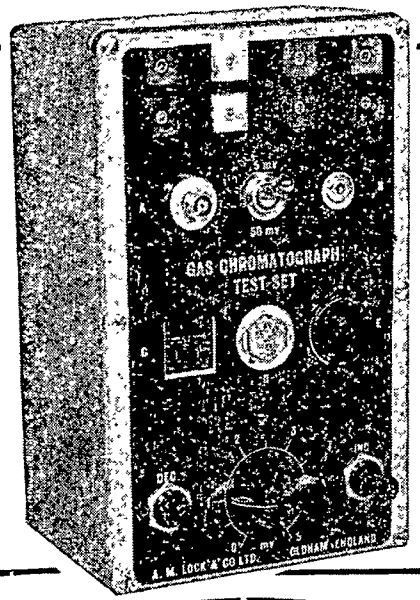
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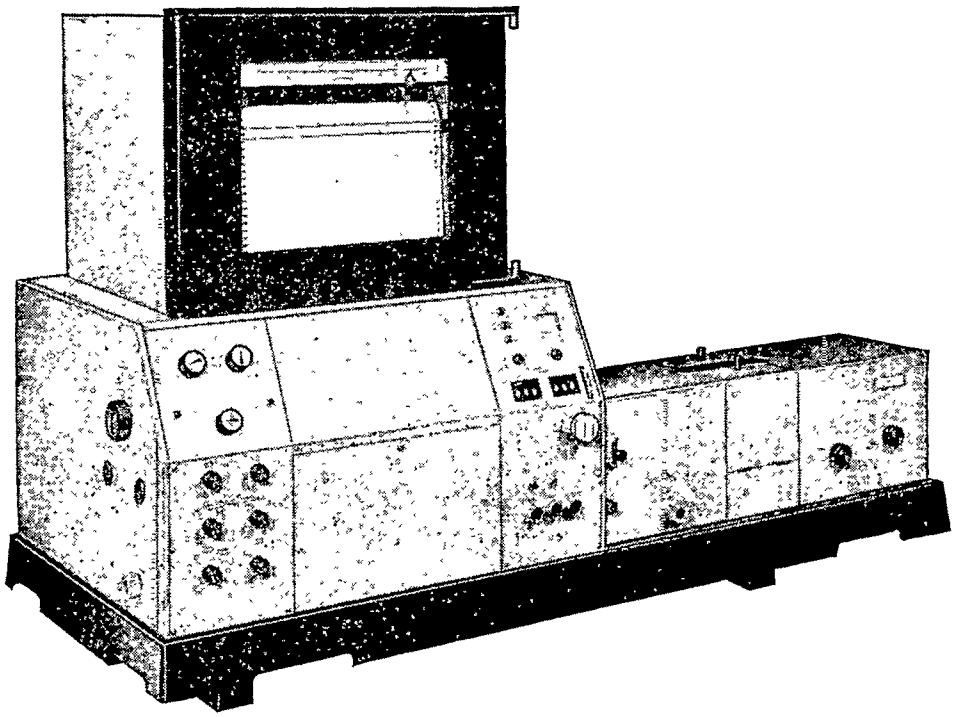
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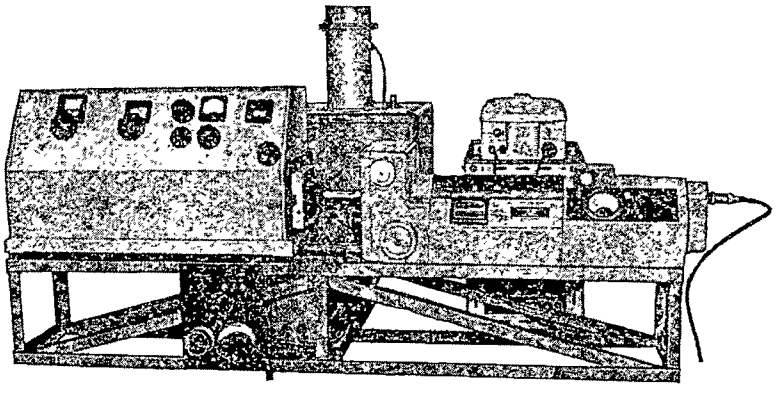
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UNIVERSITY LIBRARIES FOR THE FUTURE

IN the debate on higher education in the House of Lords on December 1, Lord James of Rusholme remarked that the money available to buy books for the new library at the University of York was less than one-half of the amount which may be available for equipping a new science laboratory for a single science subject. This absurdity is attributable to the fact that scientific apparatus seems more obviously and materially useful than books; however, as Lord James added, books are just as essential pieces of apparatus to the scientist as a cathode-ray tube or a spectrometer. Lord Holford endorsed the point in respect of the situation at the University of Kent at Canterbury, where there is no public library capable of helping out deficiencies in the central university library.

This particular point was not mentioned by the Earl of Longford in replying on the debate, and this, together with the dilatory attitude of the Committee on Libraries set up by the University Grants Committee and the Ministry of Education, and the naïve remark in the Robbins Report that "a library adequate to scholarly research is as essential to the efficient running of a university as an adequate range of computers", fully justifies the anxiety expressed recently by the Master of Sidney Sussex College, Cambridge (*The Listener*, 75, 47; 966). Universities, as Dr. D. Thomson pointed out, have managed for centuries without computers, but never without libraries. He suggested that the Government's decision not to establish further new universities for 10 years might at least offer some protection against ridiculously inadequate university libraries. His protest against the lack of realism in some of the handling of university expansion is timely, but nowhere more so than in his reference to the position of the university library. It is to be hoped that the Minister for Education and Science, who now bears the main responsibility for policy in this regard, will pay some attention to the addresses and reports of Paul Buck, director of Harvard University Library, now published by the Oxford University Press, under the title *Libraries and Universities*. There is much in the volume which the Minister might use to emphasize the futility of the Government's present attitude to university expenditure: "You cannot have a quality education without a quality library. . . . The Harvard Law School is a great school; it also has the greatest legal library in the world. These two facts are related. . . Library growth, unlike growth in most other parts of a university, is cumulative". Comments like these make nonsense of a Government policy which results in situations such as those described by Lord James and Lord Holford.

This is one context, at least, in which it is worth considering the lecture, "The Ecology of Inter-Library Loans", delivered last April by Dr. D. J. Urquhart at the Conference of the University and Research Section of the Library Association at Reading and published in the *Library Association Record* (67, 341; 1965). Dr. Urquhart was concerned not merely to describe the present position of inter-library lending, but also to indicate the direction of change and to outline new approaches. He noted, for example, that the annual output of publications in science and technology has been doubling every 15 or 20 years for the past three centuries and, even outside this field, is doubling perhaps every 20–25 years. The average audience for a learned publication, however, remains about the same, because of the parallel increase in readers, with the important consequence that the volume of rarely used material is expanding rapidly. Dr. Urquhart points out that the normal worker needs to know what publications exist on his subject quite irrespective of whether the library holds them, and he argues that sooner or later libraries must become more concerned with helping readers to discover what exists and less with describing what particular publications they hold.

When this happens, the demand for inter-library loans is likely to increase considerably. Dr. Urquhart suggests that at present few librarians are keen to advertise the inter-library lending system. He maintains that as the system developed under the National Central Library, the copyright libraries, which had most to contribute and the least to gain, held aloof. Nor have schemes of co-operative purchasing, while increasing the availability of British books, removed gaps in the national coverage. He rejects also the view of the Roberts Committee, in its report on the *Structure of the Public Library Service in England and Wales*, that most of the work of the National Central Library related to the needs of local libraries. In 1963–64 only 44 per cent of the requests received by the National Central Library came from public libraries, and a more recent survey has shown that, of publications supplied through the National Central Library, only 16 per cent came from public libraries.

Inter-library loans, according to Dr. Urquhart, are marginal to the public library service, providing only 0·1 per cent of its issues, but they are of major importance to libraries serving universities and research organizations, and provide university libraries with about 5 per cent of their issues. The National Lending Library for Science and Technology decided against a regional system, however, partly because the postal service was regarded as reasonably efficient and partly because statistical considerations indicated that it would be more efficient to centralize. A central library with five copies can provide a better loan service than five regional libraries each with one copy. The output from the National Lending Library for Science and Technology has doubled over the past 2 years and is at present about equal to the combined output of the National Central Library and the regional system.

Dr. Urquhart also questions the value of union catalogues and suggests that the question whether an adequate service could be provided if the union catalogues were more complete, or what degree of completion is desirable, requires examination. He also advocates some operational research on the regional bureaux of the National Central Library. While insufficient information for reliable decisions is at present available, he believes that no service based on union catalogues can be as rapid as one based on a central collection, as with the National Lending

Library for Science and Technology. Dr. Urquhart does not mention, however, that the latter has recently come under some criticism regarding the extent of its holdings.

Dr. Urquhart goes on to discuss the problem of creating a 'national lending library for the humanities' on the pattern of that established at Boston Spa for science and technology. The absence of a prototype such as the Science Museum Library makes the problem much more difficult than it was in the sciences, and Dr. Urquhart concludes that it is first necessary to develop a prototype for the humanities which should be accessible, so that the importance of accessibility to the shelves can be tested. Meanwhile, the existing resources should be reviewed to see what alternative action is possible.

He does not believe that a survey of library resources is required, but strongly urges the full participation of the copyright libraries in library loans. The British Museum should take the lead as the library with the major collection in the humanities, possibly developing at least a prototype for the National Lending Library for the Humanities. The new trustees, he suggests, should urgently review the possibility of such a national library becoming more actively concerned with the inter-library loan arrangements. Alternatively, he suggests that the prototype might be based on the London Library or the Senate House Library, or possibly on the National Central Library itself, if it were transferred north of Birmingham and made primarily a library.

What is done will clearly depend on how far and how fast the various organizations are prepared to move, but it will be noted that Dr. Urquhart contemplates that some of the university libraries will move towards providing an active loan service. Such a development is an argument for augmenting rather than for curtailing the services a university library can offer and the resources it can command. What is clear from Dr. Urquhart's address is that the university library should be seen both as an essential element in the effective functioning of any university institution and as an integral part of a national library service. From either point of view it is false economy to starve the university library of resources, whether in buildings, equipment, books, periodicals, new techniques, or staff.

Dr. Urquhart's choice of the term 'ecology' demonstrates the proper approach to the development of the university library as well as to library co-operation. We are concerned with a dynamic and not a static situation. Not the least of the dangers attending ill-timed 'economy' is that it prevents forward planning and may involve far greater expenditure in ineffective attempts to overtake arrears. If the order of priorities is wrong, waste and inefficiency are inescapable. Two further comments may be added. First, Dr. Urquhart is not unmindful that his proposals for concentration and for drawing in the copyright libraries might involve difficulties in the way of personal use of reference libraries. This risk should be minimized by the holding of several copies where possible, quite apart from greater use of photocopies. Moreover, as he points out, it is neither economic nor reasonable for individuals to travel long distances to reference libraries unless they have a number of items to consult, some of which would always be available.

The second point concerns the possibility of abuse of inter-library lending by some libraries seeking to borrow items which are in print and could much more cheaply be obtained that way. Dr. Urquhart rather discounts this possibility, but any system is open to abuse by those who are so minded. It should not, however, be difficult to discourage frivolous and unreasonable requests by instituting a suitable charge for each inter-library loan application form. Apart from thus increasing the incentive to buy rather than to borrow, the sum charged could be divided between the National Lending Library and the library making the loan, thus covering the loan expenses and helping the participating libraries to build up their stock of books. Given the vision and the determination which have so far been completely lacking on the Government side, Britain could easily have a library service and system in which the university libraries could play their fitting part and one entirely adequate to meet future expanding needs.

ORGANIZATION OF RESEARCH ESTABLISHMENTS

The Organization of Research Establishments

Edited by Sir John Cockcroft. Pp. 275 + 4 plates. (London: Cambridge University Press, 1965.) 63*s*. net; 11.50 dollars.

IN the final chapter of *The Organization of Research Establishments*, Sir Edward Bullard makes the significant point that as little as a half-century ago large-scale research establishments operating apart from universities were virtually non-existent. There were, indeed, Government organizations devoted to aspects of science and technology, such as the British National Physical Laboratory, the Physikalisch-Technische Reichsanstalt in Imperial Germany, and the Bureau of Standards in the United States. There were also differentiated research laboratories in a few leading industrial corporations such as the General Electric Company, the Du Pont Company, and the Bell Telephone Laboratories in the United States. But all these organizations for research and development were both comparatively small and relatively restricted in outlook. The broader tradition of scientific research was still so largely confined to university environments that many of the pioneers in the early institutes thought long and hard before they were willing to forsake those environments, from which a large share of them came.

How extraordinarily this picture has altered in recent years requires no emphasis. Even before the Second World War both industrial and Government research organizations in Great Britain had expanded greatly, in size, in numbers, and in the range and depth of their professional concerns. But it was only during and after that war that really large-scale establishments devoted primarily to research and development, formally divorced from universities and largely supported, directly or indirectly, from public funds, became familiar, at first primarily in connexion with weapons development, later in much more varied roles. Since then it has become apparent that this evolution reflected another and quite fundamental recent social change. The general public view of scientific research and development as functions of central importance both to the economic and cultural welfare of society, rather suddenly achieved, has made those activities proper—and indeed imperative—concerns of all the people, and therefore important and rapidly expanding responsibilities of Government. The implications of this shift for the organization and function of research establishments are many and profound.

Sir John Cockcroft, in his introduction to *The Organization of Research Establishments*, points out that Great Britain has been spending about £65 million a year on basic research, about £320 million a year on applied research and development, and about £245 million a year on defence, with its own requirements for research and development. In the United States increases of both total and public spending in these areas have been little less than explosive. In 1954 total expenditures for

research and development approximated 5,600 million dollars. Within a decade they increased to about 18,700 million dollars. In 1964, moreover, about 70 per cent of all the research and development performed in the nation was governmentally financed, and the Government indirectly supported about 60 per cent of the research and development undertaken by industry. Similar trends characterize other industrial nations. Sir John notes that the total volume of research and development in France and Germany comes to about half that in Great Britain, but both are expanding rapidly.

Thus organizing and administering large and complex organizations for the conduct of research and development, commonly with material support largely or entirely publicly provided, has become a skill of great contemporary importance to society. It has always been a complex and difficult art. But present circumstances have so augmented the extent and subtlety of the challenge as to create, in many aspects, virtually a novel field. Neither the task itself nor the modes of accomplishing it have ever been clearly defined. Indeed, both vary so enormously from situation to situation as to make it very doubtful that any general definition is possible. In such circumstances, a series of concrete illustrations drawn from specific cases can be far more instructive than any effort, however well conceived, to formulate abstract generalities.

It is just this quality of concreteness that makes the present work especially pertinent and valuable. The volume contains discussions of the problems of organizing and conducting research and development in eleven British establishments. They comprehend a remarkably broad range, in terms of substantive concern, of mission, and of structural size and complexity. Each is presented by an authority uniquely experienced in the organization of which he writes. Included are accounts of the National Physical Laboratory, by Sir Gordon Sutherland; the Royal Aircraft Establishment, by Dr. M. J. Lighthill; the Atomic Energy Research Establishment at Harwell, by Dr. F. A. Vick; the National Institute for Medical Research, by Sir Charles Harrington; the Medical Research Council Social Psychiatry Unit, by Sir Aubrey Lewis; the Agricultural Research Council Institute of Animal Physiology, Babraham, by Dr. J. H. Gaddum; the Empire Cotton Growing Corporation and the Organization of Research on Raw Cotton, by Sir Joseph Hutchinson; the Glaxo Organization, by Sir Harry Jephcott; Co-operative Research in the British Iron and Steel Industry, by Sir Charles Goodeve; British Railways Research and Development, by C. C. Inglish; and the Rutherford High Energy Research Laboratory, by Dr. T. G. Pickavance. There is also an account of the Bell Telephone Laboratories in the United States by Dr. J. B. Fisk, of particular interest not only in itself but for its comparative value, and one of "CERN: the European Organization for Nuclear Research", by Dr. J. B. Adams, representative of the new pattern of co-operative international effort. These thirteen essays are prefaced by the introduction by Sir John Cockcroft already mentioned, and concluded with a most interesting discussion of "What Makes Good Research Establishment?" by Sir Edward Bullard. The wide range of research and development organizations involved, their variety in size, in purpose and in substantive concerns, provides a rich inventory of comparison. Interesting contrasts abound.

Every reader will surely draw his own insights from these suggestive accounts. One element that repeatedly emerges is the continuing and critical significance of the structure and conduct of university-based research. From the beginning, the organization and the quality of university-based research have been of central importance in guiding the independent institutes and maintaining their standards. The universities have provided the orientation and the training that have qualified for his post almost every senior member of the independent research establishments, including their administrative staffs. Obviously it is of the utmost importance that the integrity and quality of university training and research be sustained in the future. Such considerations bear significantly on the issue, at present prominent both in Great Britain and the United States, as to whether it is desirable for independent research institutes to be empowered to establish training courses within their own establishments, conducted entirely independently of conventional university channels, but authorized to certify students for advanced research degrees. The question is touched on in the present volume.

The essays repeatedly emphasize by example the degree to which energy and talent devoted to administrative matters must increase relative to that dedicated wholly to substantive concerns as the size and the structural complexity of an organization devoted to research and development increase. The inherent necessity of this shift, emphasized especially in the concluding chapter, is evident. Yet there is also the ever-present countervailing danger that such an evolution, if one-sided, may jeopardize the whole tone of the effort. So the question, "Is size, *per se*, a good thing?" presents a special challenge. Some possible answers are reviewed, particularly in the chapter on the Royal Aircraft Establishment. Some of the ways in which the organizational requirements posed by size and by activities primarily directed to development, with accompanying structural articulation, and the requirements of flexibility and freedom so essential to groups concerned primarily with research can be mediated to draw maximum benefit from their interaction are particularly well illustrated in the discussion of the Bell Telephone Laboratories.

The special opportunities available for the type of research organization designed to serve a number of industrial 'clients', and some of the problems that accompany them, are well illustrated in the description of the work of co-operative research in the British Iron and Steel Industry. Similar opportunities and challenges, set in a rather different context, appear in the account of the structure and mission of the Atomic Energy Research Establishment at Harwell. The special problems and special opportunities of the research and development 'condominia' at the international level are vividly illustrated in the history and description of CERN.

A matter of unusual interest and significance, repeatedly suggested, touches the relation between age and stability in a research and development organization, on one hand, and its freshness and creative potential, on the other. The question has special contemporary relevance. For, as Sir Edward Bullard remarks, it is becoming clear that the doubling of scientific activity every 5 or 10 years to which we have become accustomed and adjusted in the immediate past can scarcely persist much longer. Deceleration may well be with us soon, and the problem of how to maintain the freshness and the creative productivity of establishments which are both becoming older and are ceasing to grow at their former rates could become very significant. Efforts to solve it could evolve more general and discerning understanding than we have at present of the deeper and more subtle elements that preserve health and promote success in the research environment.

In sum, *The Organization of Research Establishments* is unusually suggestive and valuable, and its appearance is remarkably timely. Coming when expansion and change in organization for research and development are still occurring at almost record speeds, but also at a time when those rates could begin to level sharply, the work is especially significant as a primary source.

If any minor regret is to be registered, it is that—no doubt because of unavoidable exigencies of preparation and composition—most of the accounts were apparently finished approximately four years before the appearance of the volume. Some significant changes have occurred in that time. The Department of Scientific and Industrial

Research, for example, has disappeared since the main accounts of the National Physical Laboratory and of Co-operative Research in the British Iron and Steel Industry were written. The Ministry of Science has been transformed. The account of the activities of CERN extends only to 1961. But these are minor matters indeed against the background of the scope and penetration, the quality and the potential usefulness of this book for all those concerned with the organization, the administration, and the structure of establishments devoted to research and development. CARYL P. HASKINS

TRAINING THE TECHNICIAN

Technicians Today and Tomorrow

By Dr. J. T. Young. Pp. xvii+222. (London: Sir Isaac Pitman and Sons, Ltd., 1965.) 30*s*. net.

THE timeliness of Dr. Young's book, which Sir Willis Jackson emphasizes in his foreword, is indicated not only by the establishment of the new training boards under the Industrial Training Act but also by the announcement by the Committee on Manpower Resources for Science and Technology that the triennial survey of manpower will in future cover technical supporting staff, though we may well question that Committee's view that we must await that survey before ascertaining how far the present position for educating and training is sufficient. The Carr Report and other studies of industrial training for skill have tended to concentrate on the apprenticeship system and its shortcomings, but in *Technicians Today and Tomorrow* Dr. Young does something to redress the balance. The book should do much to promote a clear understanding of the function of the technician and a wider recognition of the growing importance of the wide range of such workers. In doing so, it could also assist to alleviate another anxiety of the Committee on Manpower Resources, for such a public understanding is a powerful factor in establishing the greater esteem which could help to attract more young men and women into such careers.

Dr. Young, moreover, is fundamentally pragmatic and his clear analysis of the needs of the technician in education and training indicates the lines on which immediate steps could be taken to fill a serious gap in our provision for technical education. He provides an admirable guide, in fact, to the whole situation, for a chapter on defining the technician, followed by one giving a clear but concise historical review of the developing relations in Britain between education and industrial training, is succeeded by two in which the 1961 White Paper on "Technical Education" and the White Paper of 1962 on "Industrial Training and the Subsequent Industrial Training Act" are summarized and appraised. On this basis, he rests his detailed proposals for meeting the needs of technicians which occupy the next three chapters, and finally he considers the future position of technicians and their needs.

Dr. Young's approach is thus balanced and practical and his comments are no less sane and constructive. He faces the difficulty of defining a technician, recognizing that both the functional (or occupational) approach and the educational approach have their disadvantages. For the purposes of his book, he defines a technician as one "expert in applying specific proven techniques associated with science and technology; in particular, one who has undergone a systematic course of instruction related to those techniques". He attributes the shortage of technicians in Britain, in part at least, to the general academic nature of secondary school curricula and to prevailing attitudes towards technical education as a whole—a point which should not escape the notice of Prof. Dainton's present enquiry or of the Schools Council. He is forthright on the inadequacy of part-time education and its failure to produce results which justify its existence, and much more than the present nominal support for day release is, in his view, required before sandwich courses become extensively established in this field.

Some trenchant criticism of the British apprenticeship system is less relevant to his main theme, in which insistence on the provision of adequate finance and on the anachronism of the division between education and training recurs repeatedly. Better opportunities in technical education, whether for technicians or for others, cannot be provided on the cheap. Likewise, Dr. Young insists on the necessity of willing co-operation from both sides of industry, and neither the employers nor the trades unions escape criticism. Speedy implementation of the Industrial Training Act, coupled with a massive campaign to illumine its objectives and the urgency of the situation it has been designed to rectify, are imperative. Furthermore, when it comes to practical details, Dr. Young demonstrates that the requirements of technicians are both specific and widely different, so that they must be met by programmes adapted to those needs as well as to the nature of the educational system and the arrangements for industrial training. Here he is challenging as well as constructive, and on the transition from school to work he writes with insight and sensitivity to the needs of the adolescent. Throughout, his book is admirably designed to restore the perspective of the training of technicians which was to some extent distorted by the Robbins Report and to establish the technicians in their rightful place in industry and society, with all that that implies in terms of educational opportunity, status and financial resource. Sir Willis Jackson rightly directs attention to this matter of public esteem, and Dr. Young's book is well designed to apply some corrective to any overshadowing of the technical institutions by institutions at university level. It is a point that those over-anxious to promote equality of opportunity commonly overlook, but unless the difference is appreciated and due allowance is made for it the nation will not reap the full advantage of the services of an increasing number of scientists and technologists because they lack the support of a sufficient number of technicians. Not the least merit of the book is an admirable bibliography, while notes are appended on the emergence of the industrial technician, on the place of the National Certificate, and on the financing of technical education. R. BRIGHTMAN

COMPUTERS IN PROCESS CONTROL

Digital Computer Applications to Process Control

Edited by William E. Miller. (Proceedings of the First International Conference held September 21–23, 1964 Stockholm, Sweden, sponsored by the International Federation for Automatic Control and the International Federation for Information Processing.) Pp. xxi+595 (Pittsburgh, Pa.: Instrument Society of America, 1965 Distributed by Plenum Press, New York.) 17.50 dollars

THE first application of a digital computer to the on line control of an industrial process was made in the United States in 1958. It aroused very great interest and was followed by a wave of further applications and of activity by computer manufacturers. Generally speaking, the users were secretive about the results obtained and, at the end of 1964, it was possible to hold either an optimistic or a pessimistic view of benefits and future prospects of this development.

In September 1964 a conference was held under the joint sponsorship of the International Federation for Information Processing and the International Federation for Automatic Control to discuss the experience with these installations. A considerable body of information was released by the users, much of it for the first time, and the record of the conference, therefore, stands as an important landmark. From the published papers a better and mo

balanced judgment can be made than was possible from the previously published literature.

The picture which emerges is a mixed one. There have been the expected difficulties with hardware for measurement and actuation, and reliability was a problem in the earlier machines. The programming and engineering effort has often turned out to be greater and more expensive than was anticipated. Nevertheless, many of the users have decided that computer control already offers benefits which cannot be obtained in any other way. The financial benefits have often been good and the cost of an installation may be recovered in 18 months.

To some extent, however, the favourable economics have been due to an element of subsidy by the computer manufacturers, some of whom have recently been driven out of the field. There also remain unsolved problems in the theory of control—at present our capacity to exploit the great potential power of computers, for control is limited not only by our ignorance about the behaviour of processes, but also by the lack of effective design methods for complex control systems. One may guess that the conference marked the end of an early phase of wide-ranging experiment and the beginning of a longer, slower, but in the end more solid development.

The papers are divided into four groups, dealing respectively with chemicals and oils, iron and steel, electric power, and various other applications. In the first group are papers by Monsanto (dealing with a multi-computer system, mainly supervisory, on a large site), Imperial Chemical Industries (dealing with direct digital control), B.A.S.F. (direct digital control and optimization) and Shell (basic philosophy). The second group includes papers from the Steel Company of Wales (hot strip mill), Samuel Fox (cut-up line) and the U.S.S.R. (general survey). Power companies are represented by papers from England, France and Italy. In the fourth group are papers on a gas pipe-line network, on paper, cement, railway operation, and transport at an iron-ore mine. In addition to those mentioned, there are papers by a number of computer manufacturers on applications with which they have been concerned. H. H. ROSENBROCK

ARCH DAM STRESS ANALYSIS

Theory of Arch Dams

Edited by J. R. Rydzewski. (Papers presented at the International Symposium held at Southampton University, April 1964.) Pp. x+786. (London and New York: Pergamon Press, Ltd., 1965.) 252*s*. net.

THEORY of Arch Dams, edited by J. Rydzewski, appears on the spine of this book, the implication being that it is a text-book with a unified approach to the whole subject. In fact, the title represents the proceedings of the 1964 symposium at Southampton, which was mainly confined to elastic stress analysis of arch dams under hydrostatic loading. The twelve guineas charged by Pergamon Press seems high when compared with the sixty shillings charged for the proceedings of the conference on "Wind Effects on Building Structures" held at the National Physical Laboratory.

The eighth international congress on "Large Dams", held at Edinburgh in May 1964, brought many eminent people to the United Kingdom; advantage was taken of this to arrange the symposium between them and members of the International Association of Shell Structures, so that the usefulness of shell theory for arch dam analysis could be considered. The resulting papers present a valuable record of present-day research into the orthodox method of analysis and into attempts to apply finite difference, finite element and shell theories to arch dams. The proceedings of the symposium, therefore, do much to acquaint the dam engineer with new methods which might eventually prove useful in dam design.

Dam design is a difficult and fascinating branch of civil engineering; it requires men of wide vision, not only capable of assessing the impact of a dam on the economy of a nation, the hydrological aspects and the geophysical problems, but also possessing the technological skill to choose and design the right kind of dam (earth, rockfill, gravity or arch dam, etc.). Many factors used in the elastic analysis of dams can only be assessed in broad terms, but they can, nevertheless, have effects of over-riding importance on the results. Elastic analysis is thus only one step along the path followed by the engineer in designing a dam. A complete and elegant elastic analysis of arch dam structural behaviour does not yet exist, and it should prove of considerable help when found.

The most widely accepted method of arch dam design is the 'Trial Load Method', which the U.S. Bureau of Reclamation has developed. In essence, the dam is divided into notional arches and cantilevers, and equilibrium is satisfied while compatibility is enforced at the intersections of the arches and cantilevers only. A valuable set of papers by Copen, Beaujoint and others record recent developments in this method. There is the suspicion that sometimes the true modes of structural behaviour may be obscured by enforcing cantilever and arch action.

Probably the best way to improve the elastic analysis of arch dams is to devise techniques for solving the three-dimensional equations of elasticity and to take advantage of the ever-increasing facilities offered by digital computers. The difficulty of this is shown by Allen and Windle's solution for the simplest case of a symmetrical circular arch dam of constant thickness in a symmetrical rigid valley. They used finite difference methods with higher order corrections leading to some 450 equations, which have been solved after what must have been considerable effort. My experience is that 450 equations are not enough to represent the three-dimensional stress field with adequate accuracy, and that 2,000–5,000 equations are needed, which in turn requires special computer techniques. Otter's paper on dynamic relaxation describes one such computer technique.

A very powerful group of papers by Parme, Herzog, Tottenham and others apply the shell theories with elegance. Modes of structural behaviour other than arch/cantilever action are permitted; however, the shell theories are approximate and impose their own limitations; for example, the Kantorovitch theory does not allow any upstream deflexion. A more serious aspect, though, is the limitation of the validity of the theories to the thin zones of arch dams.

Finite element methods have been used most successfully in aircraft design; papers by Clough and Zinkiewicz give lucid accounts of the first attempts to apply the methods to arch dams.

Rocha has contributed a most valuable review paper, and the problems of rock mechanics and concrete properties are introduced by Stagg and Newman respectively. This group of papers is a timely reminder of other gaps in our knowledge, such as the need for a good failure criteria for concrete under tri-axial states of stress including uplift pressures. A. C. CASSELL

ASPECTS OF OXYGEN THERAPY

Hyperbaric Oxygenation

Proceedings of the Second International Congress, Glasgow, September 1964. Edited by I. McA. Ledingham. Pp. xi+472. (Edinburgh and London: E. and S. Livingstone, Ltd., 1965.) 70*s*.

THE second international congress on "Hyperbaric Oxygenation" was held in Glasgow during September 1964; it was very appropriately dedicated to Sir Charles Illingworth.

The programme was divided into nine parts and subjects discussed ranged from a consideration of the dangers of hyperbaric environments in general, to the use of hyperbaric oxygen in the treatment of respiratory and cerebrovascular diseases, myocardial infarction, infections and shock.

The effects of hyperbaric oxygenation on man and experimental animals as well as on aerobic and anaerobic micro-organisms were considered, from both the physiological and clinical points of view. Such fundamental problems as the design of small transportable pressure chambers and face masks were also discussed. The communications related primarily to applied aspects of the subject, with reference to human disease processes, but the response of animals with similar but experimentally induced lesions was also considered. *Hyperbaric Oxygenation* provides interesting and useful information on problems associated with working under compressed air and on methods for assessing blood-flow through particular organs or regions of the body.

The papers of Drs. Walder and Davidson on the hazards of compressed-air workers, and more particularly of 'sand-hogs' (that is, men digging under-water tunnels. where compressed air has to be used in order to keep out the water), clearly demonstrate the risks involved and the importance of expert supervision, as well as the difficulties in detecting symptomless defects of the respiratory or osseous systems during the selection of supposedly normal personnel and in the assessment of damage due to gas pressure. The adverse effects of atmospheric pollution, smoking and respiratory infections on the response of emphysema patients to hyperbaric oxygen therapy were demonstrated by Dr. Yanda, and clearly emphasize the importance of obtaining gases free from contaminants, or with only minimal contamination, for compressed-air chambers and for use in hyperbaric oxygen therapy or experiments.

In the section devoted to particular biological systems or organs, the effects of hyperbaric oxygen in experimental animals were discussed, together with reports on the use of oxygen therapy in the treatment of natural human disease processes.

The results of a clinical trial of oxygen at pressures of 1 and 2 atm. absolute in the treatment of acute myocardial infarction were presented by the Glasgow workers. No statistically significant improvement in mortality figures, as compared with those obtained by conventional therapy, was observed during the first six weeks after infarction. Furthermore, there was no significant difference in response of patients treated at the two different gas pressures. The findings of other workers in different conditions were, however, more encouraging, and Dr. Foster reported a definite improvement in results, as assessed by the sterilization of primary tumour, when radiotherapy was combined with hyperbaric oxygen at 3 and 4 atm. absolute. Good results were also reported by Prof. Hutchison and Dr. Kerr, and by Drs. Van Gool and De Jong, following the treatment of asphyxia neonatorum and retinal vascular insufficiency by hyperbaric oxygen. Final conclusions with regard to the efficacy of this procedure in the treatment of a variety of disease processes must await the results of more extensive clinical trials as well as reports on the relative merits of different programmes and techniques for oxygen administration.

Few toxic effects were reported during or after administration of oxygen at a pressure of 1–3 atm. absolute for periods of 30–60 min. For therapeutic purposes, the exposure was usually repeated on a number of occasions and after different time intervals. Using a pressure of 3–4 atm. absolute, oxygen convulsions were observed by Foster in less than 1 per cent of exposures and no permanent damage was observed.

An instructive account of the use of ^{85}Kr for the measurement of cerebral blood flow was presented by Dr. Lassen, and this provides a useful review of a technique which is at present finding wide application in the field of circulatory physiology and pathology.

The reports on the effects of oxygen at a pressure of 2 atm. absolute on bacteria are in accord with a number of previous accounts in so far as they demonstrate the ability of oxygen to inhibit the growth in a variety of anaerobes, aerobes and fungi, without destruction of the organisms. Dr. Schreiner's account of the value of the enhanced suppression of growth of *Staphylococcus aureus* in cultures subjected to both penicillin and hyperbaric oxygen also deserves to be noted, since it raises the possibility of the combined use of antibiotics and hyperbaric oxygen in the treatment of infections due to a variety of different organisms as well as in those due to anaerobes.

The editor and publishers are to be congratulated on the clear presentation and arrangement of the individual contributions, which together serve as a very useful guide to the present status and possibilities of oxygen therapy in medical practice. A particularly valuable feature of the book is the inclusion of discussions relevant to particular communications, so that many different and often divergent views are presented on this important technique.

R. E. Coupland

BRAIN OF AN APE

A Stereotaxic Atlas of the Chimpanzee Brain (Pan satyrus)

By M. R. DeLucchi, B. J. Dennis and W. R. Adey. Pp. 155. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1965.) 15 dollars; 120*s*. net.

RELATIVELY few biologists can expect to count chimpanzees among their experimental animals; price and scarcity are but two of the factors against this. On the other hand, this animal, with its relatively large brain resembling, anatomically at any rate, that of the human being to a remarkable degree, clearly offers advantages for experimental neurological studies possessed by few other living creatures.

A Stereotaxic Atlas of the Chimpanzee Brain, produced by M. R. DeLucchi and his colleagues, is based on the brains of eight chimpanzees, four of either sex, aged from about one to five years. Inevitably, the dimensions of the skulls and brains of such a group of animals may vary appreciably, and the series is clearly too small to derive precise relationships between the two which would be readily applicable to any specimen. The authors, however, show a maximum variation in length and breadth of the brains of approximately 5 per cent from the mean, that is about 5 mm, and suggest that this is small enough to give essentially the same probability of encountering a given structure stereotaxically as in the brains of smaller primates, available in larger numbers for the production of such an atlas. Variability of this kind must in any event be accepted as a necessary shortcoming of data derived from limited amounts of material.

This *Atlas* consists essentially of sixty-nine enlarged line drawings taken from sections through the brain each accompanied by a photograph of a myelin-stained and a cell-stained section at the same level. Horsley Clarke reference planes are given both for drawings and photographs. The first thirty-four drawings depict sections cut in a frontal plane, extending from the rostral part of the basal ganglia caudally to a plane passing through the medulla at the level of the inferior olivary nucleus. These figures show in labelled detail only subcortical structures, but a small outline of the whole section is also given. The second half of the volume consists of thirty-five drawings taken from sagittal sections extending laterally from the midline to a plane passing

through the claustrum. In this series the whole brain is included in the figures and again Horsley-Clarke reference planes are given.

The general production of the volume is good. The main features of the photographs are labelled, while the line drawings carry a more detailed indication of the various structures. Quite apart from the primary object of the book, the reproductions of the myelin-stained sections make it a valuable *Atlas* for comparative neurologists. It would have been even more valuable had the photographs of the cell-stained preparations been of a higher standard, but the reproductions of the frontal series are too small, and in both series contrast is poor. With this reservation, however, the volume is a useful contribution to the field of comparative and experimental neurology.

R. L. HOLMES

TRIBAL SOCIETY AS SEEN FROM MANCHESTER

Politics, Law and Ritual in Tribal Society

By Prof. Max Gluckman. Pp. xxxii + 339 + 24 plates. (Oxford: Basil Blackwell, 1965.) 47*s*. 6*d*. net.

TRIBAL society in Prof. Gluckman's new book is by and large confined to tribal Africa. This in fact constitutes a source of strength. Prof. Gluckman's chief theoretical contributions concern African societies—and this enables him to evaluate authoritatively and advocate enthusiastically the contributions of his Africanist colleagues.

Politics, Law and Ritual in Tribal Society begins with a review of the mistakes and achievements of some of the anthropological ancestors and then develops the theoretical perspective of contemporary social anthropology. Next follows a chapter on the nature of property rights and economic activity in tribal societies. This provides a background picture for the later subject-matter of the book.

Chapters 3 and 4 are concerned with the analysis of political relations, a field in which Prof. Gluckman has made some of his best-known contributions. He elucidates the point that many societies portray conflict in their organizing principles, that in fact conflict is often an attribute of society itself. In regard to societies which have no governmental structures he explains how conflicts are contained by a network of social ties, "so that men who are opposed to each other under one rule, are allied with each other under another rule".

The next problem taken up concerns order in societies with formal authority systems, especially those which constitute states. Some years ago he propounded the thesis of the 'rebellious cycle' and 'repetitive equilibrium', the main idea being that South African polities were held together because their territorial segments fought for a common prize, the kingship, and that rebellions produced no changes in political form. Prof. Gluckman has since advocated this thesis with decreasing conviction and increasing delimitation of the societies and time periods to which it may be applicable. In this book he describes how many or most states have frequently fragmented for many reasons, and puts forward an idea which he describes as 'akin' to that of Edmund Leach's (concerning Upper Burma) that regions of Africa have oscillated between large states and numerous small states. It may well be accepted in the future that concepts like repetitive equilibrium are rarely adequate abstractions of the actual dynamic facts.

The discussion of "Dispute and Settlement" (Chapter 5) is admittedly polemical. Prof. Gluckman demonstrates with admirable forensic skill that the forms in which disputes are expressed and the varied procedures adopted for resolving them make sociological sense only when they are related to the ranges of social relationships within which they operate. He introduces many concepts to distinguish between various kinds of dispute procedures and roles; perhaps not everyone, however, will share his enthusiasm that "specializing of the use of words out of the riches of English" will clarify our theoretical problems.

Prof. Gluckman's treatment of "Mystical Disturbance and Ritual Adjustment" (Chapter 6) is a disappointment. Following the Neo-Tylorian intellectualists, he distinguishes between 'mystical beliefs' and 'empirical beliefs', a distinction which the Western observer cannot easily make, let alone the primitive. The disappointment is not that he has made such a distinction, but that, having defined ritual in relation to mystical beliefs, he interprets it entirely in terms of its social effects. This reductionism has no bearing on the initial definition and distinctions made between ritual and ceremonial.

Furthermore, in the space of one chapter, he advances several disconnected views of ritual. The mystical definition of ritual has little bearing on the submission that ritual segregates roles and changes of roles and activities in small-scale societies with multiplex relations. Another avenue is explored when, following Victor Turner, he postulates the conditions under which judicial as opposed to ritual techniques for resolving social conflict may occur. Thus certain rituals are said to cloak fundamental disharmonies which arise out of conflicts of social principles. Turner made sense of Ndembu rituals of affliction in these terms; in trying to assimilate Turner's views to his own, Prof. Gluckman fails to achieve a marriage of ideas, for his own theory of 'rituals of rebellion' is of a different kind and tailored to fit different ethnographic facts. Finally, despite his own claims, Prof. Gluckman has not taken us any further in understanding why, in tribal societies, misfortunes are associated with the moral relations of the group concerned, why rituals which 'state conflict' indeed 'bless the moral order and its related natural order'.

A major theme that runs through the book is the developmental morphology of economic and political systems. This is dangerous ground to tread on and Prof. Gluckman once again demonstrates this. He works with an evolutionary scheme (the notion of two social types and transition from one to the other) which harks back to Maine, Durkheim, Toennies and others. Anthropologists have found such grand schemes of change descriptively true in a general sense but in practice too diffuse to explain the particular problems that engaged their attention. Prof. Gluckman's hypotheses show the same limitations. He explains the wide extension of kinship in tribal society as due to their undifferentiated economy, and its shrinkage to the differentiation in developed industrial society. But how does this formula help to account for the wide variations in kinship systems in tribal societies? How does the notion of tribal economy illuminate the widespread systems of ceremonial and economic exchange in, say, Melanesia which differ markedly from the African situation? Another hypothesis which stems from his morphological series is that rituals of rebellion (or 'role revealing rituals') associated with a particular kind of political and economic situation will be replaced by temple cults in more developed states where the existing structure of society is not accepted as hallowed. This is untenable, as a reference to temple cults in India and Ceylon will show, and is based on the misapprehension that temple cults lack "ritualisation of secular roles".

Prof. Gluckman has presented his book to the general reader as a statement "of how one social anthropologist, working in the full tradition of the subject, sees the general problem of rule and disorder in social life". Other anthropologists who also feel themselves to be in this full tradition may see certain questions differently, for this tradition is fuller and less consistent than he indicates.

S. J. TAMBIAH

Science and the Politician

By Marcus Goodall. Pp. viii+83. (Cambridge, Mass.: Schenkman Publishing Company, Inc., 1965.) 2.65 dollars.

CONCERN as to the extent of the possibility of planning human activities is shown both by those who welcome the increase of regulation and those who deplore it. Neither party is prepared to consider that the dispute should be subject to investigation; that we should study the extent to which planning is likely to be effective, given the means available at any time.

This is only one practical example of the type of study that Dr. Goodall calls "third science". It is characterized by attention to aims and yet by recognition of the problems that are involved in defining aims. In such respects it transcends the 'second science' of Newton, Maxwell and Einstein, which required drastic uniformizing assumptions, such as those that limit the applicability of the Second Law of Thermodynamics.

Goodall traces the beginning of the modern period back first to Darwin, then to Pierce, with his emphasis on the relationship of observer, symbol and object, and from here to Cantor, Gödel and Tarski. The connexions are only sketched out, but the effect is to provide a series of hints as to the logical problems underlying modern science and especially biology.

The book is especially concerned to point out the inadequate use that is made of modern scientific and logical possibilities by those concerned to regulate the conduct of modern affairs (and particularly 'politicians', the author's favourite aunt-sally). In this, it well represents the frustration that many feel, who see so little use made of such powerful methods. The special value of methods such as those of specification theory, games theory and the like is that it should be possible with them to suggest means to further their wider adoption for human ends. It is to be hoped that Dr. Goodall will follow this stimulating essay with more detailed proposals in which such methods are used to assess the best strategy for applying them.

J. Z. Young

Elementary General Topology

By T. O. Moore. Pp. xi+174. (Englewood Cliffs, New Jersey, and London: Prentice-Hall International, 1964.) 48*s*.

THIS very readable book provides an introductory account of general topology (but not algebraic topology) for the beginner. There is some doubt as to when topology should first appear in the training of a mathematician; but the author of this book, after several years' experience in teaching topology to undergraduate and post-graduate students, came to the conclusion that an introductory course might best follow a prior course in abstract algebra, and precede the study of advanced calculus and real analysis. Thereby, it is claimed, "maximum results follow minimum effort". So the present book has been written primarily as a text for undergraduates.

The first chapter is concerned with elementary set theory. Then follow chapters on topological spaces, mappings of such spaces, and compactness. After that, there is a general development of the properties of product spaces, metric spaces and function spaces. An important chapter, on nets and convergence, is concerned mainly with the generalization of the idea of convergence of a sequence to a point in a space. The book closes with a chapter on Peano spaces (continuous curves).

In a book of this kind there are necessarily many definitions. These are clearly stated, often illustrated, and the theorems are separated by graded exercises. At the end of each chapter there is a list of miscellaneous exercises, and, all in all, the book should appeal to students looking for a text supplementary to a course of lectures on the subject.

L. S. Goddard

Supplemental Tables of Molecular Orbital Calculations

By Prof. A. Streitwieser, Jr., and Prof. J. I. Brauman. With a Dictionary of π-Electron Calculations by Prof. C. A. Coulson and Prof. A. Streitwieser, Jr. Vol. 1: Pp. xxiv+1–860. Vol. 2: Pp. 861–1223+xxx+358. (London and New York: Pergamon Press, Ltd., 1965.) £33 net per set.

HÜCKEL calculations are now a matter of pure routine, but this does not detract from their value as a guide to the physical and chemical properties of conjugated molecules; indeed, in spite of heavy competition the Hückel theory remains the best wave-mechanical model of molecules that we have.

Supplemental Tables of Molecular Orbital Calculations presents the results of Hückel molecular orbital calculations on almost 500 hydrocarbons. The calculations have been performed on a digital computer and the output of the computer has been photographed so that the published papers are free from any errors of transcription. This makes the tables particularly reliable for use by people who do not have the necessary skill to programme molecular orbital calculations themselves. The printed results include the energies and coefficients of all the molecular orbitals of the molecules considered, their total π energies, electron densities and bond orders, and the various types of mutual polarizability. The introduction explains the tables and shows how they may be used. Volume 2 includes the "Dictionary of π-Electron Calculations" which is used also as a separate volume.

The compilation of this considerable body of data is likely to be most useful to the chemist who does not possess access to a computer; the price of the volumes, though relatively high, is negligible compared with the cost of the computing time and expertise which would be needed to carry out a calculation on a mere handful of the molecules treated.

H. C. Longuet-Higgins

Freshwater Microscopy

By W. J. Garnett. Second, revised edition. Pp. xv+376+54 plates. (London: Constable and Co., Ltd., 1965.) 42*s*. net.

THIS edition of *Freshwater Microscopy* is a great improvement on the original. The inclusion of more information, twenty-six excellent new figures and four new plates, and the bringing up to date of techniques and bibliography, all contribute to this. Apart from the slight expansion of certain paragraphs and the introduction of common species omitted from the first edition, eight of the fifteen original chapters remain substantially unaltered. Of the other seven, the chapter on microscopic equipment has been rearranged in parts to embrace modern methods and equipment, and the chapters on bacteria and fungi, the higher plants and molluscs have been rewritten and enlarged. Leeches have been gratifyingly included in the chapter on worms. The arthropods are now allocated three chapters instead of two, due mainly to the introduction of many more insect species.

While scientific inaccuracies are rare, a few do occur. For example, a single cocoon occurs in *Microdalyellia* and not *Dalyellia*; there are fourteen, not thirteen British species of leeches; leeches can have up to ten eye-spots—not up to eight; *Planorbis spiralis* should be *P. spirorbis*; and *Holopedius gibbeus* should be *Holopedium gibberum*. Though the bibliography has been extended and brought up to date, there are still obvious omissions such as "A Guide to Freshwater Invertebrate Animals" by T. T. Macan, and two recent publications on mayflies and oligochaetes in the Freshwater Biological Association series. The exclusion of the publisher and on occasion the date of publication is still rather irksome.

These are, however, but minor criticisms of an enhanced edition which admirably fulfils its purpose to "set the beginner . . . on the right path".

J. O. Young

THE DISTINCTIVENESS OF *Homo habilis*

ROBINSON[1] has questioned the validity of the new species, *Homo habilis*, proposed by Leakey, Tobias and Napier[2]. In particular, he has adduced evidence purporting to invalidate the morphological criteria which, it has been claimed, differentiate *H. habilis* from *Australopithecus*. Since Robinson's paper, at first reading, may give the impression that our claim for the existence of differences has been effectively demolished, it is necessary to look into the claimed differences more deeply.

The Dental Traits

In our diagnosis of the features of *H. habilis*, we stated that among the dental traits is "a marked tendency toward buccolingual narrowing and mesiodistal elongation of all the teeth, which is especially evident in the lower premolars . . . and in the lower molars"[2]. We maintained —and still maintain—that the lower premolars are absolutely narrower than those of *Australopithecus*

Robinson[1] regards the shape characteristics of the *H. habilis* teeth as not recognizably different from those of the australopithecines. The data on which he bases his conclusion are open to two criticisms. First, Table 1 in his paper, which shows the length/breadth indices of some of the cheek-teeth, inexplicably excludes the fourth premolar (P_4) although we had been at pains to point out that the premolars exhibit the greatest departures from the australopithecine teeth.

Secondly, the figures quoted by Robinson[1] for P_3 of *A. robustus* (*Paranthropus*) differ substantially from those quoted in his dental monograph of 1956 (ref. 3). In the earlier work, he lists the *M–D* length and *B–L* breadth of 12 Swartkrans "first premolars"; these include one tooth—*SK* 96—the measurements of which are marked with an asterisk. He then states in the text "the results of *SK* 96 have not been used in calculations because of incomplete development of the crown" (*op. cit.*, p. 69). I have therefore calculated the length/breadth indices of the other eleven P_3's and find they range from 76·0 to 92·8, with a mean of 83·25. The figures quoted by Robinson in his 1965 table for *Paranthropus*, however, present a range more than twice as great, that is, from 76·0 to 112·3. Robinson has apparently included in his latest range the incompletely developed tooth *SK* 96. For, as he states[1], the sample size for this tooth now is 12, not 11. Moreover, he explains his inclusion of the tooth *SK* 96 as follows: "The highest value listed for *Paranthropus* belongs to a tooth which had not yet begun to erupt and the final dimensions would possibly have been slightly higher than at present, although the length dimension already is very close to the upper limit for the sample of 12 specimens".

The length of *SK* 96 as recorded by Robinson[3], that is, 10·1, is indeed near the top of the range for the other eleven P_3's from Swartkrans, that is, 9·2–10·5. However, its breadth is only 9·0, which is well below the smallest value in the range of the other eleven teeth, namely, 10·6–12·9. In his earlier investigation (p. 70) he pointed out: "In *SK* 96 (incompletely developed crown) the lingual face is not strongly convex and a distal lingual groove is present. Neither of these features occurs in any of the fully developed and erupted teeth. Furthermore this tooth is appreciably narrower than any of the others. Presumably all these features are a result of incomplete development".

Elsewhere (p. 78) he calls this tooth "an incompletely developed germ". Recently, therefore, I have re-examined the original specimen in the Transvaal Museum, Pretoria, through the courtesy of Dr. V. FitzSimons and Dr. C. K. Brain. The tooth is clearly immature and its measurements cannot validly be included. At the time of death, the tooth was completely enclosed in its crypt, the most superficial part of the lamina dura being as much as 9·5 mm below the alveolar margin. The roots of the overlying dm_1 are in position and there is no evidence that resorption has begun; the whole of the distal root is visible and it is intact down to its exposed apex. When the P_3 is replaced in its correct position (indicated by part of the lamina dura and lateral mandibular wall adherent to it), there is a moderate space between the occlusal surface and lamina dura for the enamel organ, and a large space below the crown for the tooth-germ or pulp. Clearly, this tooth was far from erupting. The crown enamel is pale and highly translucent, unlike that of mature teeth from the same site. Obviously, much more enamel would have been added before the tooth had erupted. A further relevant observation was made: the crown of the tooth has been fractured and repaired; the alignment of the two moieties is not perfect, thus fractionally increasing the apparent length.

The measurements made on this developing tooth and the derived *L/B* index are thus not eligible for inclusion in the range of measures of mature teeth available for comparison. Nor is Robinson's assumption justifiable that "it is highly improbable that any slight increase in the crown dimensions [of *SK* 96] that may have occurred before eruption would alter the shape so disproportionately as to change the shape index significantly". The indices of twenty-three fully formed and erupted australopithecine P_3's from six sites range from 68·1 to 92·8; the *SK* 96 value of 112·3 suggests most strongly that if this tooth is a normal member of the same group, further development would yield a greater breadth and consequently a lower length/breadth index.

It is largely through including this immature P_3, *SK* 96, and excluding the P_4 that Robinson has provided the semblance of refuting our statements about the differences between *H. habilis* teeth and those of the australopithecines. When these errors of omission and commission are rectified, our statements remain valid.

Habiline Dental Evidence and Comparative Data

To demonstrate further the relationship between the absolute and relative dimensions of the *H. habilis* teeth and those of *Australopithecus*, I have compiled all available maxillary and mandibular dental measurements and indices on the specimens from Sterkfontein, Makapansgat, Taung, Swartkrans, Kromdraai, Garusi, Olduvai and Peninj. Many of these measurements are culled directly from Robinson's outstanding monograph[3]; others have been taken from Dart[4]; while I have personally measured a number of additional teeth from Sterkfontein, Kromdraai and Makapansgat, and all the available australopithecine teeth from Olduvai and Peninj. Data have been compiled for 23 australopithecine P_3, 22 P_4, 33 M_1, 26 M_2 and 28 M_3. Of the front teeth, there are data for 10 I_1, 8 I_2 and 20 C. Corresponding figures for maxillary teeth are 12 I^1, 15 I^2, 25 C, 38 P^3, 33 P^4, 33 M^1, 33 M^2 and 30 M^3. These figures are cited to give some idea of the

splendid samples of australopithecine teeth available for comparison. The data provided by these large samples formed the background for our statements about the divergence of *H. habilis* teeth from those of the Australopithecinae.

Similarly, I have compiled comparative tables of absolute measurements and of indices for some 136 mandibular and 66 maxillary teeth of *H. erectus* (including *Pithecanthropus, Sinanthropus, Atlanthropus, Telanthropus*, Mauer and Montmaurin). It is worthy of note that (*a*) the samples of *H. erectus* permanent teeth available are smaller than those of *Australopithecus* (170 mandibular, 219 maxillary), and (*b*) the relative numbers of upper and lower teeth discovered are reversed.

All the measurements of australopithecine and hominine teeth made by myself, including those made with von Koenigswald on original Javanese and Chinese teeth[6], were carried out with a specially prepared pair of Vernier callipers, having delicately tapering points for insertion between teeth in jaws. The technique used is considered to be comparable with that of Robinson, since numbers of his recorded dental measurements have been confirmed on the same specimens, to within a maximum margin of 0·3 mm, but a mean margin of about 0·1 mm. Each Olduvai tooth has been measured at least twice, and some thrice, by the same observer but at different times, generally with a lapse of at least 1 year between two of the measurings. Fluctuations have been small, most commonly of the order of 0·0–0·2 mm. Where successive measurements of the same dimensions differed, the mean of the two readings has been calculated and used.

In Tables 1 and 2, the mesiodistal lengths and the buccolingual breadths of the australopithecine and *H. habilis* teeth are presented. Only the *H. habilis* type and paratypes from Bed I are included, since we now consider the Bed II hominine to belong to a more advanced form[5].

The total morphological pattern of the *H. habilis* teeth obviously aligns them more closely with those of *A. africanus*. The metrical differences from the latter are therefore of most value in determining whether or not the habiline teeth belong to a different taxon. However, for the sake of completeness, we have included the dimensions of the *A. robustus* and *A. boisei* teeth.

In absolute length, only the habiline P_4's exceed the sample of 5 *A. africanus* P_4's, but in absolute breadth, both P_3 and P_4 are narrower than the entire samples of 23 P_3's and 22 P_4's. The absolute narrowing tendency of the *H. habilis* teeth is seen as well, though not so markedly, in the molars: the breadths of M_2 and M_3 lie near the bottom of the ranges for *A. africanus*, while of 33 australopithecine M_1's, only one specimen from Sterkfontein and one from Kromdraai are definitely narrower than those of *H. habilis*.

Thus, considering not merely the single type-specimen of *H. habilis*, but the whole group of mandibular teeth representing at least three different individuals from three different levels in Bed I (*MK*, *FLKNN* and *FLK* from below upwards), we may reasonably conclude that the group was characterized by absolute lengthening of P_4 and a tendency towards absolute narrowing of all the cheek-teeth, especially the premolars.

Table 3 shows that, when the 'relative' narrowing of the *H. habilis* teeth is gauged by the length/breadth index, all the *H. habilis* cheek-teeth have indices at, or above, the top of the range for *A. africanus*. The indices derived from Robinson's measurements on the type specimen, as quoted in his 1965 paper[1], emphasize this even more strikingly than those from my own measurements, for P_3 and M_1. Teeth from all individuals of

Table 1. Mesiodistal Length of Mandibular Cheek-teeth

	P_3			P_4			M_1			M_2			M_3		
	N	$\bar{x}$	Range	*N*	$\bar{x}$	Range	*N*	$\bar{x}$	Range	*N*	$\bar{x}$	Range	*N*	$\bar{x}$	Range
Sterkfontein	2	9·1	9·0– 9·2	2	9·95	9·8–10 1	5	13·9	13·0–15·1	6	15·1	14 3–16 8	8	15·2	13·5–16·7
Makapansgat	5	10·3	10 0–11·5	3	9 33	9 0–10·0	3	14·13	12·8–15·0	5	15·46	14·4–16·7	4	14·63	14·2–15·0
Taung	—	—	—	—	—	—	2	14 0	14 0	—	—	—	—	—	—
A. africanus	7	10 0	9 0–11·5	5	9 6	9 0–10·1	10	14·0	12·8–15·1	11	15·3	14·3–16·8	12	15·0	13 5–16·7
Swartkrans	11	9·72	9·2–10·5	12	11·01	10·3–12 5	18	14·88	14·0–16·1	12	16·2	15·0–17·4	12	16·8	15·4–18·5
Kromdraai	3	9·9	9·9–10 0	3	11 0	10·9–11·1	3	13 53	12·7–14·4	1	15·2	15·2	2	16·25	16·1–16·4
Peninj	2	9 55	9·2– 9·9	2	13·6	12 7–14·5	2	16 25	16·1–16·4	2	17·3	17·2–17·4	2	18·75	18·7–18·8
A. robustus/boisei	16	9·7	9 2–10·5	17	11·3	10·3–14·5	23	14 8	12·7–16·4	15	16 3	15·0–17·4	16	17·0	15·4–18·8
Total australopithecines	23	9·8	9·0–11·5	22	10 9	9 0–14·5	33	14·6	12·7–16·4	26	15·9	14·3–17·4	28	16·1	13·5–18·8
H. habilis type (*FLKNNI*)	2	9·75	9·6– 9·9	2	10·7	10 3–11·1	2	14 3	14·3	1	15·6	15·6	—	—	—
H. habilis paratype (*FLK* I)	1	9 25	9·25	—	—	—	—	—	—	—	—	—	—	—	—
H. habilis paratype (*MK* I)	—	—	—	1	10·45	10·45	—	—	—	—	—	—	1	15·1	15·1
Total *H. habilis*	3	9 6	9 3– 9·9	3	10·6	10 3–11 1	2	14·3	14·3	1	15·6	15·6	1	15·1	15·1

Table 2. Buccolingual Breadth of Mandibular Cheek-teeth

	P_3			P_4			M_1			M_2			M_3		
	N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Sterkfontein	2	11·7	11 7–11 8	2	11·7	11·6–11·7	4	12 7	11·2–13·9	6	14·0	13·2–15 3	8	13 6	12·7–14·8
Makapansgat	5	12·5	11·0–14·0	3	12·7	12·0–14 0	4	13·3	12·3–13·9	5	14·6	13 9–15 1	4	13 9	13 6–14·2
Taung	—	—	—	—	—	—	2	13 5	13·5	—	—	—	—	—	—
A. africanus	7	12·3	11·0–14·0	5	12·3	11·6–14·0	10	13 1	11 2–13·9	11	14·3	13·2–15·3	12	13·7	12·7–14·8
Swartkrans	11	11 7	10 6–12·9	12	13·1	12 0–14·5	18	14·1	13 0–15·2	12	15·0	13 9–16 2	12	14·5	12·9–16·5
Kromdraai	3	11 8	11·0–12·2	3	12 5	11·4–13·1	3	12·6	11·5–13·2	1	14 8	14·8	2	14·4	14 0–14·8
Peninj	2	13·5	13·5	2	15·0	14·8–15·2	2	15·4	15·3–15·5	2	16·1	16 1–16·2	2	16·0	15·7–16·3
A. robustus/boisei	16	11·9	10·6–13 5	17	13 2	12 0–15·2	23	14·0	11·5–15·5	15	15·1	13·9–16 2	16	14·7	12·9–16·5
Total australopithecines	23	12·0	10·6–14·0	22	13 0	11·4–15·2	33	13·7	11·2–15·5	26	14 8	13·2–16 2	28	14·3	12·7–16 5
H. habilis type (*FLKNNI*)	2	10 2	10 1–10·3	2	10·7	10·7	2	12·3	12·2–12·4	1	13·5	13 5	—	—	—
H. habilis paratype (*FLK* I)	1	9 3	9·3	—	—	—	—	—	—	—	—	—	—	—	—
H. habilis paratype (*MK* I)	—	—	—	1	11·4	11 4	—	—	—	—	—	—	1	13·0	13·0
Total *H. habilis*	3	9·9	9 3–10 3	3	10 9	10·7–11·4	2	12 3	12·2–12·4	1	13·5	13 5	1	13 0	13·0

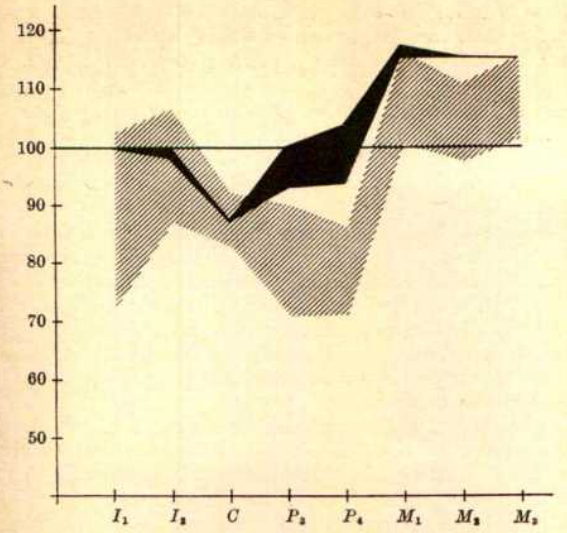


Fig. 1. Ranges of variation in the length/breadth indices of mandibular teeth of *Homo habilis* (black area) from Bed I, Olduvai Gorge, and of *Australopithecus africanus* (hatched area)

H. habilis agree in showing a high index. Table 3 provides unequivocal verification that the Bed I habilines were characterized over an extended period of time by relatively narrower teeth than were the members of *A. africanus* or of the Australopithecinae in general.

Naturally, the small sample of Bed I habilines can give but a poor picture of the variability of *H. habilis*, yet the clustering of three individuals to one side of the bigger australopithecine sample positively suggests the existence there of a population the mean dental shape of which was different from that of *A. africanus*. The mean indices for *H. habilis* and *A. africanus* confirm the differing tendencies in the two groups (Table 3), as do the graphs of the ranges (Fig. 1).

Similarly an isolated upper M^1 from the *H. habilis* type-site has a length/breadth index (102·3) which falls beyond the top of the range for 15 *A. africanus* M^1's, namely, 86·5–97·7. Another upper molar comes from the *A. boisei* type-site and its index (106·5) shows an elongate shape matched only by that of a single well-worn Makapansgat M^2 (*MLD* 24) with an estimated 107·1, while thirty other *A. africanus* upper first and second molars range from 83·1 to 97·7. Clearly, these two upper teeth from two different levels in Bed I—and which may or may not belong to the same individuals as those represented by the lower teeth from those sites—confirm the presence of the narrowing tendency in the maxillary teeth of some Bed I hominids.

The incisors likewise show a strong tendency to absolute and relative mesiodistal elongation and labiolingual narrowing. In *L/B* index, I_1 and I_2 fall at the top of the ranges for ten australopithecine I_1's and eight I_2's respectively, while I^2 greatly exceeds the top of the range for thirteen australopithecine I^2's.

Inner Contour of Mandible

The photographic reconstructions, which Hughes and I made a few years ago of the *H. habilis* type mandible, do not bear out Robinson's claim that the latter has a typically australopithecine internal mandibular contour. Marked differences are present (Fig. 2), especially when the type mandible is compared with the Makapansgat juvenile mandible which belonged to a youth of almost the same individual age.



Fig. 2. Reconstruction of the type mandible of *Homo habilis* (left) compared with the juvenile mandible (*MLD* 2) from Makapansgat, belonging to an individual of approximately the same age

The 'Phyletic Valence' of the *L/B* Index

What Robinson calls "a far more trenchant criticism" of our use of the *L/B* index is his assertion that this index is of "extremely low phyletic valence", so far as hominids are concerned. As examples, he cites his figures for one tooth, namely, P_3: but each hominid group cited by him is based on only one or at most two sites. Thus, his *H. erectus* sample is confined to the Choukoutien teeth and his

Table 3. Length/Breadth Indices of Mandibular Cheek-teeth

	P_3			P_4			M_1			M_2			M_3		
	N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Sterkfontein	2	77·5	76·9–78·0	2	85·4	83·8–87·1	4	110·2	100·8–117·0	6	108·5	104·8–111·3	8	111·5	104·7–115·9
Makapansgat	5	83·0	71·4–90·9	2	72·9	71·4–74·4	3	105·9	104·1–107·9	5	105·8	98·0–110·6	4	105·3	101·4–109·6
Taung	—	—	—	—	—	—	2	103·7	103·7	—	—	—	—	—	—
A. africanus	7	81·4	71·4–90·9	4	79·2	71·4–87·1	9	107·3	100·8–117·0	11	107·3	98·0*–111·3	12	109·4	101·4–115·9
Swartkrans	11	83·3	76·0–92·8	12	84·3	74·5–91·9	18	105·9	100·7–110·4	12	108·3	101·3–117·2	12	116·2	106·6–124·0
Kromdraai	3	84·4	81·1–90·0	3	88·3	84·0–95·6	3	107·8	102·3–110·8	1	102·7	102·7	2	112·9	108·8–117·1
Peninj	2	70·7	68·1–73·3	2	90·6	85·8–95·4	2	105·5	103·9–107·2	2	107·1	106·8–107·4	2	117·2	115·3–119·1
A. robustus/boisei	16	81·9	68·1–92·8	17	85·7	74·5–95·6	23	106·1	100·7–110·8	15	107·7	101·3–117·2	16	115·9	106·6–124·0
Total australopithecines	23	81·7	68·1–92·8	21	84·5	71·4–95·6	32	106·5	100·7–117·0	26	107·5	98·0*–117·2	28	113·1	101·4–124·0
H. habilis type† (*FLKNN1*)	2	95·6 100·1	93·1–98·0 (P.V.T.) 98·0–102·2 (J.T.R.)	2	100·0	96·3–103·7	2	116·3 117·2	115·3–117·2 (P.V.T.) 117·2 (J.T.R.)	1	115·5 114·3	115·5 (P.V.T.) 114·3 (J.T.R.)	—	—	—
H. habilis paratype (*FLK* I)	1	99·5	99·5	—	—	—	—	—	—	—	—	—	—	—	—
H. habilis paratype (*MK* I)	—	—	—	1	±91·7	±91·7	—	—	—	—	—	—	1	116·2	116·2
Total *H. habilis* (from Bed I)	3	96·9 99·9	93·1–99·5 (P.V.T.) 98·0–102·2 (J.T.R.)	3	97·2	±91·7–103·7	2	116·3 117·2	115·3–117·2 (P.V.T.) 117·2 (J.T.R.)	1	115·5 114·3	115·5 (P.V.T.) 114·3 (J.T.R.)	1	116·2	116·2

* Worn tooth.
† Two sets of indices are given for most of the type specimen's teeth, those of Dr. Robinson (J.T.R.) and those of the author (P.V.T.).

Paranthropus sample largely to the Swartkrans teeth. Conclusions about the ability of the *L/B* index to distinguish between these taxa must necessarily be based on the larger and geographically more diverse samples now available. The data I have compiled for comparison with the habiline teeth include measurements and indices for 22 *H. erectus* P_3's from no fewer than seven sites in three continents (as against thirteen from Choukoutien alone); similarly twenty-three M_1 (as against eleven) and eighteen M_2 (as against seven). Table 3 shows that, while *L/B* scarcely differentiates between *A. africanus* and *A. robustus/boisei* for P_3, M_1 and M_2, it does yield differing means for P_4 (79·2, 85·7) and for M_3 (109·4, 115·9), the two mandibular cheek-teeth omitted from Robinson's Table 1. Moreover, there are marked differences of the means between *H. habilis* and *A. africanus* for all five mandibular cheek-teeth. These figures indicate that the *L/B* index is more useful in distinguishing among lower Pleistocene hominids than Robinson believes.

Apart from these details, however, the very basis of Robinson's view is unacceptable. The taxonomic usefulness of a character in any specific comparison depends on its ability to differentiate between the two taxa considered. The fact that the *L/B* index fails to distinguish between certain teeth of *A. robustus* and *A. africanus* in no way lessens, in fact enhances, its usefulness as a mark of distinction between *H. habilis* and *A. africanus*. It is totally irrelevant to this latter comparison that some teeth of some other groups do not differ in *L/B* index. The vital fact remains that this index does show a definite difference between *H. habilis* and *A. africanus*. In our description of the type specimen, this was clearly a metrical difference to be included, in accordance with Art. 13 *a* (i) of the International Code of Zoological Nomenclature, which lays down that a new species name must be "accompanied by a statement that purports to give characters differentiating the taxon". *L/B* is one such character, which differentiates the taxon *habilis*, irrespective of whether the same character can as effectively differentiate other taxa.

The Cranial Capacity of *Homo habilis*

Robinson states: "The endocranial capacity of *H. 'habilis'* appears, on the scanty and indirect evidence available, to have differed little from that of the australopithecines with a range overlapping that of the latter substantially" (*op. cit.*, p. 122).

Let us re-examine that evidence. Associated with the type-specimen were large parts of two beautifully preserved and undistorted parietal bones. They were patently bigger than the parietal bones of any australopithecine yet discovered. The sagittal arc from bregma to lambda measures 105 mm, whereas those of five australopithecines range from 74·5 to 91·5. The lambdoid arc from pterion to asterion is 69·0 in *H. habilis*, but ranges from 55·5 to 62·5 in australopithecines. The coronal edge, although not perfectly preserved, likewise exceeds the top of the australopithecine range. Such large parietals necessarily covered a larger brain than that of any australopithecine known hitherto.

That fact was clearly acknowledged by Robinson in 1962[6]. There he endeavoured to show that early stone tools were not made by *Australopithecus*. In that investigation, he explicitly included 'pre-Zinj' (that is, the type specimen of *H. habilis*) as a larger-brained contemporary of *Australopithecus*. Thus he stated: "In fact, there is evidence throughout the entire australopithecine period either proving or suggesting the presence of a more advanced form of hominid. There are the large parietals in the 'pre-Zinj' level of Olduvai, *'Telanthropus'* at Swartkrans associated directly with *Paranthropus*, and at Sangiran *'Pithecanthropus'* and *'Meganthropus'* occur in association" (*op. cit.*, p. 102).

It was a logical and imperative step after measuring the parietals to estimate the cranial capacity. This was done[7] by using a biparietal partial endocast as a basis. The method provided a precision unattained in the wide range of estimated figures previously published for the australopithecines[8,9]. The central value (680) of the range of estimates for *H. habilis* (643–724) is about mid-way between the capacity of the biggest-brained australopithecine (562) (ref. 10) and that of the smallest-brained *H. erectus* (775). Furthermore, it is a fair inference that, had the type individual of *H. habilis* grown to adulthood, his cranial capacity would have been somewhat larger than the capacity estimated. Surely it is as valid to infer that the population range in *H. habilis* would overlap appreciably that of *H. erectus*, as to emphasize (as Robinson[1] prefers) its equally likely overlap with that of *Australopithecus*.

Hence the habiline remains, both in their dental departures and their brain-size, as well as in their inferred stone tool-making activities[11], form a population intermediate between *A. africanus* and *H. erectus*.

Where opinions may validly diverge is in the weight laid on these differences. When all presently available evidence is taken into account, we feel justified in concluding that (i) the habiline population represents a distinct taxon; (ii) this habiline taxon is more closely related to the hominines than to the australopithecines and should be placed in the genus *Homo*.

Further specimens could alter this point of view: they may spread the habiline range more towards the australopithecines or more towards the hominines, or both. In the former event, there would be no hesitation in reconsidering the classificatory position of *H. habilis*, but on present evidence a taxonomic re-alignment would seem premature. In much the same way, Mayr[12], who demoted *Australopithecus* from separate generic status in 1950 to place it under *Homo* as a *transvaalensis* species, has recently reinstated it as a separate genus[13].

Whatever designation taxonomists may finally adopt, the view I expressed at the seventh International Congress of Anthropologists and Ethnologists, in Moscow in August 1964 (ref. 11), was that the Bed I *habilis* fossils represent a form intermediate between *A. africanus* and *H. erectus*. This view is acceptable to many workers, including apparently Robinson[1]. Furthermore, the anatomical features of the Bed II hominines and *H. habilis* permit one to envisage at least a part of the habiline population of Bed I times moving forward to become the hominines of Bed II times. These phylogenetic implications of the new discoveries have been fully discussed elsewhere[14,15].

Whether recognition of an evolutionary stream from *A. africanus* → *H. habilis* → *H. erectus* justifies putting all these taxa under *Homo*, and separating them generically from *Paranthropus*, as Robinson suggests, is another matter entirely. To effect this re-alignment he relies perforce on phylogeny and ethology (implemental activities). Phylogeny and ethology may be valid sources of taxonomic inference, but it would be an unusual practice to allow them to outweigh inferences from morphology. Morphologically *A. africanus* and *A. (Paranthropus) robustus* have so much in common, that several recent international symposia on the taxonomy of fossil hominids, one at Burg Wartenstein in 1963 (ref. 16) and one at Moscow in 1964, were virtually unanimous in recognizing these two taxa as separate species of the one genus *Australopithecus*. As Mayr[13] has pointed out, the morphological differences between *Paranthropus* and *A. africanus* "are no greater than among species in other groups of mammals". My studies of the South African australopithecines, in comparison with *A. (Zinjanthropus boisei*, demonstrate more extensive areas of morphological overlap between these two South African forms than have as yet been commonly recognized. To lump *A. africanus* under *Homo*, and retain *A. robustus* in *Paran*

thropus, would be tantamount to saying that there is less difference between *A. africanus* and *H. erectus* (or even *H. sapiens*) than between *A. africanus* and *A. robustus*.

The australopithecines clearly represent a single grade of hominid organization. The group is highly variable and we may so far recognize a mesodont branch (*A. africanus*), a macrodont branch (*A. robustus*) and a megadont variant (*A. boisei*). Already it is known that these three forms show a large measure of overlap, only a few features, such as the canine/*P*3 ratio and the *P*3/*P*4 ratio, showing an absolute distinction between them. The fact that one of the three forms may bear a phyletic relationship to *Homo* is no reason to remove it from the genus *Australopithecus* and place it in *Homo*. On the other hand, *habilis* is a more advanced hominid than any of the members of the *Australopithecus* group. In fact, in teeth and jaws, it is certainly more hominized than *Meganthropus*, which in turn von Koenigswald and I believe is more hominized than the australopithecines, while neither Weidenreich[17] nor Le Gros Clark[18] could separate *Meganthropus* generically from *Pithecanthropus* (*H. erectus*), a view which Schultz[19] is also inclined to support. If further discoveries of skull, dentition and limb-bones attributable to *Meganthropus* confirm that it is not separable from *H. erectus* at generic or specific level, then *habilis* would be even more emphatically a hominine than we have claimed—and we should have to call it *Homo erectus habilis*—as D. R. Hughes[20] and Ashley Montagu[21] have already suggested. Meantime, it would be safer to continue to regard *habilis* as a species within *Homo*, thus avoiding the extreme taxonomic evaluations on either side of this middle-of-the-road position.

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In his comments on my views on the validity of the supposed taxon *H. habilis*, Tobias exhibits misapprehension of the basis of my criticism as well as confusion over some basic principles of taxonomy.

The existence of some detectable differences between the known specimens attributed to *H. habilis* and the known australopithecine material is not disputed. The point at issue is the significance of those differences.

Tobias believes that I am mistaken in thinking that the internal mandibular contour of the type specimen of *H. habilis* is of the australopithecine, not the hominine, type and provides his Fig. 2 to prove this. However, this illustration fully supports my view and disproves his. The nature of the internal mandibular contour is a character which, to the best of my knowledge, was introduced into the literature by me and I have always used it in the same way. In australopithecines the space enclosed by the internal contour of the corpus mandibulae, when seen in occlusal view, is relatively narrow, narrows rapidly as the symphysis is approached and, at the level of the P_4–M_1 contact, is narrower than the corpus thickness at this level. On the other hand, the hominine condition is one in which the internal contour is wider, does not narrow rapidly forwards and, at the level of the P_4–M_1 contact, is appreciably wider than the corpus is thick in that position. Fig. 2 of Tobias shows both the *H. habilis* and the *Australopithecus* mandibles with the same robust corpus and narrowing V-shaped contour that I have described for australopithecines. The wide U-shaped contour and more gracile corpus that I have described for hominines does not occur in either of these specimens. This is demonstrated in Fig. 1 which I have prepared. It is therefore clear that the *H. habilis* type mandible is indeed of the typical australopithecine sort with respect to this character complex.

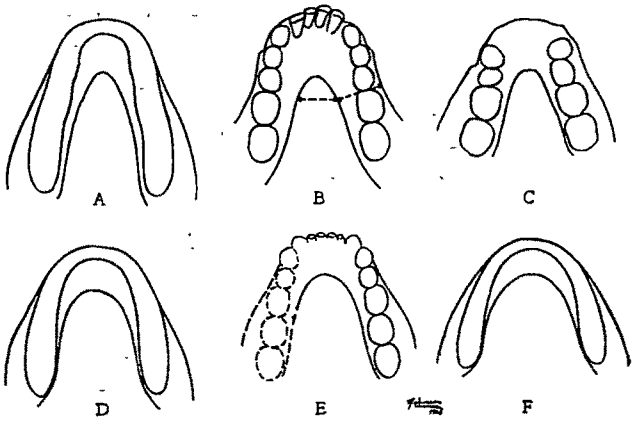


Fig. 1. Diagrammatic representation of the internal mandibular contour of: *A*, adult *Australopithecus* (*sensu stricto*) from Sterkfontein; *B*, adolescent *H. habilis* type from Bed I, Olduvai; *C*, adolescent *Australopithecus* from Makapansgat; *D*, adult *H. erectus* (equals *Telanthropus*) from Swartkrans; *E*, adult *H. habilis* from Bed II, Olduvai; and *F*, adult modern *H. sapiens*. The specimen in *E* has suffered slight damage on the left side; this has been allowed for by reflecting the right side, though most of the internal contour is undisturbed and only the portion indicated by a broken line is taken from the right side. The relevant dimensions for this character are indicated in *B*. *B* and *C* have been drawn from Fig. 2 provided by Tobias in his accompanying communication; *E* has been drawn from published photographs[4,5]

Tobias makes the explicit claim that his estimate of the endocranial volume of the skull from which the two incomplete parietals from Olduvai Bed I came represents "a precision unattained in the wide range of estimated figures previously published for the australopithecines". The best previously published estimate was that for *Sts* 5 from Sterkfontein; a skull which is almost complete and has the braincase virtually intact and apparently undistorted and empty, so that the endocranial volume can be measured directly by conventional methods. As I see it, therefore, Tobias is claiming that he can make a more accurate estimate of endocranial volume on the basis of two isolated and incomplete parietals than Broom and I could by conventional methods on a complete skull.

The difference between the passage quoted by Tobias and my later statement that the apparently greater volume of the *H. habilis* specimen does not necessarily indicate difference from *Australopithecus* (*sensu stricto*) is easily explained. Some time after writing the first statement I had occasion to re-investigate the evidence relating to cranial capacity in the australopithecines. I found that six estimates were possible for *Australopithecus* (*sensu stricto*): two very good ones and four of lesser reliability,

but based on more than parietals alone. From these estimates the probable range of variation in the population was calculated. The mean was 430 cm³ and the range, using limits of 2·5 times the standard deviation on either side of the mean, was 300–550 cm³. On comparing these figures with similar ones calculated for pongids and modern man from data, based on substantial samples, provided by Ashton and Spence[1], it became obvious to me that even limits of three times the standard deviation seriously underestimate the upper limit of the observed range for both the gorilla and man. This being the case, it is possible that the *Australopithecus* range could similarly be underestimated. Using the percentage underestimate for man as a basis for adjustment, a corrected upper value of 680 cm³ was obtained and one of just more than 600 cm³ on the basis of the gorilla discrepancy. Since the former value coincides with the estimate obtained by Tobias for a single *H. habilis* specimen, it seems to me that it is no longer by any means certain that this value is significantly larger than could be expected from *Australopithecus* (*sensu stricto*) as represented by the South African specimens. Furthermore, since there is evidence that *Australopithecus* had already embarked on the hominine type of brain expansion, though *Paranthropus* apparently had not, the possibility of finding evolutionarily slightly later forms of the former with larger brains than are now known from South Africa must be reckoned with. It therefore seems to me that a single estimate of 680 cm³ for *H. habilis*, obtained on the basis of two incomplete parietal bones alone, is narrow support for the view that the latter is not merely specifically, but actually generically, distinct from *Australopithecus*.

I do not understand Tobias's charge that my views on hominid taxonomy are a consequence of using the 'unusual practice' of allowing phylogenetic and ethological evidence to outweigh morphological evidence. This charge involves a most thorough-going confusion concerning how phylogenetic conclusions are arrived at, the use of sorting criteria in setting up the original categories into which specimens are placed and taxonomic procedure in general.

When a varied collection of fossils is being investigated for the first time, it is sorted out into groups so that finally each group includes specimens of a single species. This sorting is done on the basis of morphological criteria and in the case of fossils there are no other sorts of criteria available. Any later conclusions regarding the species, genera or other taxa which may be reached and any phylogenetic conclusions arrived at are obviously ultimately based on the sorting criteria originally used to assemble individual specimens into species groups. Since no taxon is valid unless it includes, or is included by, at least one species, no matter what level the taxon occupies in the taxonomic hierarchy, it is clear that in the long run the entire hierarchy of classification depends on the sorting criteria used to sort into species the raw material of classification, the samples obtained from Nature.

It should be clear from my papers that, for example, my separation of *Australopithecus* and *Paranthropus* into distinct genera is based fairly and squarely on morphological evidence and on inferences which are similarly based, for example, on the nature of the masticatory apparatus, but related to ecological and behavioural aspects of the genera concerned. Even the cultural aspects involved can be said to be based on morphological evidence since they concern morphology on one hand, for example, of the hand and locomotor apparatus, and on the other structural features of physical objects (artefacts) found in the deposits with the specimens.

Secondly, in so far as Tobias considers, as his comment implies, that morphology is always a more valid basis for taxonomic conclusions than is any other sort of evidence, he is, I believe, mistaken. What makes and keeps one species separate from another in Nature is not just its morphology. Similarly, the fields in which we may look for evidence of similarity, hence of probable common ancestry, are by no means confined to comparative anatomy. Clearly the entire biology of the taxa concerned is involved, and surely physiology, behaviour and ecology are as much a part of an animal's biology as is its anatomy. Any of these can provide as effective a barrier for preserving the integrity of a species as morphology can. Usually morphology provides a more practical basis for distinguishing species because of the way in which taxonomic work is normally carried out. This does not mean it is a more valid basis for taxonomic conclusions, nor is morphology always the most practical means of sorting species. This is clearly demonstrated by some examples of sibling species where behaviour provides the best criterion for distinguishing them.

I believe that Tobias is mistaken in asserting that recognition of the series *Australopithecus—H. habilis—H. erectus* as a single phyletic sequence is the basis for my suggestion that perhaps *Australopithecus* should be included in *Homo*. The main basis of this suggestion is clearly stated in the final sentence of my paper: "In this event the whole lineage in which culture is a very important adaptive mechanism is included in a single genus . . .". In other words, the genus is here based on a clearly defined adaptation which distinguishes its members from those of all other genera. This adaptation involves a complex of morphological (for example, hand, brain and locomotor), behavioural (for example, tool-making, language and hunting) and ecological (for example, diet, subsistence pattern) elements forming one of the most clearly defined and powerful adaptations in the animal kingdom. I also pointed out that the sequence appears to contain no major morphological changes, no major shifts in evolutionary direction and did not occupy a very long geological time. On the other hand, as *H. habilis* is set up in the present taxonomic definition[2], it is not easily distinguished from *Australopithecus* at the generic level or *H. erectus* at the species level.

Tobias explains that lumping *Australopithecus* with *Homo* but retaining *Paranthropus* is tantamount to saying that there is less difference between the former two than between either of them and the latter. This, indeed, is precisely what I do say and what I believe the facts indicate. For example, the size relationship of the canine to the post-canine dentition places *Australopithecus* and *Homo* together and separates them from *Paranthropus*; so does the morphology of dm_1, the cranial cresting pattern, the morphology of the palate, the supra-orbital height index, the pterygoid plate morphology, the nature of the mandibular ramus and one of the important muscular insertions on it, the ischial morphology and quite possibly also the foot and hand, though the evidence in the latter two cases is, as yet, meagre. Not all these features are unrelated, separate differences. Some clearly relate to different use of the masticatory complex and consequent differences in the stresses developed in the head. These thus concern a significant aspect of the biology of the animals. Supra-orbital height index reflects differences of significance with respect to brain evolution. The pelvic difference may reflect no more than different levels of adaptation to the same locomotor habit; though it is my opinion that the evidence actually indicates differences of locomotor habit.

Tobias criticizes on two grounds my unwillingness to accept the shape criterion of teeth as taxonomically valid. He seems to feel that both involve manipulation of data, at the very least, to suit my argument. First, my Table 1 "inexplicably excludes" P_4 of the type mandible of *H. habilis*. In the original description of this form the authors state that all teeth are relatively narrow, but that this is "especially evident" in the lower premolars and molars[2]. Nowhere is P_4 singled out as exhibiting this feature more strongly than other mandibular postcanine teeth. Since I was not undertaking a comprehensive study in the article concerned, I used representatives of both categories of mandibular teeth mentioned as exhibiting the

character especially well, choosing the most stable teeth in each. If narrowness is exhibited by mandibular premolars surely P_3 is as legitimate a choice as is P_4—indeed, since P_4 is known to be more variable than P_3 in hominids, the latter is much the better choice since it should show up any difference more clearly.

The second point of criticism involves my use of an incompletely developed P_3 of *Paranthropus*. Tobias emphasizes that the tooth crown had not completed its development, that I did not use it in calculating means in my 1956 monograph, but that I now include it in calculations. In both cases I took care to point out that the tooth had not yet begun to erupt and was not fully formed; thus no attempt was made to deceive the reader into thinking it was a fully formed crown since attention was deliberately directed to its incomplete condition. In the earlier instance I excluded it from calculations of means of absolute size since it was not complete. In the second instance I used it for determining shape, not absolute size. This, I pointed out, was done because the crown already almost matched the maximum length for a sample of eleven fully formed P_3 crowns from the same site, hence it was unlikely that there was still a great deal of growth to complete, and hence that it was improbable that the shape would change enough to alter the index significantly. Tobias believes that this would not be so since "much more enamel would have been added before the tooth had erupted". Enamel had already been laid down on this crown, but if the remainder of the enamel deposition had to alter the shape index to that of the other teeth, then with no further addition of enamel on the mesial and distal faces of the crown, the buccal and lingual faces would have had to receive a total of roughly 3 mm of additional enamel. It seems to me unlikely that enamel deposition would be so very disproportionate on different sides of the same premolar crown.

If this particular tooth is left out of the Swartkrans series, the observed range would be 76·0–92·8 for the eleven specimens. The broader of the two relevant teeth in the *H. habilis* type mandible has an index (using Tobias's own measurements) of 93·1, thus leaving a gap of 0·3 of a unit in the index between the two ranges. It should be remembered that the tooth here mentioned, which gave an index of 93·1 on Tobias's measurements, gave an index of 98·0 from my measurements and 115·8 from those of Leakey.

According to Tobias (Table 3) the observed range of the L/B index of P_3 of *Australopithecus* (*sensu stricto*) is 71·4–90·9 and that for *H. habilis* is 93·1–99·5; sample size being seven in the former and three in the latter case. Tobias believes that this is valid evidence that two distinct taxa are here involved. If we follow him in this belief, are we not in serious danger of arriving at the conclusion —quite logical from these premises—that the type mandible of *H. habilis* as measured by Leakey (L/B index 115·8, $N = 2$, for P_3) belongs to a different taxon from the same specimen as measured by Tobias? Clearly such a conclusion is not possible if a realistic approach is taken to variation and less reliance placed on very small gaps between ranges when these are observed ranges based on extremely small samples.

There seems to be some confusion in Tobias's remarks concerning my view that the phyletic valence of shape indices of teeth is low in hominids and cannot be used in the manner in which it has in the case of *H. habilis*. My point is that there is so much overlap in this character in the hominid group that it cannot be used as a sorting criterion. Tobias criticizes this in two respects. First, that I have used relatively small samples to demonstrate my point instead of using all the available material. Had I been attempting to demonstrate non-overlap, hence validity of the criterion, then clearly the largest possible samples should have been used since small samples are an obvious danger to the case. Since I was concerned with overlap the case is very different: I cannot see how increasing sample sizes can remove overlap which is already present on the basis of small samples. The very fact that small samples from restricted areas have been used and nevertheless proved overlap improves the effectiveness of the demonstration that the criterion is useless for sorting purposes. The *H. habilis* material is from Olduvai alone; by design I used similar local samples (which, because they are local and small, are very unlikely to be as variable as pooled samples from widely scattered sites) to show that the ranges nevertheless overlap very greatly.

Secondly, Tobias maintains that the very basis of my view is unacceptable since, "The taxonomic usefulness of a character in any specific comparison depends on its ability to differentiate between the two taxa considered. It is totally irrelevant . . . that some teeth of some other groups do not differ in L/B index. The vital fact remains that this index does show a definite difference between *H. habilis* and *A. africanus*". In other words, my comparisons between *Paranthropus*, *Australopithecus* and *Homo* are beside the point. In this view I believe that he is seriously mistaken. What two taxa are being compared by Tobias? According to him *H. habilis* and *A. africanus*; two species, that is. But these two species, according to Tobias, belong in different genera; hence when comparing them one is in fact comparing the genera in which they belong. By placing the species in two different genera one is, in effect, saying that the main difference between them are the differences between the genera *Australopithecus* and *Homo* since the only legitimate way in which the two species concerned can get into different genera is by the one possessing the sorting characteristics of *Homo* and the other possessing those of *Australopithecus*. To insist that one is here comparing only the two species and not the genera to which they belong is to misunderstand a fundamental aspect of taxonomy. In view of the position which Tobias is attempting to defend, he needs to demonstrate that the dental shape characteristics of the genus *Australopithecus* (which according to him includes the material I place in *Paranthropus*, hence this must also be included) differ significantly from those of the genus *Homo*. If he wishes to maintain that the shape index is a valid taxonomic criterion in this instance, then it is necessary that he demonstrates that by using it he can sort any specimen belonging to either of these genera into the correct genus. Clearly, if the ranges of this feature for the two genera overlap, then this requirement cannot be met.

The inability of this criterion to sort *Australopithecus* specimens from those of *Homo* is clearly demonstrated in the table given in my paper on this subject[3]. In this the most stable teeth in the groups mentioned (mandibular premolars and molars) as showing this difference most clearly are compared and it is seen that for *Australopithecus* and a large sample of *H. erectus* the means are not very different and the ranges overlap almost completely. In Tobias's tables he has added some specimens to the *Australopithecus* sample, and this, as could have been predicted, makes the overlap even greater so that scarcely any of the specimens could be sorted into the correct genus by this criterion. As was pointed out, adding modern samples of *Homo* makes the observed range for his genus considerably greater.

It might be argued that even though the tooth shape criterion is of no value at the generic level, it might be at the species level and would thus allow *H. habilis* to be distinguished from other species of *Homo*. The enormous variation in this index in modern man, all within a single species, which includes the known range for *H. erectus* as well as the ranges for the genera *Australopithecus* and *Paranthropus*, rather clearly suggests that this is not possible either.

The shape index therefore cannot be used as a sorting criterion of the known hominid genera and, on available evidence, it is improbable that it could be used at the species level either, within the genera. Consequently I

conclude that it has very low phyletic valence and that it does not serve the purpose for which Tobias is attempting to use it. He is therefore very wide of the mark in claiming that the only semblance of validity my objection has stems from the use of the one incompletely developed crown from Swartkrans. The confusion of the generic and species levels in his comparisons further invalidates his taxonomic conclusion.

My criticism of the establishment of the species *H. habilis* is that (1) variation has not been treated adequately or realistically; (2) the sorting criteria used are not valid; (3) there has been some confusion between the genus and species levels in the taxonomic analysis.

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GEOGRAPHY IN CANADIAN UNIVERSITIES

By Prof. W. G. V. BALCHIN
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THE *Commonwealth Universities Yearbook, 1965*, lists a total of fifty-five universities and colleges in Canada with the power to grant degrees. Of these, thirty-eight are exercising all their powers, thirteen are holding some or all of their powers in abeyance while in federation or affiliation with another university, one (Guelph) is a new university formed from colleges previously affiliated to other institutions, and three (Brock, Trent and Simon Fraser) are new universities with full powers but founded so recently that their first students have yet to graduate. More than a third of the universities of Canada have received their charters since 1940, mainly during the past decade.

The rate of change and of expansion in university facilities in Canada in the period since the end of the Second World War has been phenomenal. An impressive illustration is afforded by Simon Fraser University in Vancouver. It was conceived of and approved in 1963 and an architectural competition was then held for a design which would fit the 1,200-acre site selected. The Erickson-Massey plan was chosen from 71 entries late in the same year and this scheduled an opening in 1965 with 2,000 students after an expenditure of 18 million dollars on buildings. In fact the University opened on schedule in October 1965 with 2,400 students and further rapid growth is expected.

Matching this post-war explosion in university development there has been an even greater growth in the facilities for teaching and research in geography. Apart from the Universities of Toronto and Montreal, the subject was almost non-existent in Canadian universities at the outbreak of the Second World War. To-day it is well established in all the major universities and in more than half the degree-awarding institutions, a development which has largely occurred in the past decade and which doubtless owes much to the survey on the state of geography in Canadian universities undertaken in 1951 by Prof. (now Sir Dudley) Stamp for the Canadian Social Science Research Council.

An understanding and appreciation of the place of geography in the academic structure of Canadian universities involves a background knowledge of the general pattern of university evolution within the Dominion. The story begins in 1635 when the priests of Quebec City, capital of the French colony of Canada, founded the Collège des Jésuites. The Séminaire de Québec came next in 1663 and from the amalgamation of these two institutions there grew the whole system of French-Canadian university education. Laval University was the first to emerge in 1852, and secondly, in 1920, the University of Montreal, which was established as a "succursale" of Laval in 1876. Many classical colleges, each associated with one of the universities, were established during the nineteenth century and institutions giving instruction in the French language spilled over the provincial frontiers. The Collège de St. Boniface, for example, was established in Manitoba in 1818, the bi-lingual University of Ottawa in 1848 and St. Joseph's of New Brunswick in 1864.

Colleges and universities in the English-speaking tradition came later than the French foundations and it was really the American revolution which brought English-speaking education to Canada. The Empire loyalists who streamed north into Canada soon established educational institutions most of which were Anglican and appropriately named "King's College". King's College at Windsor, Nova Scotia, was founded by provincial charter in 1789 and received a royal charter in 1802. It is now located at Halifax as the University of King's College and is closely associated with Dalhousie University. In New Brunswick the Provincial Academy of Arts and Sciences became the College of New Brunswick by Provincial Charter in 1880, received a royal charter as King's College, New Brunswick, in 1828, and became the University of New Brunswick in 1859. In 1827 "King's College at York" was founded and from this emerged the University of Toronto when the name of the city was changed from York to Toronto. As part of the same trend came Queen's College at Kingston, four years after the accession of Queen Victoria. Catholic and Anglican sponsorship of universities was followed by support from other denominations, for example, Mount Alison (United Church) and Acadia (Baptist). McGill University in Montreal is an example of an institution founded by a wealthy individual without either Church or State support.

During the twentieth century the support of universities by the Provinces became predominant, for example Alberta in 1906, Saskatchewan in 1907 and British Columbia in 1908. The massive growth in facilities to match the demand for university places after the Second World War has seen the increased importance of provincial aid and also the addition of Federal Government funds. Federal sources include the National Research Council and the Social Science Research Council. The latter operates on a limited scale, but co-operates closely with the semi-independent Canada Council, which also gives financial assistance to geography on the Arts side. The Canada Council was founded in 1957 when the Federal Government allocated 50 million dollars of death duties

derived from the James Dunn estate. The capital sum has since been supplemented by other large gifts. In order to qualify for grants from certain of these sources many institutions have changed from a sectarian to a non-sectarian basis while others have amalgamated or federated into new universities.

Ontario in particular has experienced the explosion in facilities. In 1939 it had six universities—McMaster, Ottawa, Queen's, Toronto, Waterloo-Lutheran and Western Ontario. To-day it has seventeen if we include Osgoode Hall Law School, which has degree-granting powers. Even more extraordinary is the fact that most of the expansion has taken place in the past 6 years. New charters were authorized at Windsor in 1953 and Carleton in 1957, and these were followed in rapid succession by Waterloo 1959, Royal Military College 1959, Laurentian 1960, Lakehead 1962, York 1963, Trent 1963, Brock 1964, and Guelph 1964.

In western Canada the early concept of a unified provincial university is changing as a result of population pressure. British Columbia has recently chartered the new universities of Victoria (1963), Simon Fraser (1964) and the Notre Dame University of Nelson. Alberta and Saskatchewan now operate two campuses each (at Edmonton and Calgary, and at Saskatoon and Regina respectively) and these may well emerge before long as four separate universities. The rate of change is indeed so great that some of these foundations are not recorded in the *Commonwealth Universities Yearbook, 1965*.

It is against this general background of university expansion that one must set the development of geography as a discipline. Geographical courses were introduced in Toronto in 1906 and in the University of Montreal in 1910, while the universities of British Columbia, McMaster and Western Ontario followed in the 'twenties and 'thirties. A major step forward came in 1935 with the establishment of a separate Department of Geography within the University of Toronto under the direction of Prof. Griffith Taylor. It was the Second World War, however, that demonstrated to Canada, as indeed the First World War had to Britain, that geography was important and valuable both as a university discipline and as a practical subject. As a result departments of geography were founded in quick succession in many of the existing universities and were also incorporated without question in nearly all the new foundations. The sequence began with McMaster in 1942 and was followed by McGill (1945), Laval (1946), British Columbia (1946), Western Ontario (1946), Montreal (1947), Ottawa (1951), Manitoba (1954), Edmonton (1955), Carleton (1957), Waterloo-Lutheran (1959), Queen's (1960), Saskatoon (1961), Waterloo (1961), York (1962), Victoria (1962), Calgary (1963), Brock (1964), and Simon Fraser (1964). Geography is also being taught at the undergraduate level in a further ten Canadian universities, but in these full departmental status has yet to be granted. Progress has still to be made in some localities especially in the older-established universities in the Maritime provinces.

First Degrees

The vast majority of Canadian students are registered in general rather than honours degree courses. General degrees are made by passing a certain number of courses each year, that is, collecting credits. Thus the B.A. degree normally comprises five full courses each year, the B.Sc. degree six or more. Sessional examinations are held on the year's work in each course and a pass standing is required for admission to the following year. This pattern can produce very large undergraduate geography registrations in those institutions where certain geography courses are regarded as basic to some of the degree structures, or where geography courses prove popular among both arts and science students. In the United States there has been considerable criticism of the credit system of qualifying for a degree, especially when the degree is obtained by piling up credits in totally unrelated subjects, but this situation rarely obtains in Canada, where various systems of required courses make a co-ordinated pattern of undergraduate work.

The honours degree course requires concentration on either one or two subjects and sometimes means an additional year of study. The attainment of an honours level in the examinations is also a requirement for both entry to and continuance in an honours course. Many of the smaller universities and colleges have not yet progressed to the stage of offering honours degrees in geography.

Although the credit system dominates the degree structure, in many other respects it is not difficult to see the influence of the universities of England and Scotland —especially Oxford, Cambridge, Edinburgh and London —in the English-Canadian universities and departments of geography. In the French-Canadian universities, however, the traditional French pattern is strongly in evidence. The *baccalaureat* with its well-rounded classical education usually precedes specialist and university studies. This instruction is given by approximately 30 associated colleges under the supervision of universities such as Laval and Montreal. Thereafter the student enters a university where specialist training in geography is available in an '*Institut*'—which in Canada resembles the normal department of geography. Both Laval and Montreal have maintained a close contact with French universities and some professors of geography have held chairs simultaneously in Canada and France.

Higher Degrees

The situation regarding higher degrees is still in process of evolution so far as geography is concerned. Generally in English-Canadian universities a master's degree demands at least a year of study in certain prescribed courses beyond an honours bachelor's degree, and an acceptable thesis. The Ph.D. degree requires at least two additional years, prescribed courses, examinations and the presentation of a comprehensive thesis. Experience shows that studies extending over 2 and 3 years respectively are more normal. Most universities offering honours courses for the first degree also offer masters' degrees, but owing to faculty affiliation these are more often available in arts (thirteen institutions) than in science (five institutions). The Ph.D. degree in geography is awarded in nine universities. Doubtless this situation will be remedied in the course of time as the number of honours students and the volume of postgraduate work increase.

In the case of the French universities the M.A. is often a first degree and the *Diploma d'Études Supérieures en Géographie* may be more equivalent to a master's degree. There is little similarity of system between one university and another and it is valueless to try to relate the *baccalaureat, licence, diploma* or *doctorat* with supposed English equivalents. The situation is perhaps less confusing for the British with their own anomalies as between Oxford, Cambridge, Edinburgh and the civic universities, than for academics from other countries where more uniform practice prevails. There is, however, at present a great deal of discussion under the auspices of the Quebec Ministry of Education on the subject of greater standardization throughout the Province.

Staffing and Equipment

The staff-student ratio in Canadian universities is akin to that which prevails in Britain and with ratios of 1:12 common would be regarded by many as quite favourable. However, the credit and course system means that each student may be attached to several departments and that departmental staff-student ratios are much less favourable in practice. Geography departments are expanding rapidly and many have adopted the interesting

policy of trying to secure overseas nationals as their regional experts. British, American, French and European geographers are much in evidence and doubtless will remain so until the newly emergent schools of geography begin to produce Canadian geographers in quantity. At present the opportunities for highly qualified geographers are widespread in Canada, and the Dominion also suffers a 'brain-drain' south across the border to the United States.

The very large number of students taking general courses in geography at the undergraduate level has led to the establishment of a grade of graduate assistants in most departments in order to help with practical classes, laboratories and field work. Graduate assistants normally study for higher degrees at the same time. Good honours degree students from Britain are especially sought for these posts and there should be a steady and continued demand for such for many years to come. The posts are financed in a variety of ways. Most universities award their own graduate fellowships and assistantships with stipends of 2,000 dollars and more. Commonwealth fellowships may be held in Canada and Provincial fellowships are available, notably in Ontario and Alberta, while the National Research Councils and the Canada Council also award scholarships and fellowships. Application for many of these awards may be made by United Kingdom subjects.

For the young geographer anxious to acquire overseas experience, research training and a higher degree, Canada offers a wide range of possibilities. Intending migrants should, however, match their research interests with appropriate localities and/or departmental interests. While most departments are broad-based, some have developed specialist interests or can offer specialist facilities. Each year most departments issue brief descriptive pamphlets which can be obtained on request.

The physical facilities available to geography in Canadian universities are rapidly catching up with, and in some cases moving ahead of, those available in the United Kingdom. Completely new buildings for geography have recently been occupied or are under construction in most universities where the subject is represented—notably Alberta (Edmonton and Calgary), Carleton, Manitoba, McGill, McMaster, Simon Fraser, Toronto, Victoria, Waterloo and Western Ontario. Canadian geographers, like their British colleagues, have given a good deal of thought and attention to departmental buildings over the past 5 years and they recently requested Prof. Hans Carol of York University, Toronto, to act as a central clearing-house for planning details. It is interesting to note that, whereas in the 1951 report on geography in Canadian universities Prof. Stamp included a plea for map laboratory space as an essential requirement, this is now taken for granted and many departments have included specialist laboratories for physical geography, climatology, cartography, photogrammetry and pedology. Technical, secretarial and draughting support does not yet match that available in the United Kingdom, but this can be more easily remedied.

Field Work

Unlike laboratory provision, field work in Canadian universities tends to lag behind that in Britain. There are in fact a number of inhibiting factors such as the length and rigour of the winter which admittedly makes field work difficult during much of the academic session, and this is aggravated by the early termination of the session in May. Field work is also not easy to fit into course and credit systems, and students who are working their way through college find it difficult to attend long-vacation field courses. Only honours and postgraduate students can be conveniently managed in the field when general groups run into the hundreds, while the immense and uniform extent of many physical and cultural landscapes makes comparative studies difficult and encourages the widespread use of air photographs as substitutes.

Canadian geographers, however, are grappling with this problem and in many cases with notable success. Courses on field work and field techniques are being introduced into the schemes of study either on a voluntary basis (for example, Carleton) or half credit basis (for example, Western Ontario), while in other departments (for example, Toronto, McMaster, Waterloo and Alberta) excursions are organized to areas of geographical interest in Canada or the United States. The province of Quebec has a long-standing field tradition with weekly excursions at Montreal and ten excursions during the session at Laval. The McGill Department of Geography has established field stations at Schefferville, Northern Quebec; at Barbados in the West Indies; at Rupununi in British Guiana; and at Axel Heiburg Island in the North West Territories. In this respect Canadian geographers have leapt ahead of their colleagues in Britain. Mention should also be made of the pioneer field studies being undertaken by the Geographical Branch of the Department of Mines and Technical Surveys. The field activities of this Government Department include expeditions involving substantial transport resources such as helicopters and light aircraft; organized as a regular and normal part of the work of the Geographical Branch, these Government activities are without parallel in Britain.

Summer Schools

Another feature of considerable importance is the summer school. Although summer schools are not held annually by all departments there are several normally available each year usually lasting from 6 to 8 weeks in July and August. The summer school has many functions in Canada. In some cases it grants credits towards a degree, in others it is a valuable refresher course for teachers. The summer school can also function as an alternative to sessional field work, which, as we have seen, can be difficult in the Canadian winter. The summer schools attract not only Canadians but also Americans, while some schools, notably McGill at Stansted and British Columbia at Vancouver, have become internationally known. It is customary at most summer schools to invite leading British, American and European geographers to contribute specialist courses in support of those given by the resident staff. Details of the summer schools are published by the organizing departments and are available on request.

Research

The full and continuing development of a subject depends ultimately on active research and publication. The steadily improving facilities and increasing number of geographers at work in Canadian universities in the past decade have greatly increased the volume of research work. In addition the Geographical Branch of the Department of Mines and Technical Surveys undertakes a great deal of research work; it is one of the six branches of the Department which is a complex federal research organization specializing in the earth sciences and related fields. The Provincial Government of Quebec also maintains a geographical research establishment known as the Centre d'Études Nordiques at Laval University.

Canadian geographers are now well served for the publication of their research. Pioneer work in this field was undertaken by the Société de Géographie de Québec, which published a Bulletin from 1880 until 1937 and, jointly with the Société de Géographie de Montréal, from 1942 until 1944. This gave place to the *Revue Canadienne de Géographie* which is the organ of both the Société de Géographie de Montréal and of the Institut de Géographie de l'Université de Montréal. The oldest uninterrupted series is the monthly *Canadian Geographical Journal* which has been published by the Royal Canadian Geographical

Society since 1930. In 1951 the newly-formed Association of Canadian Geographers began issuing the *Canadian Geographer* as a quarterly. It also produces a valuable annual report which lists geographical research publications and statistics relating to geography in Canadian universities. This association now has a membership of more than six hundred of whom half are associate or student members. Its British Columbia Division has been publishing *Occasional Papers* since 1960.

The Geographical Branch issues a range of publications including an annual *Bibliographical Series* and an irregular series of *Geographical Papers*, both since 1950, the *Geographical Bulletin* since 1951 and memoirs since 1953, as well as the Canadian atlases and gazetteers and various coloured maps. The *Geographical Bulletin* became a quarterly in 1965. The *Cahiers de Géographie de Québec* have been published by the Institut de Géographie de l'Université Laval since 1952 and the Centre d'Études Nordiques has produced a series of *Travaux Divers* appearing irregularly since 1963. Mention must also be made of *The Cartographer* which made its début as a twice-yearly publication during 1964. Although it is published by the Ontario Institute of Chartered Cartographers, it is strongly slanted to the cartographic interests of the geographer. Papers in Canadian periodicals are not necessarily in the same language as the title of the journal. English articles are often supported by a French summary and vice versa.

There are almost limitless possibilities for geography in Canada. Covering an area of 3·75 million square miles, its population is still only 20 million. Much of the territory has yet to be investigated and there are numerous problems in the fields of both physical and human geography awaiting attention. Hand in hand with the opening up of the country there is a growing demand for experts in geographical education, land use assessment, town and country planning, climatology, cartography, pedology, marketing and intelligence work—avenues of activity and careers into which the appropriately trained geographer fits with ease.

OBITUARIES

Prof. N. I. Grashchenkov

On October 8, 1965, one of the most outstanding Soviet neurologists and neurophysiologists, Prof. Nikolai Grashchenkov, died at the age of sixty-five at the height of his creative activity.

From the first, Prof. Grashchenkov strove in all his scientific activity to establish the physiological method in neurology. He understood clearly that the extremely subtle experiments which Nature performs by effecting the central and peripheral formations of the nervous system in man considerably enlarge the perspectives of creating real human physiology. Throughout his scientific activity, N. I. Grashchenkov carried these principles into practice. As part of his training, Grashchenkov spent 2 years at the most advanced physiological laboratories of the world at the Universities of Cambridge, New Haven, New York and Boston. When he returned to the Soviet Union, he placed experimental investigations at his offices on a broad footing.

During the Second World War, Prof. Grashchenkov turned his investigations in clinical neurophysiology towards the war effort, and at the same time he became consulting physician at one of the Field Forces. It was there that he formulated the hypothesis of the malfunction of the synaptic structures in the central nervous system being responsible for reversible disorders. He founded the doctrine of functional asynapsy in some pathological conditions of the brain and spinal cord, which still has great theoretical and practical importance.

In his investigations of synaptic processes in the central nervous system, Grashchenkov concentrated his attention on the role of bio- and physico-chemical interrelations in some forms of nervous pathology.

Together with his collaborators he published a large number of books, articles and monographs dealing with the role of the hypothalamus in the neuro-humoral-hormonal regulation of brain functions. These investigations were of great importance in the elucidation of the problem of homoeostasis in physiology and in clinical neurology. He showed that laboratory, clinical or physiological examinations alone did not give a reliable indication of the status and functions of healthy or sick organisms and of the work of different organs or physiological systems. These problems could be solved only by means of consistent dynamic study of physiological and biochemical parameters.

One of the features of the investigations carried out at Prof. Grashchenkov's laboratory was the application of dosed-out functional load, making it possible to find homoeostatic limits and in each case to expose the links most open to injury in the system of homoeostatic forces of the organism. This approach made it possible to put into practice a number of new therapeutic methods in the treatment of some forms of hypothalamic pathology.

Grashchenkov paid much attention to the physiological analysis of traumatic injuries of the brain and to the problem of muscular diseases, particularly myasthenia. The most important problems of neurophysiology, such as the questions of function localization in the brain, of sleep and arousal, behaviour and the orientating reaction, lay within the range of his interests. Grashchenkov's works on virus encephalitis are also well known, and his early results on this subject still retain their value and scientific significance.

Grashchenkov combined his scientific and pedagogical work with great organizational activity in the field of public health in the U.S.S.R. He held a number of responsible posts in the Ministry of Public Health, and for a number of years he was the president of the Byelorussian Academy of Sciences. In recent years, also, he was one of the leaders of the Physiological Department of the U.S.S.R. Academy of Sciences.

Grashchenkov's life shows the possibilities open to people of talent in Soviet society. The son of a peasant, a village cowherd, he took part in the Civil War and became one of the leading scientists not only in home but in world medicine. He was, for some years, assistant director-general of the World Health Organization; he repeatedly represented the U.S.S.R. in different international organizations, and participated in many international scientific symposia. In the person of Nikolai Grashchenkov, Soviet medicine has lost one of its most gifted and versatile representatives. G. Kassil

Dr. I. L. Chaikoff

Dr. Israel Lyon Chaikoff died on January 25, 1966, in Berkeley, California. He was born in London on July 2, 1902, and he grew up in Toronto. He graduated from the University of Toronto in 1924 and immediately embarked on his career of research in physiology, which started in association with the late Prof. J. J. R. Macleod, in the department where insulin had recently been discovered by Banting and Best. The impact of the discovery had many effects; one of these was to shape

the lives of contemporary young research workers in the medical sciences. One of the most distinguished of these was I. L. Chaikoff. He received the degrees of Ph.D. in 1927 and M.D. in 1930 from the University of Toronto, after which he joined the faculty of the University of California at Berkeley. Here he became a full professor in 1942. His laboratory maintained an unbroken flow of eminent contributions to biochemical endocrinology and metabolism. Many of his students continued their work in these fields as prominent members of the faculties of other universities.

Chaikoff was an originator in metabolic research, with the broadest of interests in biochemistry, medicine and nutrition. He was one of the first to introduce radioisotopes in biology and medicine, and he pioneered with iodine-131, phosphorus-32 and carbon-14 in research on the thyroid, the pancreas, and the liver. For years he investigated the biochemical changes following experimental pancreatectomy, especially with regard to the aetiology of fatty infiltration of the liver. He made numerous investigations in arteriosclerosis, phospholipid metabolism, cholesterol biosynthesis (including the role of squalene as a precursor), lipogenesis, the role of insulin in intermediary metabolism, fatty acid oxidation, carbohydrate metabolism, the action of oestrogens, anterior pituitary hormones, adrenal steroids, and other fields involved with the function of the body.

He shunned publicity and public appearances, preferring to devote himself to his laboratory, to the teaching and training of students, and to the encouragement of his large staff of scientific associates and collaborators, among whom he inspired many loyal friendships. He objected strongly to any form of pretentiousness and he had wide literary interests. He was a man of the highest standards in research and scientific writing and he was keenly conscious of the history and progress of the biological sciences. He was a prolific contributor to the leading scientific journals in his field, with a list of more than 400 publications in them. He held a Guggenheim fellowship in 1941–42, and a Miller research professorship during 1963–65. He was a Harvey lecturer in 1952, he received the Medal of the Endocrine Society of the United States in 1958, and he was faculty research lecturer at the University of California in 1958. He was married in 1949 to Isabelle Rawls, who survives him.

T. H. Jukes

NEWS and VIEWS

The Royal Society of Edinburgh

Sir Lawrence Bragg has accepted the invitation of the Royal Society of Edinburgh to deliver the James Scott Lecture, 1966, at the Society's ordinary meeting on March 6, 1967. The Keith Prize for the period 1963–65 has been awarded to Dr. Reinhold Fürth, formerly reader in theoretical physics, Birkbeck College, University of London, for his paper on the statistical thermodynamics of liquids published in the *Proceedings* of the Society during the period of the award and in recognition of his many valuable contributions in the same field.

Geology in the University of Cambridge : Prof. O. M. B. Bulman, F.R.S.

Prof. O. M. B. Bulman resigns from the Woodwardian chair of geology in the University of Cambridge in October; the chair is the oldest geological endowment in Great Britain. Having been successively University demonstrator in geology (1931–34), lecturer in palaeozoology (1934–44), and reader in palaeozoology (1944–53), he was elected to the chair in 1955. He had entered the University of Cambridge as an 1851 Exhibition Senior Student at Sidney Sussex College in 1926 following Ph.D. studies at the Imperial College of Science and Technology under Prof. W. W. Watts, and he returned to Cambridge in 1931 after holding teaching appointments in zoology and geology at Imperial College. Throughout his researches, Prof. Bulman has been predominantly a student of the Graptolithina, a group of fossils extensively used in classifying the Ordovician and Silurian strata. He has applied ingenious techniques and gifted powers of illustration to the better understanding of the morphology and evolutionary paths of these fossils. He and his research students have given remarkable impetus to studies of the group and of its occurrences in various parts of the world. Prof. Bulman has devoted much time to benefit the activities of scientific organizations and has held the office of president of Section C (Geology) of the British Association for the Advancement of Science (1959), the Palaeontological Association (1960–62) and the Geological Society (1962–64). In addition, for more than thirty years he has been a co-editor of the *Geological Magazine*. He is at present a member of the Natural Environment Research Council's Geology and Geophysics Committee and a Trustee of the British Museum (Natural History). At Cambridge he has developed a more diversified and adequately accommodated research school and has recently taken a prominent part in the reorganization of the Natural Sciences Tripos and postgraduate studies there.

Prof. H. B. Whittington

Prof. H. B. Whittington, who will succeed Prof. O. M. B. Bulman in October as Woodwardian professor of geology in the University of Cambridge, will take up that appointment with a record of world experience more varied than that of any previous occupant. A graduate, Ph.D. and D.Sc. of Birmingham, he went to Yale as a Commonwealth Fellow in 1938 and in 1940 moved to Rangoon to teach at Judson College. During the Second World War, he became attached to an ambulance unit in Burma, whence he proceeded to Ginling College, in Chengtu. In 1945 he returned to Birmingham, but the invitation which he accepted in 1949 to go to Harvard University as a visiting lecturer was rapidly turned into the offer of a more permanent appointment and for years he has been professor of geology at Harvard and curator of invertebrate palaeontology in the Museum of Comparative Zoology. His copious list of publications includes a worthy number relating to the stratigraphy of the British Isles and America and even one or two on the physiology of Szechuan, but it is as a palaeontologist, and especially as an expert on the trilobites, that he has achieved an international reputation. In 1955 he became secretary of the Palaeontological Society of America and he has just completed his term as president of that body. The Geological Society of London awarded him the Bigsby Medal in 1957. It is a testimony to his international reputation that he has been asked by the Geological Survey of Canada to direct excavations in the Middle Cambrian Burgess Shales of British Columbia which revealed to Walcott so much that was previously unknown and unsuspected in the animal world. Continuation of this direction after next summer will presumably be from Cambridge.

Applied Mathematics in the University of Manchester: Prof. L. Mestel

Dr. Leon Mestel, University lecturer and Fellow of St. John's College, Cambridge, has been appointed to a new chair of applied mathematics in the University of Manchester. Early in his research career, Dr. Mestel attracted notice by new ideas which he put forward on the energy source of white dwarf stars. Difficulties had arisen if the source were attributed to nuclear processes; Dr. Mestel showed that the difficulties were overcome if the energy came from a cooling process. Subsequent research has amply confirmed this theory. In 1954, he was awarded a Harkness Commonwealth scholarship. While at Princeton University he worked with Prof. Lyman Spitzer on problems of star formation. In their joint work, important new ideas on the significance of an interstellar magnetic field and of interstellar grains were put forward. These ideas have been incorporated in all subsequent discussions of the star formation problem. Returning to Cambridge, Dr. Mestel was appointed University lecturer in mathematics. In 1957 he was elected a Fellow of St. John's College. His interests in cosmic electromagnetism have continued, and in recent years he has been much concerned with applying this subject to problems of cosmogony and of stellar magnetism.

Mechanical Engineering in the University of Sussex: Prof. F. J. Bayley

Dr. F. J. Bayley has been appointed the first professor of mechanical engineering in the University of Sussex. Dr. Bayley has been reader in gas dynamics at the University of Newcastle since 1959, in the Department of Mechanical Engineering where he had previously been a lecturer since 1955. After graduating at Newcastle in 1948, he specialized in fluid mechanics, thermodynamics and heat transfer. For his original contributions to these subjects he was awarded successively the degrees of M.Sc. (Dunelm) in 1951, Ph.D. (Dunelm) in 1955, and D.Sc. (Newcastle) in 1964. After taking his first degree he was appointed scientific officer at the National Gas Turbine Establishment at Pyestock, where he remained for 3 years until he was appointed a research engineer at the Pametrada Research Station in 1951. Two years later he returned to the then King's College, Newcastle, where he held a Clayton research fellowship from the Institution of Mechanical Engineers until he was appointed a University lecturer. Dr. Bayley has become well known in recent years as a consultant on special regenerator and heat-exchange problems and has advised a number of firms and institutions in regard to the design of such equipment, both in Britain and in the United States.

William Whewell, F.R.S.—a Versatile Scientist

Among a number of scientists who have been eminent in more than one field, William Whewell, who died 100 years ago on March 6, was particularly remarkable. This Master of Trinity had so many activities as tutor and lecturer at Cambridge and as professor of mineralogy, as well as a science writer and author of full-scale texts, that it is surprising to look back on all his other work. The son of a master carpenter, Whewell went to Cambridge in 1812 and took various prizes, including the Chancellor's Medal for English, while all the time he was apparently a mathematician. He became second wrangler, wrote texts on mechanics, and always strove to improve mathematics teaching at Cambridge. Yet, after travels abroad, Whewell was equally noteworthy for dissertations on architecture, while in mineralogy and crystallography his papers in the *Philosophical Transactions of the Royal Society* as well as of the Cambridge 'Phil'—of which he was a founder member—were such that it seemed no surprise to find him elected to the chair of mineralogy in 1828. Whewell was a 'universalist', as his contemporaries put it. He naturally had critics, since he had a finger in many a pie, so much so that Sydney Smith commented: "Science is his forte, omniscience his foible". Yet though a theorist and philosopher rather than an experimentalist, Whewell benefited many of the branches of science in which he was interested. In geology, Whewell helped Lyell in nomenclature, while in electrical science students to-day scarcely realize that it was Whewell who introduced such everyday terms as 'ion' in his association with Faraday. Whewell was an acclaimed expert on the theory of tides, with fourteen memoirs in the *Transactions* of the Royal Society. President of the Geological Society and of the British Association for the Advancement of Science, and author of a classical three-volume *History of Inductive Sciences*, Whewell could write as well on astronomy as he could (after ordination) on theology. To appreciate the full breadth of his interests one must turn to the *Dictionary of National Biography*, in which whole columns are devoted to his publications alone.

British Government Aid Overseas

In reply to questions in the House of Commons on February 8, the Parliamentary Secretary to the Ministry of Overseas Development, Mr. A. E. Oram, said that British Government disbursements of economic aid were provisionally £189 million in 1965, compared with £160 million in 1963 and £191·6 million in 1964, the latter figures being 0·59 and 0·66 per cent of the gross national product, respectively. About £147 million of the 1965 total went to the Commonwealth. The Government's target for gross official aid in 1966–67 was £225 million. In a written answer, the Minister, Mr. A. Greenwood, stated that financial assistance was not being given to any African country which supported the illegal régime in Rhodesia. Britain's total bilateral economic aid to independent African countries in 1964–65 was £69·6 million, of which £17·1 million was for technical assistance; of this £63·7 million and £16·8 million, respectively, was to Commonwealth countries. In a written answer on February 10, Mr. Greenwood gave bilateral advances (including Exchequer advances to, but not total overseas investment by the Commonwealth Development Corporation) as £139·2 million in 1963, £173·2 million in 1964 and £172·3 million in 1965. Of these totals, £119·6 million, £153·7 million and £151·7 million, respectively, were for Commonwealth countries, and £22·5 million, £23·6 million and £28·6 million, respectively, for technical assistance to Commonwealth countries.

Articulated Ship

In reply to a question in the House of Commons on February 8, the Joint Parliamentary Secretary to the Ministry of Technology, Mr. R. Marsh, said that an articulated ship had been devised by a firm of naval architects. The National Research Development Corporation had already announced its willingness to consider sharing the cost of development of this ship as a joint venture if a suitable industrial partner could be found. The Corporation had for some years been supporting the development of *Dracones*, flexible containers that could be towed on water and used for the bulk transport of liquids. *Dracones* were already in production and operating successfully in several parts of the world.

Computer Programme Library

In reply to another question on February 8, Mr. Marsh said that the Computer Programme Library of the European Nuclear Energy Agency collected and distributed magnetic tapes on which were recorded data related to fundamental scientific and engineering problems in the nuclear field. The Library did not handle material relating to the evaluation of nuclear installations or

costings. Since it became operational in May 1964, the Library had received 299 computer programmes, of which 32 were from the United Kingdom. The Library was now nearing the end of its first three-year term and proposals relating to the Centre were being considered by the Government which would then decide on its policy towards the Centre.

Patent Office

In a written answer in the House of Commons on February 10, the Minister of State, Board of Trade, Mr. G. Darling, stated that six organizations had made representations to the President against moving the Patent Office from London. The President intended to arrange to move the Patent Office to a suitable site in Croydon, but the Library would remain in Central London and the Printing and Sales Branch at St. Mary Cray.

Committee on the Civil Service

In a statement in the House of Commons on February 8, the Prime Minister said that the Government accepted the recommendation of the Estimates Committee that a committee should be appointed to examine the structure, recruitment and management, including training, of the Home Civil Service. Lord Fulton had agreed to be chairman of this committee. The other members would be Sir Philip Allen, Sir Edward Boyle, Sir William Cook, Sir James Dunnett, Sir Norman Kipping, Prof. Lord Simey, Mr. W. C. Anderson, Mr. N. Hunt, Mr. R. Nield, Mr. J. Wall and Mr. S. Williams. The very broad terms of reference would require a fundamental and wide-ranging enquiry in the tradition of the great enquiries of the past, such as Northcote–Trevelyan in 1853 and the Tomlin Commission in 1931, and he hoped that its recommendations would enable the Civil Service to meet Britain's needs for many years to come. Mr. Wilson emphasized that the decision to set up this committee did not mean that the Civil Service had in any way been found lacking by the Government in its present operations. On the contrary, it was the experience of Ministers that the Service met the demands put on it with flexibility and enterprise. Nor did the willingness to consider changes in the Civil Service imply any intention of the Government to alter the basic relation between Ministers and civil servants. Civil servants remained the confidential advisers of Ministers, who alone were answerable to Parliament for policy: no change was envisaged in this fundamental feature of the parliamentary system. In reply to Mr. Heath, who welcomed the statement, Mr. Wilson said that it would be within the terms of reference and competence of the committee to enquire into all aspects of the functioning of and recruitment to the Civil Service. An important working party was already considering training, and the result of that enquiry would be made available to the new committee. While welcoming the report of the Estimates Committee on recruitment to the Civil Service as an extremely valuable contribution to the investigation of this subject, Mr. Wilson added that he did not necessarily accept all the conclusions of its report. He also added that it would be right for the committee to enquire into the question of exchanges between the Civil Service and the universities, business and local government. As regards wider recruitment, in the past 18 months there had been considerable success in attracting from universities and industry highly suitable persons to help with planning and with scientific and technological work, and there had been the greatest possible effort to widen the field of recruitment. The committee would also consider the possibilities of secondment and part-time service. A similar statement was made in the House of Lords, also on February 8, by the Earl of Longford, who, in reply to Viscount Dilhorne's question, assured him that the Legal Civil Service fell within the terms of reference of the committee.

British Government Aid Overseas

In the article appearing under the above heading on p. 1153 of the December 18, 1965, issue of *Nature*, the figures given as "British Government contributions in 1964–65" are, in fact, the contributions over the period 1946–47 to 1964–65. The figures for the year 1964–65 are, in fact, £9 million to the International Development Association, £3·6 million to the United Nations Expanded Programme of Technical Assistance and Special Fund, £1·9 million to the United Nations Relief and Works Agency, £0·3 million to the United Nations Children's Fund, £0·2 million to the United Nations Assistance to the Congo, £0·6 million to the World Food Programme and £3·2 million to the Indus Basin Development Scheme.

Medical Education in the United States

A conference to consider curriculum, programming and planning in medical schools and teaching hospitals was held by the New York Academy of Sciences during March 1965, and supported in part by grants from the Alfred P. Sloan Foundation, the Commonwealth Fund, the Russell Sage Foundation and the New York Chapter of the American Institute of Architects. A record of the conference has been published and includes papers on medical schools and teaching hospitals; curriculum, programming and planning; the mainstream of American medical education, 1765–1965; development of a new medical school; past experiences and considerations for the future; research in medical education participation of faculty and students; experimentation in medical education; the student, the patient and the university; biophysics and medical engineering; the relationship between medical education and medical practitioners; teaching and research; behavioural science in the medical curriculum; the science of nutrition in the medical curriculum; the humanities in the medical curriculum; budgeting the operation of the medical school; introduction to physical programming and planning; criteria for planning; long-range planning of the medical centre; and architectural programming and planning for the medical centre (*Ann. N.Y. Acad. Sci.*, 128, Article 2: *Medical Schools and Teaching Hospitals: Curriculum, Programming and Planning*. By Walter H. Blucher and 33 other authors. Pp. 457–720. New York: New York Academy of Sciences, 1965. 5 dollars).

Supply of Anti-serum in India

An indication of the problems in public health which confront an over-populated sub-continent such as India is shown in the annual report of the King Institute of Preventive Medicine, Guindy, for 1962–63 (By Dr. S. Govindarajan. Pp. 8+2. Guindy, Madras: King Institute of Preventive Medicine, 1965). The stock of anti-serum at the Institute was not satisfactory during the year under review, since no foreign exchange was released during the previous two years towards the purchase of foreign sera. Sera from indigenous sources were several times costlier and were not available in adequate quantities whenever they were required. The manufacture of anti-serum at the King Institute covers only a small part of the requirements, and thus purchases have to be made from abroad or from indigenous sources to supplement local manufacture. There are few institutions and firms in India which actually manufacture anti-sera. The majority of the firms which supply anti-sera are merely representatives of foreign firms, arranging the supply from other countries through the State Trading Corporation. The price of sera fluctuated widely and the sera were not available in adequate quantities. The time of supply was uncertain and could not be relied on. Such a situation created a heavy burden on the Department of Anti-toxins at the Institute.

Radiochemistry of Chromium and Rhodium

THE Sub-committee on Radiochemistry, one of several sub-committees working under the Committee of Nuclear Science within the National Academy of Sciences–National Research Council, is responsible for the publication of a series of monographs, each of which collects into one volume pertinent information required for radiochemical work with an individual element. These monographs are revised from time to time to bring the information up to date. Monograph *NAS-NS*-3007, issued in December 1964 and compiled by J. Pijck (University of Ghent), deals with chromium, and *NAS-NS*-3008, issued in May 1965 and compiled by J. C. Armstrong (Florida State University) and G. R. Choppin (Hazelton Nuclear Science Corporation, Palo Alto, California), with rhodium (*NAS-NS*-3007. *Radiochemistry of Chromium*. By J. Pijck. Pp. v+64. 75 cents. *NAS-NS*-3008: *Radiochemistry of Rhodium*. By James C. Armstrong, jun., and Gregory R. Choppin. Pp. vi+75. 1 dollar. Washington, D.C.: National Academy of Sciences–National Research Council. Available from the Clearing-house for Federal Scientific and Technical Information, National Bureau of Standards, U.S. Department of Commerce, Springfield, Virginia, 1965). Both monographs are revised and expanded versions of earlier editions. They include reviews of the nuclear and chemical properties of the elements, discussions of methods of sample dissolution and of separation reactions, descriptions of counting techniques and a compilation of radiochemical separation procedures.

Building Economics and Cost Planning

CERTAIN trends in the building industry, not only in Britain, but also in many other countries, are discernible in modern developments designed to break away from strictly traditional procedures, to establish firmly a more rational code of building economics and a new concept of what has come to be known as 'cost planning'. Contemporary studies of building conditions to-day have conclusively shown that purely technical problems of construction in developed countries are relatively insignificant when compared with problems of cost and labour estimating, budgetary control at all levels, and an urgent need to improve communications throughout the industry. This theme is the subject of a stimulating paper by T. W. Miners entitled "Some Aspects of Building Economics and Cost Planning" (National Building Research Institute, South African Council for Scientific and Industrial Research. CSIR ref. R/Bou 122. Reprinted from *South African Architectural Record*, 50, No. 2; February 1965). "Cost planning, in its widest sense, may be seen as an attempt to rationalize the cost aspects of a building project, from conception of the idea to build, through the design and construction phases and then finally throughout the life of the building until the period of economic obsolescence has been reached."

Mr. Miners discusses the different types of economic investigations undertaken in co-ordinating national interests, what he calls 'fringe' interests, and those of builders, group interests (such as local authorities), manufacturers, investors, and, last but not least, the interests of the client: ". . . he is the most important person to the industry". In the paragraphs on "Cost Planning and the Design Process", the technique of 'cost planning' implies the method used during the design process of keeping the cost of a building project within a predetermined sum; it includes preparation of the 'cost plan', and also the following stages of 'cost checking'. Fundamentally, this scheme of 'cost planning' is envisaged as a bridge between national, design, construction and investment interests; these are closely interrelated and dependent on each other. Some re-organization of the industry along these lines would appear to be inevitable and this essay makes a strong case for its serious consideration. Fully aware of the potential advantages of 'cost planning', the National Building Research Institute has already embarked on a programme of research in this field, including building economics in the wider sense, in collaboration with the Industrial Economics Division of the Council for Scientific and Industrial Research.

Arctic Stratigraphy

The Triassic Stratigraphy of Svalbard is an example of the major advances being made in many parts of the Arctic from the previous reconnaissance level of geological knowledge (Norsk Polarinstitutt. Skrifter Nr. 135. By S. H. Buchan, A. Challiner, W. B. Harland and J. R. Parker. Pp. 92. Oslo: Norsk Polarinstitutt, 1965. 15 kr.). It is the result of work carried out by the group directed in the University of Cambridge by W. B. Harland. It contains a review of many scattered earlier publications, but consists mainly of new and detailed observation, much of it clearly presented in diagrammatic measured sections and summarized in a fence diagram. A new scheme of stratigraphical subdivisions is proposed in line with modern international usage. A lengthy "Index to Svalbard Triassic Fossils" includes all published fossil determinations and provisional determinations of the new Cambridge material. Probably the most interesting section for the non-specialist reader is one comparing these Triassic rocks with those of other Arctic areas. This demonstrates the key role of Svalbard in working out the complex pattern of movements between the various Arctic–Atlantic landmasses.

Life Insurance Medical Research Fund of Australia and New Zealand

APPLICATIONS are invited by the Life Insurance Medical Research Fund of Australia and New Zealand for financial assistance to conduct research broadly related to cardiovascular function and disease. The Fund will award grants-in-aid to non-profit institutions in Australia or New Zealand, equipped with the basic facilities for research, to support a specific programme of fundamental or clinical research under the direction of an experienced full-time investigator. Such grants will be made initially for one to three years. The Fund is also offering research fellowships to university graduates to engage full-time in fundamental or clinical research. Such fellowships will be of two types: (*a*) fellowships to work in Australia and New Zealand will be granted initially for one year to medical graduates, who need not have previous research experience; (*b*) travelling fellowships will be granted for a period of two years abroad and a third year in Australia or New Zealand to graduates with at least two years' experience in research. Travelling expenses will be provided for the fellow and his family. Further information and forms of application can be obtained from Dr. H. M. Whyte, medical director, Life Insurance Medical Research Fund of Australia and New Zealand, Box 4134, G.P.O., Sydney, New South Wales. The latest date for receipt of applications is July 1, 1966.

Travelling Research Fellowships to Denmark and Sweden, 1966–67

APPLICATIONS are invited for the Carlsberg–Wellcome and Wellcome–Swedish travelling research fellowships, 1966–67. The object of these is to encourage working visits, on an exchange basis, between Danish and British, and Swedish and British, investigators in any branches of the natural sciences which have a bearing on human and animal medicine. One of each fellowship is awarded annually to a graduate from the United Kingdom for a year's work in the exchange country, and one Swedish and one Danish candidate annually may work in the United Kingdom. The stipend may range from £1,000 to £2,000 per annum; travelling and some incidental

expenses are provided in addition. The Carlsberg–Wellcome fellowships are arranged with the Carlsberg Foundation in Copenhagen; the Wellcome–Swedish fellowships are provided jointly by the Medical Research Council of Sweden and the Wellcome Trust. Candidates in the United Kingdom can obtain further information from the Secretary, the Wellcome Trust, 52 Queen Anne Street, London, W.1. Completed applications must be submitted by April 9, 1966.

Guinness Awards for Science and Mathematics Teachers

The Guinness Awards for Science and Mathematics Teachers are now being extended to Africa. They are being carried out in association with the Department of Education in Tropical Areas of the University of London Institute of Education, Malet Street, London, W.C.1, together with the assistance of a number of Departments and Institutes of Education in Africa, Ministries of Education and the British Council.

Nature Subscriptions

It is regretted that the charges for direct postal subscriptions to *Nature* printed on the back page of the index for Volume 207 are incorrect. The present charges are:

	Inland	Overseas
12 months	£14 0 0	£13 15 0
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Payment from abroad should be made by International Money Order or by cheque payable to Macmillan (Journals), Ltd. (Macmillan (Journals), Ltd., is now at 4 Little Essex Street, London, W.C.2.)

Organization of the Scientific Civil Service

It has been brought to the Editor's notice that the fourth paragraph of the review of the Report of the Tennant Committee, published on p. 113 of the December 18, 1965, issue of *Nature*, is open to misinterpretation. The figures given in the first sentence of the paragraph relate to the unestablished staff referred to in the preceding paragraph. Thus, in the scientific and experimental officer classes taken together, 80 per cent of the officers serving in a temporary or unestablished capacity have been employed in the Civil Service for 5 years or less, and in the scientific officer class 60 per cent and in the experimental officer class 50 per cent of the unestablished staff have been employed for 2·5 years or less.

University News:

Edinburgh

Dr. R. M. Cormack has been appointed senior lecturer in statistics. The following lecturers have also been appointed: Dr. C. P. Mayers (pathology); Mr. D. A. Williams, Mr. C. W. D. Peare and Mr. E. F. Harding (statistics).

London

A chair and Research Group in Traffic Studies have recently been established at University College, London, and Prof. R. J. Smeed, formerly deputy director (traffic and safety) of the Road Research Laboratory, and more recently chief scientist at the Ministry of Land and Natural Resources, has been appointed holder of the chair and director of the Group.

Manchester

Prof. D. H. Valentine, professor of botany in the University of Durham, has been appointed George Harrison professor of botany and director of the Experimental Grounds in succession to Prof. C. W. Wardlaw, who is retiring on September 30. The following appointments have also been made: *Readers*, Dr. A. Allsopp (cryptogamic botany); Dr. K. J. Dormer and Dr. E. W. Simon (botany); Dr. W. C. E. Higginson, Mr. O. S. Mills, Mr. N. L. Paddock and Dr. G. F. Smith (chemistry); Dr. F. P. Stainthorp (chemical engineering); *Senior Lecturers*, Dr. F. R. Jevons (biological chemistry); Dr. L. Barden (soil mechanics); Dr. R. A. J. Shelton (metallurgy); Dr. G. A. Lumb (medical chemistry); Dr. G. Taylor (immunology); Dr. G. C. Wood (chemical engineering); Dr. W. D. Humpage, Dr. D. C. Northrop, Dr. L. M. Wedepohl, Dr. T. E. Broadbent and Dr. D. Tipping (electrical engineering); Mr. S. V. Fagg (mathematics); Mr. N. D. Whitehouse (mechanical engineering); Dr. I. G. Edmunds (physics); *Lecturers*, Dr. S. Dunstan, Dr. M. G. Barlow and Mr. M. J. Ware (chemistry); Dr. G. S. Taylor (botany); Dr. J. A. Joule (organic chemistry); Mr. D. G. Buchanan, Mr. B. E. Jones and Mr. P. Swarbrick (electrical engineering); Dr. J. M. Anketell and Mr. J. Esson (geology); Mr. R. D. Gregory, Mr. J. A. King-Hele, Mr. J. T. Smith, Dr. G. Christensen and Dr. D. A. Lavis (mathematics); Mr. P. Wilkes (metallurgy); Dr. J. M. Irvine (theoretical physics); Mr. J. E. B. Ponsonby (radio-astronomy); Dr. R. T. W. L. Conroy (physiology); Dr. A. J. Barson, Dr. A. Garner and Dr. R. S. Kirk (pathology); Mr. G. H. West (polymer and fibre science); Dr. N. Jones and Mr. J. T. Turner (mechanical engineering).

Announcements

Mr. J. H. Nicholas and Mr. M. E. Burt have been appointed deputy chief scientific officers at the Road Research Laboratory: Mr. Nicholas has been appointed head of the Construction Division, and Mr. Burt head of the Design Division.

Mr. H. Ll. D. Pugh and Mr. A. H. Low have been awarded the W. H. A. Robertson Medal and Premium for 1965 by the Council of the Institute of Metals, for their paper, "The Hydrostatic Extrusion of Difficult Metals", which was published in the March 1965 issue of the *Journal of the Institute of Metals*.

The 1966 congress of the Institute of Animal Technicians will be held in the University College of North Wales during March 31–April 1. Further information can be obtained from Mr. A. E. Mundy, 5 Ouse Bridge Drive, Carlton, Nottingham.

The first meeting of the newly formed North East Section of the Society for Analytical Chemistry will be held in Durham on March 24. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A symposium on "Scottish Estuaries", arranged by the Institute of Biology (Scottish Branch), will be held in the University of Strathclyde during March 31–April 1. Further information can be obtained from Dr. E. J. Perkins, Department of Applied Microbiology and Biology, Biology Annexe, University of Strathclyde, George Street, Glasgow, W.1.

A joint meeting of the North of England Section of the Society for Analytical Chemistry and the Leeds University Union Chemical Society will be held in the University of Leeds on March 18. Prof. C. L. Wilson will deliver a paper on "Analysis Reborn". Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A conference on "Nuclear Structure and Elementary Particles", arranged by the Nuclear Physics Sub-committee of the Institute of Physics and the Physical Society, will be held in the University of Oxford during March 30–April 1. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A symposium on "Tape Recording of Biological Signals", organized by the Scottish Section of the Institution of Electronic and Radio Engineers and the Scottish Electronics and Control Section of the Institution of Electrical Engineers, will be held in Edinburgh on March 29. Further information can be obtained from Mr. P. M. Elliott, 21 Craigmount Loan, Corstorphine, Edinburgh 12.

NATIONAL RESEARCH DEVELOPMENT CORPORATION

THE sixteenth annual report of the National Research Development Corporation*, covering the year ended June 30, 1965, again emphasizes a marked increase in the Corporation's activities; to cope with the anticipated increase, major changes were made in the organization of the Corporation. The two Departments of Applied Science and Engineering have been divided into groups for Bio-Sciences, Industrial Chemistry and Scientific Equipment, and for Electrical Engineering and Electronics, Computers and Automation, Mechanical Engineering, and Production Engineering, respectively, and a Department of Planning and Policy Co-ordination has been established. Financial assistance was provided for more than thirty-five new projects (representing an initial investment of £5,759,000) compared with thirty in the previous year, as well as additional support of about £2 million for existing projects including hovercraft. Actual expenditure on development was £591,339 compared with £457,366 in the previous year, and forward commitments now amount to £8,653,000. Nine items of development expenditure totalling £128,000 were written off, and ninety-five development projects were in being at the end of the year compared with sixty at the end of the previous year. During the year, fifty-six new licence agreements with firms in the United Kingdom and overseas were completed, and nineteen terminated, compared with seventy-one and thirty-three, respectively, in the previous year, making a total of 593 licence agreements in force in the United Kingdom and 56 overseas (561 and 58, respectively, in the previous year).

Two major changes during the year noted in the report were the transfer in February 1965 of statutory responsibility for the Corporation from the Board of Trade to the Minister of Technology and the extension of the Corporation's financial resources and the increase of its scope under the Development of Inventions Act, 1965, passed in June. The Corporation believes that its future business will continue to be concerned mainly with supporting technological development, but that work of a high-risk, long-term, or applied-research nature will be more extensively supported where this is judged to have a probable commercial advantage for an industrial user. Even more extensive may be the support of innovation in industry, and for this purpose the Corporation's experience in furthering the exploitation of its own projects may be more generally applied. The Corporation also affirms its belief that in all significant developments where Government spending with industry is involved, it has much experience to offer in the conduct of joint ventures with firms. It considers that Government departments could well profit from closer contact between the research establishments and itself. It also believes that further steps should be taken to increase the awareness of industry of the help which the Corporation could give. The report reiterates the view that in future the proportion of projects in which industry and the Corporation share the risks will increase and that a large increase in the number and scope of applications for assistance from industry can be expected.

Of the 1,122 inventions communicated to the Corporation during the year, 276 were from Government departments and Research Councils, 168 from universities, nine from industrial research associations, 93 from private firms and 510 from private individuals in the United Kingdom. Of 183 patent rights assigned to the Corporation during the year, 134 were from Government departments and Research Councils, thirty-four from universities and two from industrial research associations. At the end of the year, the Corporation held 377 United Kingdom patent applications and 817 granted patents, as well as 912 overseas patent applications and 1,346 granted patents. A further 113 United Kingdom patent applications and forty-nine granted patents were held by the Corporation's subsidiary development companies, as well as 443 overseas patent applications and 432 granted patents. The 1,226 United Kingdom patents and patent applications which are the subject of exploitation include some awaiting formal assignment. Receipts from exploitation amounted to £436,853. The Corporation is represented on the Co-ordinating Committee on Technology for Developing Countries and has supported projects intended to meet the specific needs of the developing countries. The Joint Development Committee with the Department of Scientific and Industrial Research no longer exists.

Among new projects started during the year under the Bio-Sciences Group are those for a bollworm attractant, a process for a component of human follicle stimulating hormone, fish-drying equipment, a locust attractant from grass and a typhoid vaccine. Under the Industrial Chemistry Group the report notes new projects for bonded boron carbide, metal polishes and a plastic sealing process; and under the Scientific Equipment Group, for an endo-microscope, a histogram plotter, membranes for heart–lung machines, and a project for a library of teaching programmes on industrial training for use with teaching machines.

The Computers and Automation Group records new projects for the automatic process and plant control system known as *ARCH*, for developing computer hardware and software, and for a magnetic tape recorder and a magnetic tape store. The Electrical Engineering and Electronics Group gives details of new projects for a beta-backscatter gauge and a high-background radiation monitor and reports that the first batch of six instruments for a photosonic blind aid has been completed. Work continues at the Imperial College of Science and Technology on the fundamental aspects of two systems of television bandwidth compression although support from the Corporation was terminated at the end of 1964. New projects in the Mechanical Engineering Group are for the development of a large Diesel engine of advanced and novel (opposed piston) design, and epicyclic gears. The Production Engineering Group reports schemes for a new type of tunnel kiln (hoverkiln) for firing clay and glazed ceramics and pottery, and a numerically controlled turning machine. At the time when the report was published the development and test programme for flexible containers—the *Dracone* project—was almost complete and the original fuel cell programme was expected to be completed by the end of 1965. Satisfactory progress is reported with the oil well drilling rig project, and further progress with the development of hovercraft is also recorded, including the initiation of a joint programme of research at the National Physical Laboratory.

* National Research Development Corporation Sixteenth Annual Report, 1964–65. Pp. 53+8 plates (London: H.M.S.O., 1965) 5s. net

THE LEVERHULME TRUST

THE fifth report of the Leverhulme Trustees* covers the years 1962–64, in which commitments totalled £1,230,950, of which £591,650 was to the universities and centres of higher education, £130,950 to learned societies and research institutions, and £193,700 to medical research; these figures compared with £885,350, £371,150, £46,550 and £184,900, respectively, in the triennium 1959–61. Among the grants to universities may be noted £3,750 to the Department of Zoology, University of Cambridge, for taxonomic work; £11,000 to the Department of Sociology, University of Essex, for a survey of journalism; £1,300 to the University of Keele for research into municipal government; and £18,500 to the University of Lancaster for research into higher education. A further £3,000 went to the University of Leeds for research posts in the history and philosophy of science, and £24,000 for the proposed diploma course in development administration for overseas students. The University of Liverpool received £34,000 for fellowships in general research; the Institute of Education, London, £18,000; the London School of Economics, £12,950 for scholarship and research awards and £2,400 towards a conference on the philosophy of science. The University of Manchester received £2,700 for a research fellowship in the Department of Chemistry on the metabolism and biosynthesis of unsaturated fatty acids, £4,000 for an investigation of income distribution in the United Kingdom before and after tax, and a final grant of £6,000 for the Jodrell Bank Experimental Station. The Department of Chemistry, University of Newcastle upon Tyne, received £3,600 for an investigation of the enzyme, catalase, in the decomposition of hydrogen peroxide, and the University of Nottingham £8,900 for a project in adult education through the medium of television. A grant of £35,000 to Queen's University, Belfast, was for staff for the School of Librarianship, while £11,550 goes to the University of St. Andrews for industrial health courses at Queen's College, Dundee, and £22,500 to the University of Southampton for the Department of Econometrics. Overseas grants included £18,000 for tutorial staff for University College, Dar-es-Salaam; £28,000 for Royal Society Leverhulme visiting professorships for India; £24,500 for the Inter-University Conference, Hong Kong; £15,000 to University College, Nairobi, for adult education; and £18,000 for tutorial staff at University College, Makerere.

The Association of Commonwealth Universities received £6,600 for travel grants for the ninth quinquennial conference, the British Association £3,500 for analysis of mathematics teaching projects, the British Museum £3,500 for a research fellowship on mammals, the Institute for Strategic Studies a further £4,500 for a research assistant, the Joint Mathematical Council for the United Kingdom £6,000 for research into teaching mathematics, and the Institute of Mathematics and its Application another £8,000 for the same purpose. £70,000 went to the Royal Society for Leverhulme Scholarships for young scientists, and £3,000 to the Scott Polar Research Institute for a research assistant.

Awards to schools, chiefly for scholarships, totalled £36,400; to architecture, £48,600, including £9,000 to the Royal Institute of British Architects for an industrialized building study; and miscellaneous grants included £15,000 to the Administrative Staff College, £20,000 to the Centre for Educational Television Overseas for English Language Teaching, £15,000 to Voluntary Service Overseas, and £5,000 to the Overseas Development Institute for an economic survey of Uganda. Among those for medical research may be mentioned £22,500 to the Department of Electrical Engineering, Imperial College of Science and Technology, for a one-year course for post-graduate medical students; £20,000 to the Liverpool School of Tropical Medicine for a visiting lectureship, £25,000 to the Middlesex Hospital Medical School for scholarships at the Institute of Clinical Research; £32,400 to the Eastman Dental Hospital for visiting Indian and Pakistani studentships; £13,500 to the Royal College of Surgeons for research assistantships at the Institute of Basic Medical Sciences; £8,300 to the Institute of Orthopaedics for a research fellowship in the Department of Biomechanics; and £6,600 to the Department of Mental Health, University of Aberdeen.

* The Fifth Report of the Leverhulme Trustees, 1962–1964. Pp. 93. (London: The Leverhulme Trust, 1965.)

SELECTION OF CLAY BUILDING BRICKS

DEFECTS in brickwork, when these occur, are not necessarily due to faulty design or construction, but often probably because the properties of the bricks used in a particular building or other project are not given enough serious consideration in the first place, relative to the proposed conditions which they are destined to withstand. For example, constructional brickwork should not, be or remain, excessively wet, since clay building bricks differ in their tolerance of exposure to wet conditions; wet brickwork lends itself to risk of sulphate attack on the mortar, to efflorescence, and to frost damage; sulphate expansion of mortar rarely occurs except in parapet and retaining walls subject to continual water infiltration; where sulphate expansion of brickwork in normal external walls does occur, this is often due to the bricks themselves containing excessive soluble sulphates, for example, potassium sulphate. This advice is among the many practical hints given in *The Selection of Clay Building Bricks*—1*.

Criticisms are often voiced on the admittedly ugly, white, powdery growth or encrustations revealed on brickwork, especially on new buildings, to the effect that this is due to use of newly manufactured bricks, not properly kilned, or possibly not long enough 'seasoned' or to use of inferior raw materials, and so on; actually, as this pamphlet is at pains to emphasize, efflorescence is normally a temporary springtime occurrence on new brickwork and is generally harmless; it happens because very small amounts of alkali salts, usually derived from cement in the mortar, are latent to produce this blemish given the right conditions. "Examples of destructive efflorescence, where crystallization of soluble salts causes crumbling of brick surfaces or displacement of plaster, are rare—probably rarer than they were a generation ago, to judge from enquiries received at the Building Research Station." Efflorescence can recur every spring for many years, so long as water can find ways of percolating through the brickwork; in parapet walls this often implies faulty damp-proof courses; none the less, it is on all occasions unsightly, but difficult to eradicate completely; beyond remarking that the precautions on site necessary to reduce

* Building Research Station Digest (Second Series), No. 65 (December 1965): *The Selection of Clay Building Bricks*—1. Pp. 4. (London, H.M.S.O., 1965) 4*d*

or avoid efflorescence are tedious and seldom observed in practice, no suggestions are forthcoming on how best to eradicate this trouble; superficially this may be done by various expedients, but the result is seldom permanent, if conditions inside the brickwork are unfavourable or if there is recurrent reaction between water and vulnerable constituents in the cement.

On the question of frost damage to bricks, this appears to be rare in normal external walls between damp-proof course and eaves, although not uncommon in parapets, free-standing walls, and retaining walls, especially where insufficient care has been exercised in selection of special quality bricks. Thermal, and particularly moisture, expansion of bricks are important factors; the former happens with all building materials, its effects are well known and usually allowed for at the design stage; the latter form of expansion is not so generally recognized, this property only receiving scientific attention in recent years. The practical implications of moisture expansion, as set forth in this publication, may be summarized thus: bricks should not be used straight from kiln, or on site unless stacked for at least one week; special attention must be paid to storage before use if bricks are to be laid in high-strength mortar, which will become rigid very quickly; short returns in brickwork, specially vulnerable to cracking by expansion, are to be avoided; in long walls, expansion joints should be provided capable of accommodating an expansion of $\frac{3}{8}$ in. in 40 ft. Quality control testing of bricks, envisaged in the new British Standard 3921 : 1965, is here strongly and rightly urged; acceptance of 20-year-old test reports, still occasionally proffered by manufacturers of bricks, should be refused.

MINERALS OF THE SEA

A SURVEY and understanding of the geology of the sea-floor, implying the four oceans and the shallower seas that feed them, equally a knowledge of the organic and inorganic constitution of sea-water itself, are not new concepts in the realms of exploration or exploitation of the resources of the Earth, but much more is to-day being heard and learnt of this fascinating subject because of the universally intensified research efforts being made in its contemporary study. From the original *Challenger* expedition to the present *Mohole* project, the story of the oceans and that greater part of the Earth's crust which they conceal, has been slowly but very surely growing. Scientific interest, in stature, far outweighs that of economic, commercial investigations into the problems of the oceans, but that state of things is probably destined to be upset, if not reversed, in the not-too-distant future. All over the world the energy, food and mineral resources of the oceans are being re-appraised, explored and, where feasible, exploited in the interests of mankind. Britain is by no means behind in the quest, and this does not mean simply 'North Sea oil and gas prospects'.

In September 1965 there began a relatively little publicized investigation into the possibilities of long-term exploitation of the oceans and their resources, sponsored by the Commercial Oceanology Study Group, a consortium of six British companies: British Petroleum, Richard Costain, Hawker Siddeley Group, Imperial Chemical Industries, Rio-Tinto Zinc Corporation, and Unilever. This joint project is expected to last at least a year. According to an article entitled "Minerals of the Sea" (*BP Magazine*, No. 17; Autumn 1965. The British Petroleum Co., London), each company is already working in different but related fields of oceanology; each will ultimately contribute its own particular knowledge and experience to the study. The article itself is an extract from the book, *World Beneath the Oceans*, by Dr. T. F. Gaskell (courtesy of Aldous Books, London, 1965); Dr. Gaskell, with Mr. D. C. Ion, both of British Petroleum, are members of this Study Group.

This particular article is devoted specifically to the minerals of the sea, the salts it carries and deposits, and the natural cycle of meteorological and geological events which, through millions of years of time, have given rise to them. Salinity measurements through the entire width and depth of the oceans have been going on during the past eighty years or so, and the chemical composition of sea-water is well established; the problem is, how are we going to extract or mine some of the more valuable products it contains on an economic scale? As Dr. Gaskell rightly points out, shortage of terrestrial fresh-water, as world population increases, must inevitably be made good by water processed from the sea; in fact, water will become one of the most important products of the sea. Other examples are magnesium (there are 4 million tons of magnesium dissolved in every cubic mile of sea-water), for which the process is already perfected; bromine, for the photographic industry and for producing anti-knock compounds for petrol; gold, the pipe-dream of men for years past: ". . . there is more gold in the sea than there is in use now", but commercially this problem has not yet been solved. Iodine is obtained from plants and animals of the sea; lobsters collect cobalt; the oyster gathers stray atoms of copper; and so on. The origin of petroleum, actually still an unsolved mystery, is in modern theories intimately bound up with the sea; sooner or later this secret will be unfolded, perhaps when we know more about the ocean floor itself.

THE MOLE: ITS ADAPTATION TO LIFE UNDERGROUND

THE increased economic importance of the mole (*Talpa europaea*) as a farm pest has engendered renewed research into its behaviour and structure. The Ciba Foundation guest meeting on "The Mole: its Adaptation to an Underground Environment", held on January 5 and 6, was attended by approximately thirty persons and eleven papers were contributed covering a wide variety of topics. There were also several films, tape recordings and demonstrations, providing a very varied programme.

The first day of the meeting was mainly concerned with behaviour. At the beginning of the symposium the mole was considered to be almost exclusively subterranean, continually burrowing in search of food. By the end of the meeting this picture had changed greatly. Dr. K. Mellanby showed that different moles used established burrows over and over again. Natural flooding proved that they could migrate over considerable distances and return after flood waters had receded. Mr. A. T. B. Rudge had succeeded in developing a trap for catching live moles. He had constructed monitored artificial burrows of wire mesh and shown that moles had periods of activity lasting 2–3 h, followed by rest periods of 3–4 h. This rhythm continued day and night. These findings were confirmed in the field by tagging moles with radioactive silver or cobalt sources attached to their tails. In their active periods they were continually running along their burrows (maximum speed up to 2·5 m.p.h.), only stopping for food that had fallen by chance into their runs. Moles did not often tunnel except while making

repairs. Mr. P. Morris showed, by investigating food taken by various animals (for example, species of owls), that moles must often come to the surface, probably mostly at night, to be caught by these predators. Other investigations in France under freak drought conditions gave supporting evidence, as many moles were caught in traps placed on the ground surface. Dr. F. Raw gave details of the food theoretically available to moles. Earthworms, wire-worms, molluscs and insect larvae seemed to form the possible basic diet. Mr. D. W. Yalden finished the day's session by describing the skeletal adaptations to locomotion. The session on behaviour thus presented a very different view of the behaviour of the mole to that normally assumed. It habitually uses preformed tunnels, during its waking periods it is continually searching for food, which it finds by chance encounter as it moves through its tunnels, and it is occasionally a surface dweller, probably mainly at night.

The second session, on microscopy, began with an account of the mole's pituitary gland by Prof. R. L. Holmes, who showed that there were no basic differences from the pituitary gland of other animals. The senses of the mole were described by Dr. T. A. Quilliam. The eyes had a very small number of receptor elements and there was a cellular lens. The mole had a snout packed with Eimer's organs that probably served for tactile reception. There were also innervated vibrissae around the snout and in the tail. The ear was little different to that in other animals, although there were less than two turns to the cochlea. The fine structure of the olfactory mucosa was considered by Dr. P. Graziadei. He showed that the olfactory mucosa was large in extent (as were the olfactory bulbs of the brain). The receptor cells were ciliated, although some of these cells bore only one cilium and were not so closely packed together as in other mammals. The mucosa contained more supporting cells. Drs. R. Lund and J. Lund considered the central visual pathways and showed that they were poorly developed although moles could be taught to discriminate between light and dark. Dr. B. H. Crawford outlined physical methods that were available to investigators to allow recordings to be made in the mole's tunnel, of light, sound, vibrations, heat and some other modalities, to see what sensory clues of the surroundings were available. Dr. M. Lobban outlined her studies on Arctic coal-miners and explained how the circadian rhythms were affected by the unnatural conditions under which the men work, in some ways analogous to those experienced by the mole. She pointed out that moles would be very useful animals for investigation of circadian rhythms. It seemed from the papers in this section that the mole has eyes that have little discriminatory abilities, a very well-developed olfactory sense, an auditory sense and complex tactile receptors in the vibrissae and in Eimer's organs in the snout. Whether the latter organs could detect other environmental features was left uncertain.

The symposium is eventually to be published in full as a supplement to the *Journal of Zoology, Proceedings of the Zoological Society of London.*

V. C. Barber

SCATTERING OF ELECTROMAGNETIC WAVES BY ELECTRO-ACOUSTIC PLASMA WAVES

By P. F. LITTLE and S. M. HAMBERGER

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THIS article reports some preliminary measurements of the scattering of an incident beam of microwaves by self-excited plasma waves in a mercury arc column; from these results we derive an experimental dispersion curve for the plasma waves which we compare with a dispersion curve measured directly with Langmuir probes in the column. In this manner we identify the waves responsible for the scattering as propagating electro-acoustic waves, which are known to be present in the positive column of low-density arcs. Both self-excited and externally excited waves of this type have been observed[1,2]. Their phase velocity is approximately $c_s = (KT_e/M)^{\frac{1}{2}}$, where KT_e is the electron thermal energy and M the ion mass: in our plasma $c_s \approx 10^5$ cm/sec.

Let us write for the incident electromagnetic wave the frequency and wave vector ω_1 and $\mathbf{k}_1$; the fluctuations in plasma density may be represented by a spectrum of

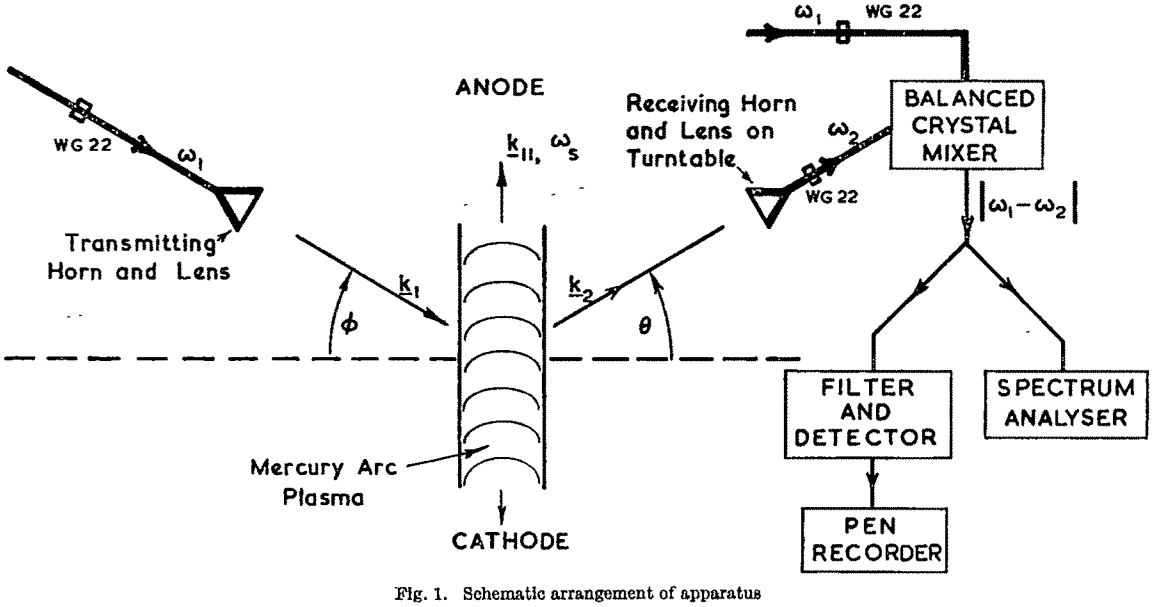


Fig. 1. Schematic arrangement of apparatus

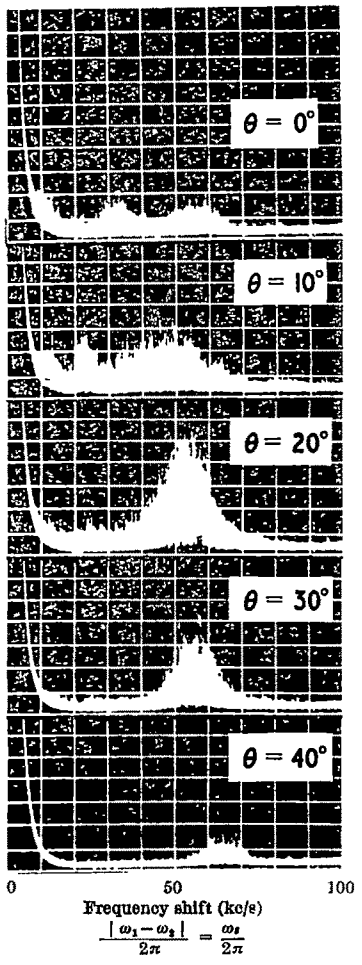


Fig. 2. Spectrum of scattered radiation. Each photograph consists of twenty superimposed traces showing the scattered intensity as a function of frequency shift. The sequence shows how the received spectrum depends on the angle of observation θ for φ = 0

frequencies ω_s and wave vectors $\mathbf{k}_s$. Then, as a result of non-linear interactions in the plasma, the scattered microwave spectrum will be described by typical quantities ω_2, $\mathbf{k}_2$ such that

$$\omega_1 \pm \omega_s = \omega_2 \quad (1)$$

$$\mathbf{k}_1 \pm \mathbf{k}_s = \mathbf{k}_2 \quad (2)$$

From equation (1) we may equate the frequency shift $|\omega_1-\omega_2|$ on scattering to the scattering frequency ω_s. From equation (2) we would expect the scattered wave observed at a particular angle to be due to a particular value of $\mathbf{k}_s$. More specifically, since we are interested in $k_{\|}$, the component of $\mathbf{k}_s$ along the column, equation (2) becomes (see Fig. 1):

$$k_1 \sin\varphi \pm k_2 \sin\theta = k_{\|}$$

or, since $k_1 \approx k_2 = 2\pi/\lambda_0$, where λ_0 = free space wavelength of the incident waves:

$$k_{\|} = \frac{2\pi}{\lambda_0}(\sin\varphi \pm \sin\theta) \quad (3)$$

In our experiment the plasma was the column of a 5-cm-diameter low-pressure mercury arc, as previously described[1], which was operated at 9-amp discharge current and a pressure of 0·4 mtorr. A beam of millimetre microwaves (λ_0 = 8·65 mm) at a power level of a few mW was used to illuminate a short section of the arc (Fig. 1). The receiver could be oriented to observe the same section from different angles. A single klystron provided both the probing signal and the local oscillator drive to the balanced mixer, the output of which therefore consisted of a signal at a frequency $|\omega_1-\omega_2|$, that is at ω_s, which could be observed directly with a low-frequency spectrum analyser.

Fig. 2 shows the spectrum analyser display of the amplitudes of the various frequency-shifted components of the scattered radiation at several different angles to the column, when illuminated at normal incidence (φ = 0). It is obvious from the sequence of spectra shown that there is a large signal around 52 kc/s which has a maximum intensity when the scattering angle θ is about 20°. To obtain a more accurate measure of the optimum scattering angles for a wide range of frequencies ω_s the mixer output was passed through a filter, rectified, and plotted on a chart recorder, while the angle θ was varied between +30° and −50° by rotating the receiver on a turn-table driven by a synchronous motor. Disregarding some spurious maxima associated with the optical system (which did not change with the selected frequency) a maximum or sometimes two maxima corresponding to the ± signs in equation (3) could be found for each frequency in the range 20–70 kc/s. Fig. 3 shows the result obtained for a filter tuned to accept 50 kc/s. From values of θ corresponding to the maxima the values of $k_{\|}$ were calculated from equation (3). The results are shown as a dispersion plot in Fig. 4 for normal incidence (φ = 0) and for φ = 20°.

For comparison we also give in Fig. 4 the measured values of ω_s and $k_{\|}$ for electro-acoustic waves excited in the same plasma by an external coil and detected by a Langmuir probe[3]. A third independent set of observations is obtained by applying the correlation technique used with photomultipliers in earlier work here[1] to the signals from two Langmuir probes, one fixed and one moving. This is an analysis of self-excited waves in the column, leading to the results shown. Within the limits of experimental accuracy all the points lie on a common curve except those for microwaves incident at φ = 20°. For these the dispersion plot appears to be displaced by a small systematic error possibly due to refraction at the tube walls.

Using the theory of Woods[4] and the measured electron temperature, $KT_e = 3{\cdot}4 \pm 0{\cdot}2$ eV, we derive the theoretical dispersion curve for the (0,1) mode shown in Fig. 4. It is in excellent agreement with observation at low frequencies, but at higher frequencies lies above the experimental values, as previously reported[3].

It should be noted that a small but finite value of $k_{\perp}$ is essential to satisfy equation (2) with this experimental arrangement unless φ = θ. The finite size of the plasma alone makes $k_{\perp} \neq 0$, and in addition the wave fronts of

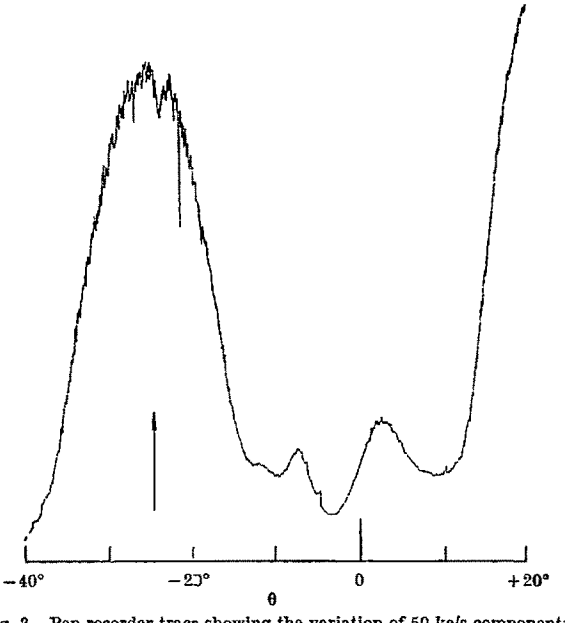


Fig. 3 Pen-recorder trace showing the variation of 50 kc/s components of the mixer output with angle of observation θ. The peak at θ = −24° corresponds to a value of $k_{\|}$ = 2·9 cm^{-1}. The structure at θ = 0° appears at all frequencies and is attributed to geometrical effects

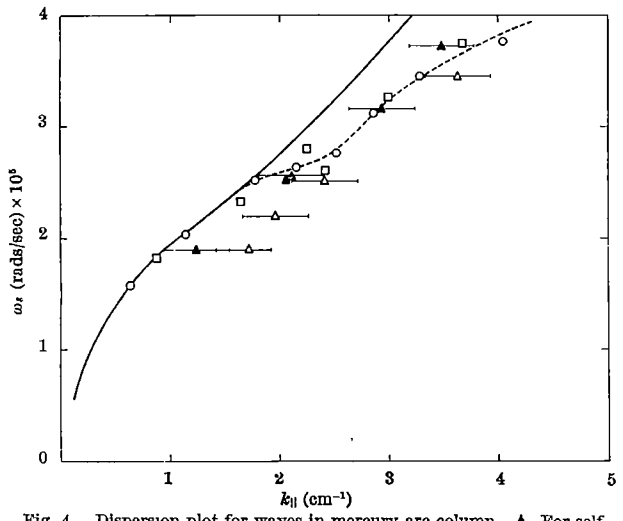


Fig. 4. Dispersion plot for waves in mercury arc column. ▲, For self-excited waves using microwave scattering $\varphi = 0$; △, for self-excited waves using microwave scattering $\varphi = 20°$; □, for self-excited waves using Langmuir probes; -○-, for externally-excited waves using Langmuir probes; —, theoretical curve for (0,1) mode, KT_e = 3·4 eV

the acoustic waves are curved[3]. A range of $k_{\perp}$ values, different for each frequency ω_s, is needed to describe such waves. Hence values of $k_{\perp}$ to satisfy equation (2) can always be found.

These experiments depend on non-linear interaction between certain plasma waves and electromagnetic radiation leading to a frequency-shift on scattering. Other experiments demonstrating this effect have been reported[5]. However, our studies of the angular dependence of the scattered wave intensity provide information also about the wave-length spectrum in the plasma, thus enabling us to identify the scattering wave. This non-perturbing technique may find application in other plasmas.

Such observations of coherent scattering should be contrasted with those in the ionosphere: there the scattering results in a continuum of frequency shifts (line broadening) rather than the appearance of satellite lines. The reason is that acoustic waves in the ionosphere are highly damped, since $T_e \sim T_{ion}$, and the amplitudes of a wave-vector k_s vary stochastically, rather than harmonically, with time[6]. The result is incoherent rather than coherent scattering. On the other hand, electron plasma oscillations at ω_p should lead to satellites at a frequency $\omega_1 \pm \omega_p$, but these have not as yet been observed. We thank Mr. H. G. Jones for valuable assistance with the experimental work.

[1] Little, P. F., and Jones, H. G., *Proc. Phys. Soc*, **85**, 979 (1965).
[2] Crawford, F. W., *Phys. Rev. Lett.*, **6**, 663 (1961). Alexeff, I., and Neidigh, R. V., *Phys. Rev.*, **129**, 516 (1963).
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DETECTION OF CIRCULAR AND LINEAR POLARIZATION IN THE OH EMISSION SOURCES NEAR *W3* AND *W49*

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FOLLOWING the detection of anomalously excited OH emission at 1,665 and 1,667 Mc/s from the vicinity of several thermal radio sources by Weaver, Williams, Dieter and Lum[1], Weinreb, Meeks, Carter, Barrett and Rogers[2] reported observations of linear polarization in the 1,665-Mc/s spectrum of the OH source near *W3*. Zuckerman, Lilley and Penfield[3] were unable to detect any linear polarization greater than 8 per cent in two of the features in the spectrum of *W49*.

Prior to the publication of these results a search was begun at Jodrell Bank for the linearly and circularly polarized emission which might be expected from the Zeeman splitting of the narrow lines at 1,665 Mc/s emitted from *W3*, *W49*, *W51* and Orion *A*. The small width of the lines is ideally suited to this search. The present article reports the detection of strong circular polarization in the spectra of the OH sources near *W3* and *W49*.

The narrow band six-channel receiver designed for hydrogen line Zeeman measurements was used for this experiment. The crystal filters have a bandshape equivalent to a gaussian with a half-intensity width of 0·60 kc/s. The receiver has a parametric amplifier front-end which gives an overall system noise of 280° K. For polarization measurements the 250-ft. Mark I telescope was slightly under-illuminated by a crossed dipole and reflector combination. The signals from the dipoles were combined through appropriate line lengths and a hybrid to give either both planes of linear polarization or both senses of circular polarization. The receiver was then switched between the two linear or the two circular polarizations by a diode switch. The output of each of the six channels was recorded on punched tape as the sum and the difference of the two polarized intensities.

A separate investigation was made of the extent and position of the OH sources near *W3* and *W49* using a rather narrower beam obtained with a dipole and shaped reflector plate on the 250-ft. radio telescope. The beam-width in this case was 15′ arc. This investigation confirmed that the OH source was significantly displaced from *W3* in the position $\alpha = 2\text{h } 23\text{m } 29\text{s}$, $\delta = +61° 38'$ (1950·0) given by Weinreb, Meeks, Carter, Barrett and Rogers. The OH source in *W49* coincides in position with the low right ascension component of *W49* as found by Zuckerman, Lilley and Penfield.

The *W3* Source

The OH source near *W3* has a simpler emission spectrum than that of *W49* and the preliminary results of the investigation to be reported here will be largely concerned with the former source. Fig. 1*a* shows the main emission features in the spectrum of the source near *W3* plotted as the mean of the opposite senses of polarization. This spectrum shows much detailed structure, some of which is very narrow. The feature at −8·5 kc/s and the sharp edges at −1·5 kc/s and +8·5 kc/s indicate root mean square velocity spreads of 0·10, 0·09 and 0·08 km/s. The narrow structure is also reflected in the circular polarization data.

The difference between left- and right-hand circular polarization intensities is plotted in Fig. 1*b*. The sense of polarization is that seen when viewed along the direction of propagation. Strong circular polarization effects are evident over the whole emission spectrum. The data have been corrected for systematic effects amounting to 10 per cent of the profile intensity which had been carefully measured in separate observations of strong radio sources. It is believed that Fig. 1*b* represents the true circular polarization intensity to better than 10 per cent of the

profile intensity. The peak-to-peak noise on the plot is ±1° K. The circularly polarized components at −19·5 kc/s, −8·5 kc/s and +7 kc/s are nearly 100 per cent polarized. The most intense circularly polarized feature at −0·5 kc/s lies on the low-frequency side of the main emission peak of Fig. 1*a* and indicates 60 per cent circular polarization at this frequency. In the frequency interval +1 kc/s to +6 kc/s the polarization structure is complex and indicates the presence of several polarization features. Similarly, in the range −24 kc/s to −2 kc/s there is a low, but significant, level of circularly polarized structure.

Linear polarization measurements were made at three selected feed angles at which systematic effects were less than 10 per cent. Observations at different hour angles provided a range of position angles (pa) to give a measurement of the polarization temperature and position angle every 0·5 kc/s throughout the spectrum. These results are shown in Figs. 1*c* and 1*d*. Significant linear polarization effects are observed in regions within the spectrum. A maximum polarization temperature of 5° ± 2° is observed at the centre of the strong line at +0·5 kc/s corresponding to 25 ± 10 per cent polarization. The features at −8·5 kc/s, −3·5 kc/s, +6 kc/s and +21·5 kc/s also show significant polarization effects. On the other hand, features such as those at −6 kc/s, −2·5 kc/s, +3·5 kc/s and +8 kc/s show linear polarization temperatures less than ~1° K which for these features corresponds to <30, <12, <15 and <12 per cent polarization, respectively. The position angle remains constant over most of the linearly polarized features except in the case of the region from −1·5 kc/s to +2·5 kc/s which shows a gradual variation from position angle = 55°–120°. This result should be taken into account in any interpretation of the data. There appears to be general agreement between the present results and the more limited linear polarization data published by Weinreb, Meeks, Carter, Barrett and Rogers[2].

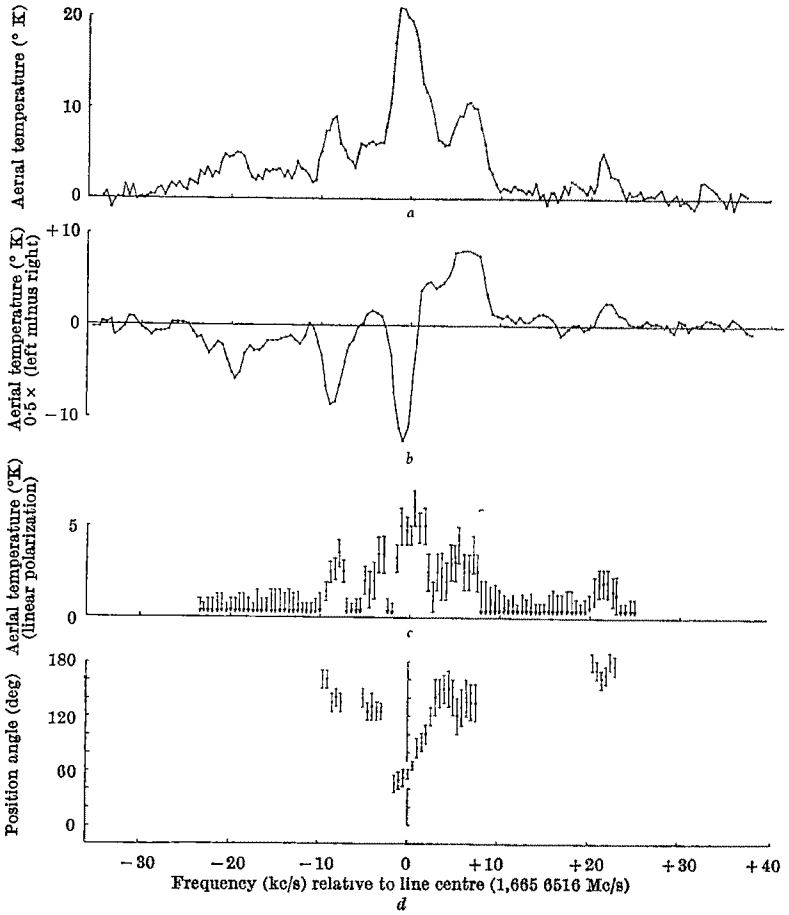


Fig. 1. Observations of the OH emission source near *W*3. *a*, The emission spectrum. The frequency is given relative to the centre of the main feature at 1,665·6516 Mc/s. This spectrum is the mean of the orthogonal polarization intensities. *b*, The difference in intensity between left- and right-hand circular polarization. *c*, The intensity of linear polarization measured as the amplitude of variation of intensity with position angle (equals 0·5 (*T* max–*T* mn)). The errors indicated are ~1 *S.D.* Systematic effects which would double these errors are probably present. *d*, The position angle of the linearly polarized component

The W49 Source

Extensive circular and linear polarization measurements were also made of the 1,665-mc/s emission from the OH source near *W*49. Because of the more complex spectrum of this region, only preliminary results will be reported here. They indicate polarization phenomena very similar to those found in *W*3. The major difference between the two OH sources is that the broad central complex of *W*49 shows rather weaker polarization effects. The emission spectrum and the difference between left- and right-hand circular intensities are shown in Figs. 2*a* and 2*b*.

In the *W*49 OH source the component at −116 kc/s is strongly circularly polarized. Narrow polarization components can be distinguished in the central complex. Linear polarization measurements show that the features at −116, −99 and −94 kc/s are less than 20 per cent polarized.

Interpretation of the Results

With the detection of strong circular polarization in the emission spectra of the *W*3 and *W*49 OH sources it would seem reasonable to look first for an explanation of these effects in terms of the Zeeman effect. Zeeman splitting is possible in the four hyperfine splitting lines of the $^2\pi_{3/2}$, $J = 3/2$ Λ doublet state of ^{16}O ^1H centred around 1,666·3 Mc/s. In the case of the 1·665 Mc/s line the Zeeman splitting is 1·17 $\mu_0 H$, where μ_0 is the Bohr magneton. This corresponds to a splitting between the σ components of 3·3 kc/s for 10^{-3} G. For a magnetic field directed away from the observer the right-hand circularly polarized component is displaced to a lower frequency in an emission spectrum. The observations will now be discussed in terms of a possible Zeeman effect.

The relatively simple emission spectrum of the OH source near *W*3 is split into a rather complex pattern of circularly polarized components. It can be shown that the components in Fig. 1*b* can be arranged into pairs of oppositely polarized σ components. Moreover, within the limits of errors of the results the intensity of left-hand circular polarization integrated over the entire spectrum is equal to the intensity of right-hand circular. This is as expected for the Zeeman effect.

The linear polarization data should be compatible with the circular polarization results, having σ components at the same frequencies as the circular σ components. In addition the linearly polarized components for a single feature should have a constant position angle with a sharp 90° position angle break between the π and σ components. Such a sharp position angle step is seen in Fig. 1*d*. However, as already noted in the range −1·5 kc/s to +2·5 kc/s, there is a gradual change in position angle. Such an effect would result from the addition of the linearly polarized Zeeman patterns of two distinct features which were phase coherent; alternatively the result is consistent with an additional component at 1·5 kc/s. Indeed, in this region of the spectrum the circular polarization data also require more than

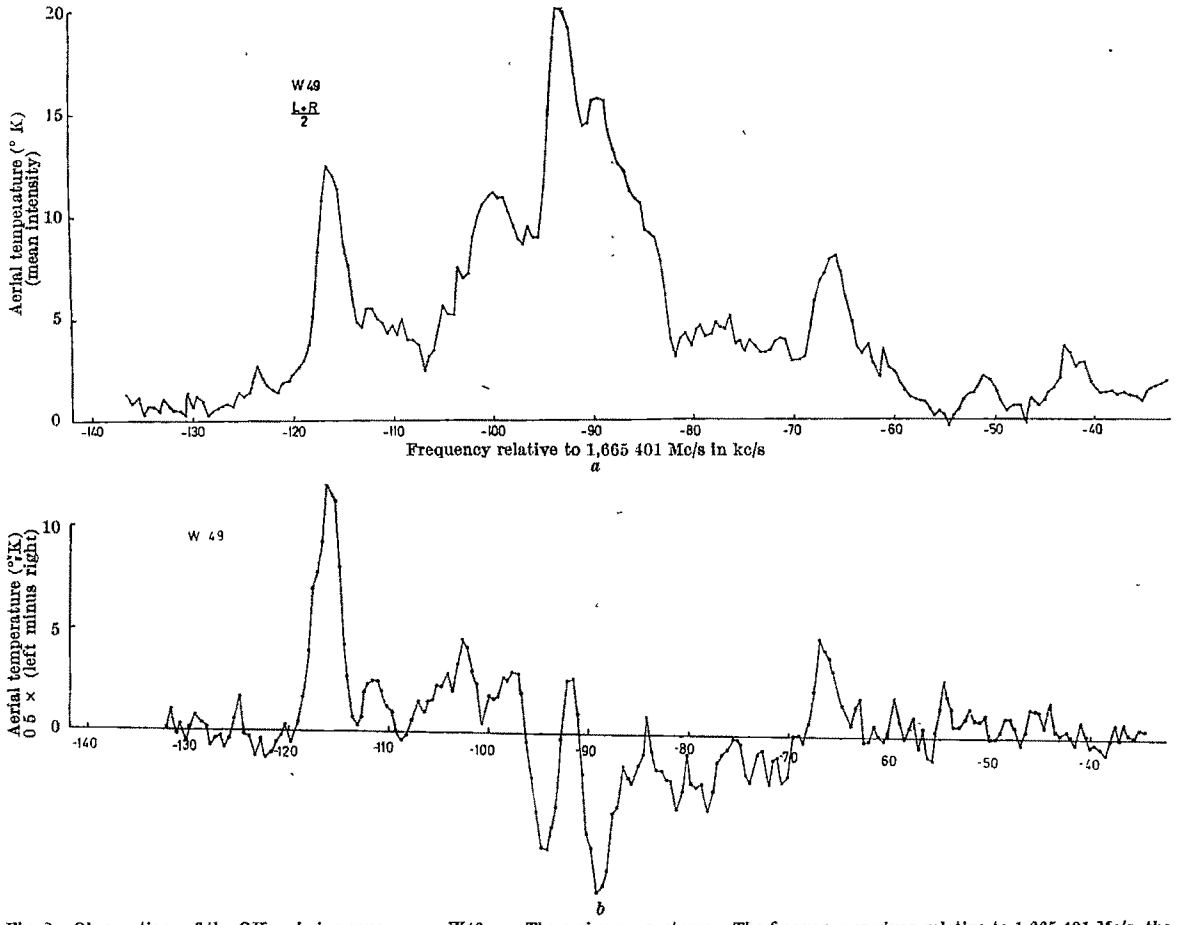


Fig. 2. Observations of the OH emission source near *W*49. *a*, The emission spectrum. The frequency is given relative to 1,665·401 Mc/s, the rest frequency. The spectrum is the mean of the opposite senses of circular polarization. *b*, The difference in intensity between left- and right-hand circular polarization

one Zeeman-split feature. As would be expected with the Zeeman effect, the features which show the largest percentage circular polarization (for example, −19·5 kc/s, −9 kc/s, +8 kc/s) show little or no linear polarization. Moreover, in a complex of features with overlying π and σ components the smaller (by half) frequency separation of the linearly polarized components will result in a greater cancellation of the linear polarization than the circular polarization.

The complete resolution of the circular and linear polarization data on the basis of Zeeman splitting into features with varying but related amounts of linear and circular polarization has not yet been made. However, a first attempt shows that the stronger components can be fitted to a self-consistent Zeeman pattern. A solution is suggested in Table 1. Other combinations of fields may possibly be fitted to the data particularly for the weaker features listed under *D*. A good agreement in the direction of the magnetic field is indicated by the results of Table 1. All features show that the major component of

Table 1

σ	π	σ	
−0·5 kc/s R.H. $T_a = 12°$	+0·5 kc/s	+1·5 kc/s L.H. $T_a = 12°$	Feature *A* Field $0{\cdot}6 \times 10^{-3}$ G directed away from observer.
Linear is weak			
−8·5 kc/s R.H. $T_a = 8°$	−1 kc/s	+6 kc/s L.H. $T_a = 8°$	Feature *B* Field $4{\cdot}2 \times 10^{-3}$ G directed away from observer. pa of field = 50° projected on to the plane of the sky.
Linear pa = 140° $T_a = 2·5°$	pa = 50° $T_a = 5·0°$	pa = 140° $T_a = 2·5°$	
+1·5 kc/s R R.H. 8%	+4·5 kc/s	+7·5 kc/s L.H. 8%	Feature *C* Field $1{\cdot}8 \times 10^{3}$ G directed away from observer.
Linear is very weak			
−19 kc/s R H. $T_a = 3·5°$	+1·5 kc/s	+22 kc/s L.H. $T_a = 3·5°$	Feature *D* Field $12{\cdot}4 \times 10^{-3}$ G directed away from observer pa of field = 85° projected on to the plane of the sky.
Linear confused	pa = 85° (Inferred from +22 kc/s component)	pa = 175° $T_a = 1·7°$	

the field is directed away from the observer. Even if *D* is taken as two features, corresponding to about half the field strength so that the other σ components lie in the complex near 0 kc/s, the fields in both would again be directed away from the observer. The magnetic field in features *A* and *C* is closely aligned in the line of sight. In feature *B* the field is inclined at 30° to the line of sight and its projection on to the plane of the sky lies at position angle 50°. The field in feature *D* is also inclined at about 30° to the line of sight and its projection on to the sky lies at position angle 85°.

Conclusion

The foregoing considerations indicate that it is possible to interpret the present circular and linear polarization data as a Zeeman splitting of the OH emission line. The magnetic fields so derived for the *W*3 OH emission source are extremely high in comparison with the fields believed to exist in the galactic plane as investigated, for example, in normal interstellar neutral hydrogen clouds (see, for example, Davies, Shuter, Slater and Wild[4] and Verschuur and Davies[5]) which are at least 10^2–10^3 times weaker. The circular polarization results for *W*49 would indicate similar magnetic field strengths.

The present observations clearly demonstrate the existence of strong circular polarization in the anomalous OH emission spectrum at 1,665 Mc/s from the OH sources near *W*3 and *W*49. If the interpretation of the data in terms of the Zeeman splitting suggested here is correct, then unexpectedly large magnetic fields are involved which raise many problems in connexion with the OH lines requiring further discussion.

We thank Prof. A. G. Gaydon, of the Imperial College of Science and Technology, and Dr. A. E. Douglas, of the National Research Council of Canada, for indicating the

structure of Zeeman splitting expected for the OH lines. Dr. H. E. Radford supplied us with exact values of the Zeeman splitting. Dr. R. J. Long and D. Williams assisted with the modification and testing of the feed system for this experiment. We also thank Sir Bernard Lovell for his interest and encouragement in this project.

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TRITERPANES IN OPTICALLY ACTIVE PETROLEUM DISTILLATES

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THE identification in petroleum of two isoprenoid hydrocarbons, phytane and pristane[1,2], lends additional support to the theory that petroleum, like coal, is derived from a biological source material. Further support for this theory springs from the knowledge that certain petroleum distillates[2,3], and some from coal[4], are optically active. In each case this activity has been associated with saturated polycyclic hydrocarbons, and the frequent occurrence of optical activity in biochemical systems is well known. Several authors[5–7] have suggested that these polycyclic hydrocarbons might be derived from naturally occurring steroids and triterpenoids.

We have examined distillates, boiling in the range 340°–550° C (taken under reduced pressure so that the temperature of the sample being distilled did not exceed 300° C), from several crude oils and have paid particular attention to such distillates from Nigerian crude oil. The specific rotation of these Nigerian distillates increases from $(\alpha)_D^{19}$ + 1·08° at boiling-point 410° C (at 760 mm, corrected) and reaches a maximum $(\alpha)_D^{19}$ + 6·9° at boiling-point 478° C. After this, the activity diminishes progressively until distillates boiling at about 500°–550° C show only marginal activity; $(\alpha)_D^{19}$ + 0·3°.

A detailed examination of the Nigerian distillate boiling at 340°–480° C was carried out, and liquid adsorption chromatography over silica gel yielded 71–72 per cent by weight of a naphthene plus paraffin portion. Mass spectra indicated the presence of high concentrations of the hydrocarbon series C_nH_{2n-6} and C_nH_{2n-8}, with carbon numbers in the range C_{27} to C_{31}, and a prominent fragment at *m/e* 191. Half this saturate concentrate formed an adduct with urea, and 66 per cent by weight of the adducted hydrocarbon proved to be *n*-alkane (*n*-C_{19} to *n*-C_{34}). The remaining 34 per cent of adducted hydrocarbon appeared from gas-liquid chromatograms to be mainly 2- and 3-methylalkanes[8].

Thermal diffusion[9], in two stages, of the urea non-adduct yielded two materials [$(\alpha)_D^{18·7}$ + 17·6° and $(\alpha)_D^{20}$ + 14·5°, in cyclohexane, respectively]. These materials were combined and, after a preliminary separation by liquid-adsorption chromatography over alumina, yielded a resin (approximately 1 per cent weight on the crude oil). A portion of the gas-liquid chromatogram of this resin (later shown to be a concentrate of pentacyclic naphthenes) is reproduced in Fig. 5. Prominent peaks are lettered and the hydrocarbons eventually isolated from this resin are in turn lettered so that they are equivalent to the corresponding peaks in the gas-liquid chromatogram. This resin was re-chromatographed over alumina to give sixteen fractions. Fraction 3 (0·4072 g), after crystallization from a variety of solvents, yielded 5 mg of hydrocarbon '*F*' (purity by gas-liquid chromatography > 98 per cent weight, melting-point 232·5°–234·5° C corrected) and eventually 1–2 mg of hydrocarbon '*D*' (purity > 90 per cent, melting-point 169°–180° C corrected). Fraction 9 (0·1790 g) likewise yielded some 2–3 mg of hydrocarbon '*E*' (purity > 98 per cent, melting-point 259·5°–260·5° C corrected). The analytical data determined on the three solid hydrocarbons are listed in Table 1. There was unfortunately an insufficient sample for the determination of the optical activities of the two pure hydrocarbons '*E*' and '*F*', or of '*D*'. The analytical data, included in Table 1 and discussed briefly below, suggest that these are pentacyclic triterpanes (the term triterpane has been used to denote the fully saturated hydrocarbon analogues of the triterpenes). High resolution mass spectrometry (Associated Electrical Industries '*M.S.* 9') confirmed an increase in concentration of the hydrocarbon series C_nH_{2n-8} at C_{27}–C_{31} in the resin from thermal diffusion. Prominent fragments in the mass spectrum at *m/e* 205, 177, 137, 123 and 109 were now dominated by the very intense fragment at *m/e* 191. Djerassi *et al.*[10] note the frequency with which this fragment at *m/e* 191 occurs in the mass spectra of triterpanes. In the mass spectrum of lupane two different fragment species contribute to this peak at *m/e* 191 making it by far the most intense fragment in the upper part of the spectrum. One of these species, which contains rings *A* and *B*, is classed by these authors as the most characteristic fragmentation product of pentacyclic triterpanes. The mass spectra of our hydrocarbons '*D*', '*E*' and '*F*' are compared with that of authentic lupane in Figs. 1–4. These are seen to be similar; each contains a strong parent peak, *M*, and a significant peak at *M*-15 as well as prominent fragments at *m/e* 191, 137, 123 and 109. Hydrocarbon '*D*' shows in addition a strong peak at *m/e* 177. The accurate masses of the molecular ions (listed in Table 1) indicate that hydrocarbons '*E*' and '*F*' each contain five saturate rings and thirty carbon atoms, and a molecular skeleton based on perhydropicene is thus one of the types which must be considered.

The infra-red spectra of the resin (obtained by thermal diffusion of the urea non-adduct), and the hydrocarbons '*E*' and '*F*' isolated from it, were very similar to that of lupane. The spectra demonstrated that these materials were free of hydroxyl, carbonyl and double bonds.

Authentic lupane exactly reinforces peak *J* shown in the gas-liquid chromatogram (Fig. 5) and the possible identity of *J* with lupane cannot be excluded at this stage. All of the triterpanes that we have examined (zeorinane, friedelane, heterolupane and γ-onocerane) have retention volumes which occur in the region covered by peaks *C*–*P* in the gas-liquid chromatogram, but none of these reinforces any of the peaks in the chromatogram. The presence of a peak at *I* in Fig. 5 is not obvious, but one was discovered between peaks *E* and *D*, when the chromatograms of the sixteen fractions separated by liquid adsorp-

Table 1. PHYSICAL PROPERTIES OF HYDROCARBONS '*D*', '*E*' AND '*F*'

Hydrocarbon and gas-liquid chromatogram peak	Accurate mass of molecular ion *M*	Empirical formula	Nuclear magnetic resonance data: Protons in methylene + methine to methyl ± 10%	Nuclear magnetic resonance data: No. of methyl groups per molecule	Gas-liquid chromatographic data: Relative retention volume *n*-C_{30}* = 1·0	Purity (% wt.)
D	398·391	$C_{29}H_{50}$	—	—	0·93	> 90
E	412 406	$C_{30}H_{52}$	1·1	8	0·95	> 98
F	412 408	$C_{30}H_{52}$	1·1	8	1 10	> 98
J	—	—	—	—	1 11	—
Lupane	—	$C_{30}H_{52}$	1·2	8	1·11	> 98

* RRV data from Fig 5.

tion chromatography over alumina; as already described, were examined.

The nuclear magnetic resonance spectra of hydrocarbons '*E*', '*F*' and lupane are all very similar and each contains eight methyl groups per molecule (Table 1).

Hydrocarbon '*E*' is being examined by X-ray crystallography. Measurement of the unit cell dimensions and density gave a molecular weight for '*E*' of 411, in good agreement with the value determined by mass spectrometry (Table 1). Inspection of the Patterson syntheses and the Fourier maps suggests a nucleus, probably based on perhydropicene, in which four of the five rings (*A*, *B*, *C*, *D* or *B*, *C*, *D*, *E*) are clearly seen to be six-membered. The conformations of these four rings are all chair and the ring fusions are all *trans*. The conformation of the fifth ring is not yet fully established nor are the locations of all of the eight methyl groups. The detailed structure of hydrocarbon '*E*' will be published elsewhere when the X-ray investigations are complete.

The cumulative evidence leaves little doubt that hydrocarbons '*E*' and '*F*' are pentacyclic triterpanes. From the scanty data so far obtained, '*D*', with five rings and twenty-nine carbon atoms (Table 1), would appear to be a *nor*triterpane.

The occurrence of these triterpanes is not limited to Nigerian crude oil. Similar resins from Kuwaiti, Iranian and Libyan crude oils yield gas-liquid chromatograms with the same peaks *A*–*D*, *G*–*H* and *K*–*P*, but the proportions in each crude are different. The peaks *E*, *F*, *I* and *J*, prominent in the gas-liquid chromatogram of the Nigerian resin, are, however, not seen in the chromatograms from the resins of the other three crude oils. The mass spectrum of each resin showed a prominent fragment at *m/e* 191 and a high concentration of the hydrocarbon series C_nH_{2n-8} at C_{27}–C_{31}.

Our preliminary examination of a tetracyclic concentrate (which was also prepared from the thermal diffusion of the urea non-adduct, mentioned earlier) shows that the hydrocarbon series C_nH_{2n-6} in this material lies in the carbon number range C_{26}–C_{31}. The mass spectra show fragmentation patterns which suggest a mixture of steranes incompletely separated from the triterpanes.

Possibly some of the optically active hydrocarbons isolated by Oakwood *et al.*[8], and the hydrocarbon of melting-point 292°–297° C separated from a Colorado shale oil by Cummings and Robinson[11] which has also been referred to by Jones and Dickert[12], are similar in structure to those that we have isolated. Compounds related to picene and hence to pentacyclic triterpenoids have already been isolated from petroleum. Carruthers and Watkin[13] have reported the isolation of 1,2,3,4-tetrahydro-2,2,9-trimethylpicene from an American crude oil, and the triterpene lactone, oxallobetul-2-ene, has been identified in Kuwaiti crude oil by Barton, Overton and Carruthers[14].

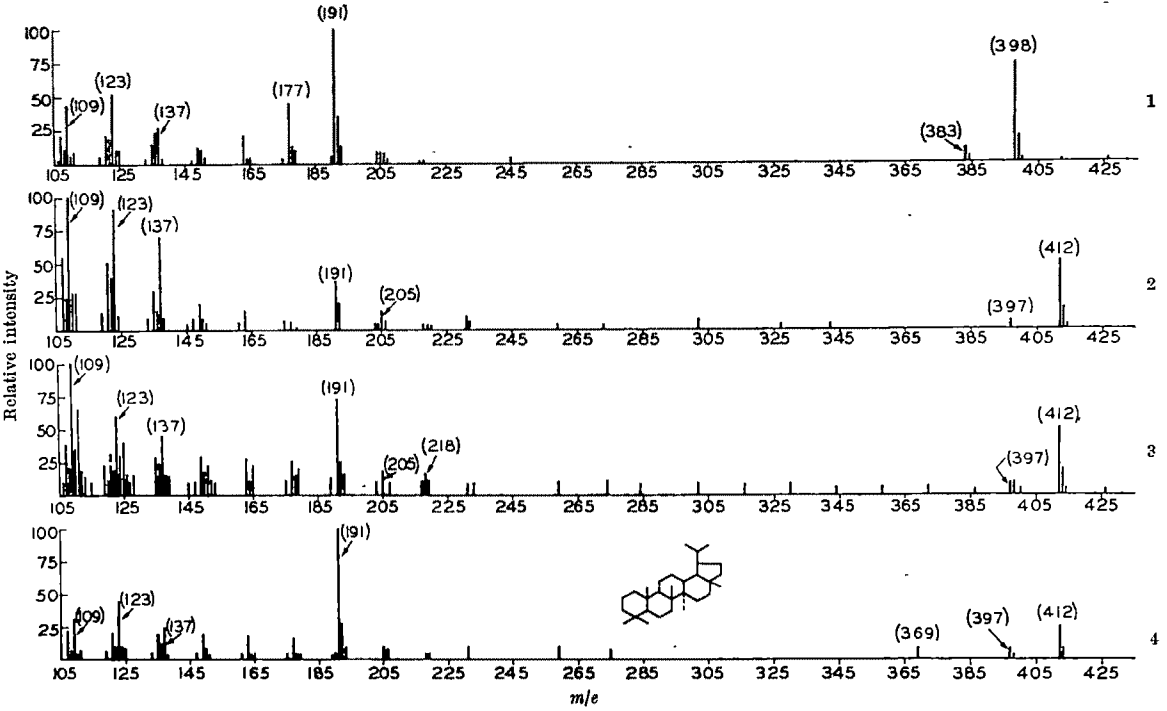


Fig. 1. Mass spectrum of hydrocarbon '*D*'

Fig. 2. Mass spectrum of hydrocarbon '*E*'

Fig. 3. Mass spectrum of hydrocarbon '*F*'

Fig. 4. Mass spectrum of lupane

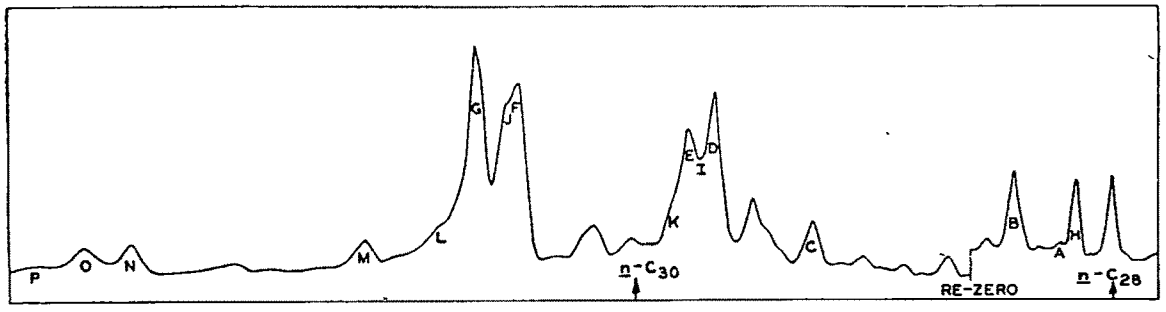


Fig. 5. Portion of gas-liquid chromatogram of pentacyclic naphthene concentrate with added n-C_{28} and n-C_{30}. 148-ft. glass capillary column, 0 01 in. internal diameter, coated with '*S.E.* 30' gum. Carrier gas: hydrogen at 252° C. Flame ionization detector. Injection point to methane peak: 3 min 20 sec. Methane peak to n-C_{28}: 51 min 2 sec. Methane to peak *O*: 134 min 39 sec

Meinschein[6] has identified sterane and terpane hydrocarbon types in sediments and petroleum. The triterpanes that we have isolated may be derived from pentacyclic triterpenoids which are abundantly distributed in the plant kingdom but occur only rarely in the animal kingdom[15]. Animals appear unable to synthesize pentacyclic triterpenoids but build instead the tetracyclic systems derived from lanostane. Our present knowledge of the distribution of the triterpenoids in the plant kingdom[15] is obviously far from complete. It does, however, suggest that pentacyclic triterpenoids are abundantly distributed in terrestrial plants, especially in the more highly specialized phanerograms, whereas they are rare in aquatic plants. Steroids, on the other hand, appear to be abundantly distributed in both aquatic and terrestrial flora. Thus the certain recognition of individual steranes and triterpanes in petroleum may enable the petroleum geochemist to relate this evidence to the flora from which the crude oil most probably originated and recognize a marine or terrestrial origin for each particular crude oil. The evidence reported here indicates that part of Nigerian crude oil may well have had a terrestrial origin.

We thank Prof. D. H. R. Barton, Dr. T. G. Halsall and Dr. G. Eglinton for samples of triterpanes.

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POSSIBLE ABIOGENIC ORIGIN OF SOME NATURALLY OCCURRING HYDROCARBONS

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A GROWING body of evidence supports the theory that many compounds of biological significance were formed by the action of various forms of energy on the Earth's early atmosphere before the appearance of life[1–3]. Although such abiogenic processes could have given rise to hydrocarbons, many geologists are inclined to the view that hydrocarbons found in sediments are the result of the degradation of biological material[4]. The possibility of a dual origin, abiogenic and biogenic, has also been suggested[5]. A few hold the extreme view that all hydrocarbons, in deposits ancient or modern, resulted from abiogenic synthesis[6–8].

The analysis of carbonaceous chondrites has revealed the presence of a number of organic compounds which are found in living organisms[9,10]. Similar results have been obtained in the study of Pre-Cambrian sediments[11–13]. Although the question of the origin of these compounds has not been completely solved, the characterization of hydrocarbons present in them has contributed to the understanding of the problem. Results of such enquiries will not only throw light on the path of chemical evolution on earth, but will also lay the guidelines for the analysis of returned lunar samples.

Several criteria have been adopted for the biogenic nature of hydrocarbons whether in ancient sediments or extraterrestrial samples. Considerable importance has been attached to the existence of pristane and phytane[14]. The preponderance of normal hydrocarbons has also been judged to be a factor related to biogenicity[15]. Since hydrocarbons are supposed to be derived from biogenic fatty acids which have an even number of atoms, by the loss of one carbon atom, the excess of odd-numbered hydrocarbons is also regarded as a sign of biological origin[5,16].

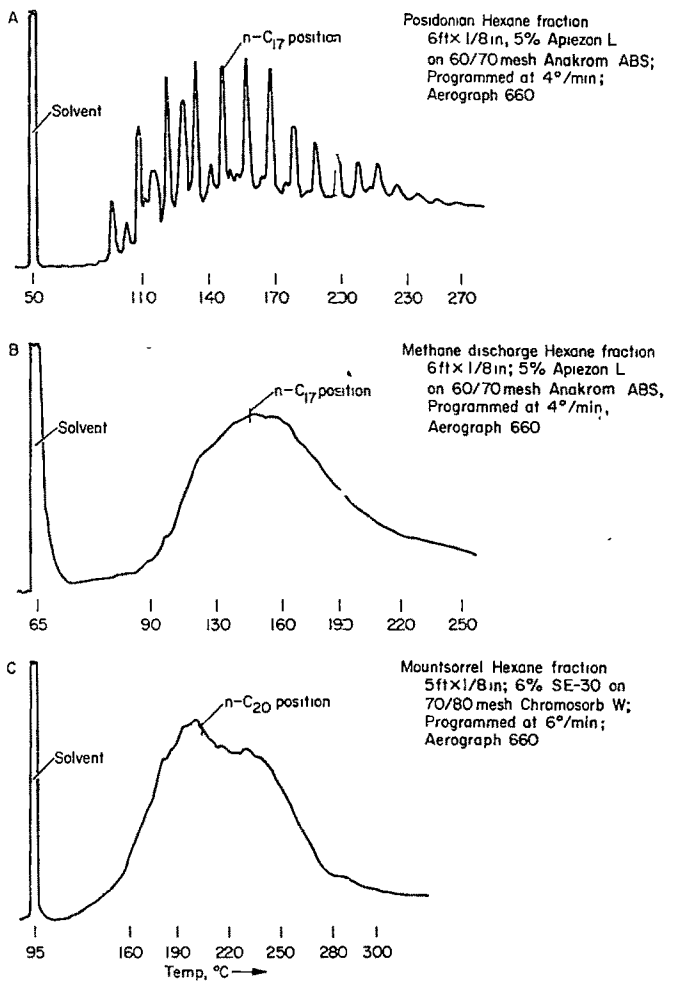


Fig. 1. Gas chromatograms of hexane fractions: (*A*) Posidonian; (*B*) methane discharge; (*C*) Mountsorrel

In order to understand the true significance of such criteria, we decided to compare hydrocarbons synthesized abiogenically with those found in some terrestrial samples. Our previous investigations showed that a variety of hydrocarbons could be synthesized by the action of a spark discharge on methane[17]. The same experiments demonstrated that there is a difference in the nature of the hydrocarbons produced, depending on the type of discharge and the distribution of energies involved. Our earlier analytical studies did not extend beyond hydrocarbons having more than ten carbon atoms.

During the course of this investigation our attention was directed to the existence of hydrocarbons of possible abiogenic origin in the Mountsorrel formation[18,19] near Leicestershire, England. On this site, bitumen occurs in mineral veins. It is found in a tract running from Mountsorrel in Leicestershire to Castleton in Derbyshire, and occurs in a basalt dyke which cuts the granodiorite. The geological evidence put forward by Sylvester-Bradley suggests that these hydrocarbons were deposited during the time that the rocks were formed. The granodiorite itself is Caledonian in age[20]. The possibility that some of these hydrocarbons may have migrated from another source, such as sedimentary rock, should not be discounted. However, according to Sylvester-Bradley, this theory is difficult to support. The oil deposits are found underlying sedimentary rock and, because oil floats on water, it is unlikely that the oil migrated downwards to the igneous rock. Mueller[21] outlined the alternative theory that the mineralizing solutions must have contained hydrogen but not carbon, and that the carbon would have migrated from organic sources in the surrounding sedimentary rock. However, in some places the veins cut through igneous rock which cannot possibly contain carbon. According to Mueller's hypothesis, the carbon must have migrated downwards from overlying sedimentary rocks.

In our present investigations we compared the hydrocarbons from the Mountsorrel formation, claimed to be abiogenic, with hydrocarbons synthesized by the action of a spark discharge through methane, and the hydrocarbons from the Posidonian shale[22], which contain fossils of marine organisms and are generally accepted to be biological in origin. Analysis of the hydrocarbons by gas chromatography and mass spectrometry would highlight similarities and differences among the three samples.

The method used for the synthesis of the hydrocarbons was a slight modification of the one described earlier[17]. The semi-corona head consisted of a 'Pyrex' tube with a 500-ml. flask attached to it. The inside electrode was made of 6-mm-thick stainless-steel tubing extending almost to the bottom of the flask. The narrow head of the flask was covered with a metal foil which served as the outside electrode. The energy sources used were exactly as described before. After a week's discharge through the methane, the deposit on the sides of the flask had the appearance of an oil film.

The Posidonian shale (kindly supplied by Dr. I. A. Breger, U.S. Geological Survey, Washington) was collected from the Bohrung Lingen 330 at a depth of 1,151·0–1,159·5 m. This shale is considered to be Jurassic in age. The specimen analysed did not appear to show any weathering. The exterior of a large piece was carefully washed with water. The sample was then broken and air-dried on a glass plate and the fragments ground in a mortar to 60-mesh size.

Samples of Mountsorrel were collected (under the guidance of Prof. P. C. Sylvester-Bradley) from an area of the Mountsorrel quarry which had been blasted within 48 h of collection. The fresh surface of the rock was further chipped to obtain samples that had not been exposed to the air. The possibility of contamination was thus reduced to a minimum. The samples were canned in air-tight containers and were not opened until the time of analysis. Although the bitumen at Mountsorrel is found in a variety of forms[18]—compact masses, vein-like bodies, globules completely enclosed in dolomite, oily films in calcite geodes, or associated with the dolomite—only the last was used in our analysis as it was judged to be representative of the general composition and was readily ground to a powder of uniform size.

The materials to be analysed were extracted with distilled hexane. Twenty-five grams of the Posidonian and 3 g of the Mountsorrel were each placed in glass soxhlets and extracted for 6 h. In the case of the spark discharge, 100 mg of product were dissolved in hexane. The resulting material, which had a brownish colour in the case of the Mountsorrel and the Posidonian, was evaporated to dryness under a stream of nitrogen. This residue was chromatographed on a 10-cm alumina column. The material on the column was then eluted with 100 ml. of distilled hexane. The aliphatic hydrocarbon fraction which was eluted by the hexane was used for the analysis.

One fraction of the hexane extract was subjected to gas chromatographic analysis on columns of 5 per cent *SE*-30 and 'Apiezon *L*'. A second portion of the

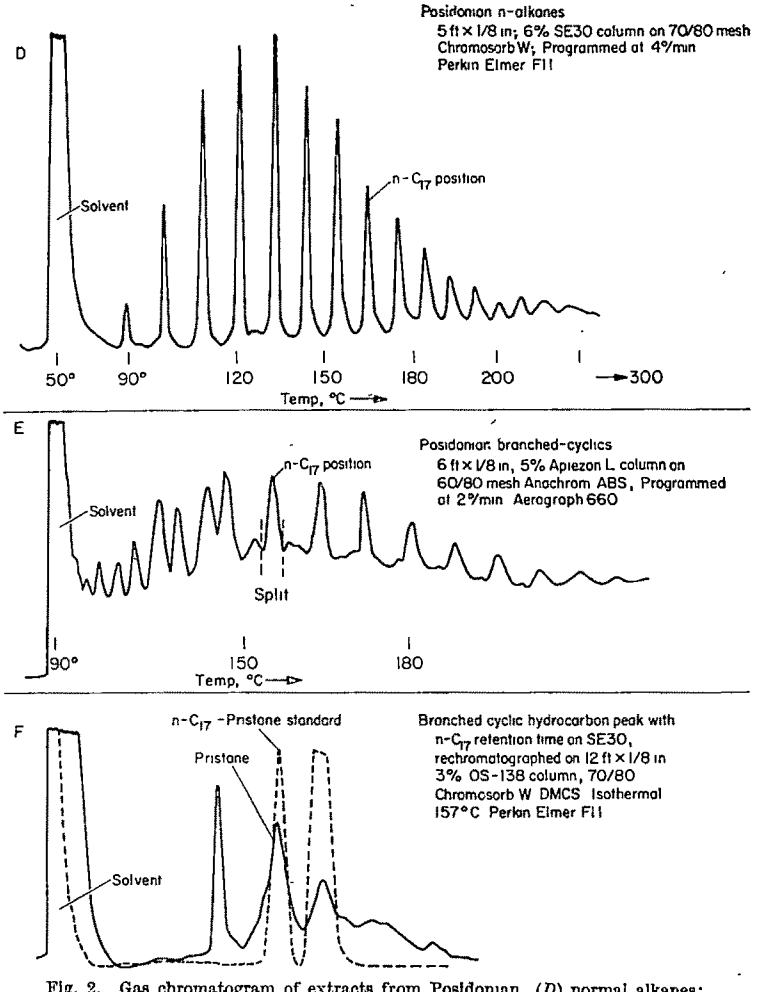


Fig. 2. Gas chromatogram of extracts from Posidonian. (*D*) normal alkanes; (*E*) branched-cyclics; (*F*) pristane fraction

extract was then treated with a 5 Å molecular sieve in order to separate the normal alkanes from the branched-cyclic alkanes. The molecular sieve was activated by heating at 200° C for 2 days in the presence of phosphorus pentoxide in a vacuum. The ratio of sieve to sample was 20 : 1. After sieving for 50 h in dry, distilled benzene, the benzene solution was decanted and the sieve washed with benzene for 1 h. The washings of the sieve were combined with the decanted solution. In order to extract the normal hydrocarbons absorbed by the sieve, it was dissolved in dilute hydrogen fluoride. The normals were recovered by extracting the hydrogen fluoride solution with three times its volume of hexane.

Fig. 1 is a comparative study of the three fractions extracted from the Posidonian (*A*), spark discharge (*B*), and Mountsorrel (*C*). The chromatogram *A* reveals that both normal and branched-cyclic alkanes are found similar to those in hexane extracts of biological shales[12]. The chromatograms *B* and *C*, however, are very clearly unresolved.

The striking feature is the contrast between the chromatograms of the Posidonian and those of the Mountsorrel or the spark discharge. This points out very clearly that the conditions which are sufficient to separate hydrocarbon mixtures obtained from materials which may be described as biological, are not sufficient to resolve the hydrocarbons from either the Mountsorrel or the spark discharge. For *A* and *B*, we used an 'Apiezon *L*' column, but for *C* we used an *SE*-30 column, which could withstand higher temperatures.

Fig. 2 illustrates the effect of molecular sieving on the Posidonian hydrocarbons. The chromatogram of the normal alkanes (*D*) indicates a distribution of normal alkanes ranging from C_{12} to C_{30}. There appears to be no preponderance of the odd numbers over the even. The second chromatogram (*E*) shows the branched fractions obtained after molecular sieving. This illustrates the presence of pristane as determined by its retention time. The pristane fraction was then collected and re-injected on to an *OS*-138·12-ft. column and the result is shown on the third chromatogram (*F*). The identity of the pristane peak is clearly established here by the use of a standard and the superimposition of the standard over the C_{17} peak. Further analysis of the total Posidonian alkane fraction, similar to Fig. 1*A*, on a 100-ft. 0·01 in. 'Apiezon *L*' capillary column, confirmed the presence of both pristane and phytane.

While the use of the molecular sieve very clearly separated the normals in the Posidonian from the branched-cyclic fractions, molecular sieving failed to isolate any such fractions from either the Mountsorrel or the spark discharge. The lack of any detectable amount of normal alkanes in both the Mountsorrel and the spark discharge emphasizes their difference from the Posidonian. A further attempt to resolve the chromatograms of the Mountsorrel and the spark discharge by the use of a 100-ft. capillary column programmed at 2° a minute did not improve the situation. While the large humps shown in Figs. 1*B* and 1*C* spread out to numerous smaller peaks, there were no peaks which stood out significantly. The use of capillary columns emphasized that both the Mountsorrel and the spark discharge material contained a wide variety of hydrocarbons.

Since gas chromatography did not resolve the hydrocarbons in either the Mountsorrel or the spark discharge mixtures, we examined the mass spectra of all three of the extracts. A comparison of fragmentation patterns is illustrated in Fig. 3. An overall comparison of the three mass spectra once again shows very clearly that both the Mountsorrel and the spark discharge hydrocarbons are different in nature from the Posidonian hydrocarbons.

The fragmentation patterns obtained from the Posidonian exhibit rather high intensities at *m/e* of 141, 155, 169, and 183 (*G*). These fragments have been associated with isoprenoid mass spectra[23]. On the other hand, the fragmentation patterns of the Mountsorrel (*I*) and the spark discharge (*H*) show only very minor intensities at these ion masses. In fact, the distribution of peaks occurring in these spectra is scarcely indicative of the presence of any significant amount of saturated normal and isoalkanes, The spectra point to the presence of highly unsaturated compounds of a highly ordered nature. Preliminary evidence from bromination appears to confirm a high degree of unsaturation in both the Mountsorrel and spark discharge hydrocarbons.

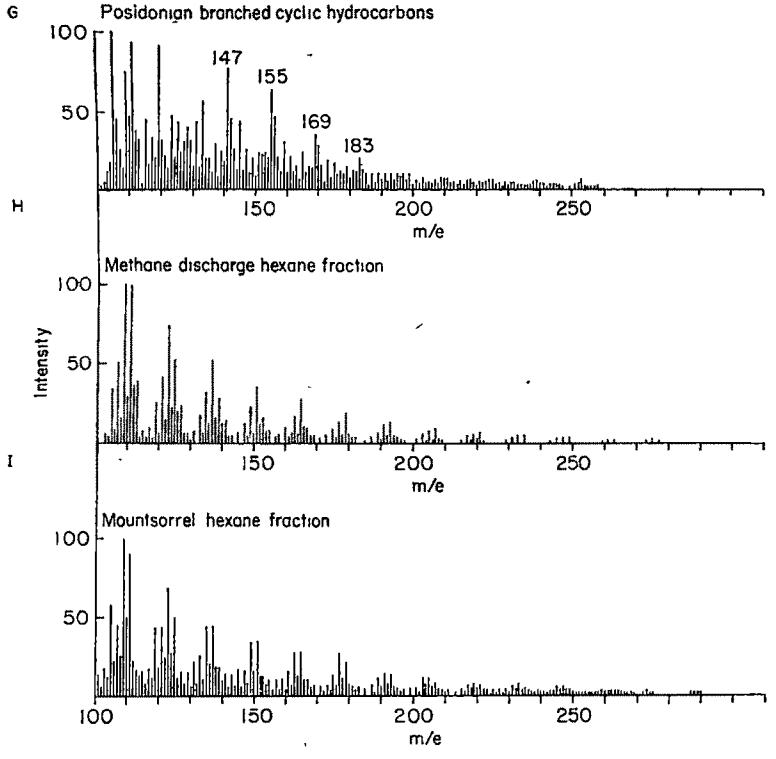


Fig. 3. Mass spectra of, (*G*) Posidonian branched cyclic hydrocarbons; (*H*) methane discharge hexane fraction, (*I*) Mountsorrel hexane fraction

Elemental analysis of the Mountsorrel formation showed that there was scarcely any nitrogen present in the compound, and there was no evidence of porphyrins[24]. A preliminary analysis for fatty acids by gas chromatography and thin-layer chromatography produced negative results. Infrared spectra also pointed to the absence of any carbonyl function. The presence of porphyrins, of fatty acids, and some carbonyl function is generally associated with biological oils.

The evidence presented from the gas chromatographic and mass spectrometric analysis shows very clearly that the hydrocarbons from the Posidonian shale, which is recognized to be associated with marine sediments, are strikingly different from those in both the Mountsorrel and the spark discharge material. The chemical evidence for the biological nature of the Posidonian revolves around the presence of (*a*) porphyrins[22,25]; (*b*) normal alkanes; (*c*) saturated branched chain isoprenoid-type compounds, especially pristane and phytane.

The hydrocarbons produced by the spark discharge on methane are truly abiogenic. They show no normals or saturated branched chain isoprenoids. The Mountsorrel

extract does not exemplify any of the characteristics associated with biogenicity illustrated by the Posidonian, but shows a marked similarity with the hydrocarbons synthesized by the spark discharge. On the basis of the chemical evidence presented, one is led to infer that the hydrocarbons in the Mountsorrel formation were produced by a process very different to that which produced the hydrocarbons in the Posidonian and are possibly abiogenic in origin.

We thank Dr. I. A. Breger for samples of the Mountsorrel and Posidonian; Prof. P. C. Sylvester-Bradley and Mr. R. J. King for valuable discussions and help in collecting samples from the quarry in Mountsorrel; Dr. A. L. Burlingame for use of his mass spectrometer; Dr. B. Nagy for help with the thin-layer chromatography analysis of the extracts for fatty acids; and Mr. F. Woeller for providing the hydrocarbons synthesized by the semi-corona discharge.

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EUROPEAN MID-DEVONIAN CORRELATIONS

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In recent palaeontological literature, much attention is being devoted to the structurally complex group of atrypoid brachiopods, many species of which locally serve as valuable index fossils. Taxonomic revision on a generic level has not kept pace with the rapidly increasing number of new species, and a number of new genera need be proposed. The introduction of the atrypoid genera *Mimatrypa* Struve 1964 (ref. 1), *Spinatrypina* Rzhonsnitskaya 1964 (ref. 2), and *Pentlandella* Boucot 1964 (ref. 3) has filled part of the gap. Another genus, restricted in distribution largely to one type of sediment and fauna, is added in this article.

The new genus *Atryparia* (Latin suffix *arius, a, um*, pertaining to) is closely related to and probably derived from the genus *Atrypa* Dalman 1828. It belongs to the subfamily Atrypidinae, family Atrypidae under the classification at present accepted. The new genus contains a number of new species generally obscured under the name *Atrypa globosa* Leidhold 1928 (ref. 4) (non *Atrypa reticularis* var. *globosa* Barrois 1889—ref. 5) in Eifelian stratigraphy. *Atrypa globosa* Leidhold is shown elsewhere[6] to be synonymous with *Mimatrypa desquamata* (Sowerby 1840). *Atryparia* n.gen. is easily separated in all stages of ontogeny from associated specimens of *Atrypa* Dalman, although it is rarely as abundant.

Atryparia n.gen.

Type species: Atryparia instita n.gen., n.sp. *Range:* Nohn beds to Ahbach beds, Eifelian, Middle Devonian (Fig. 1). *Distribution:* Germany, Belgium, France, Algeria, Morocco.

Diagnosis: Large, generally widely frilled, biconvex atrypids with coarse, strongly divergent, shallow and undulating ribs. Frills hug the shell wall and do not cause the interruption of ribs into 'rows' as in *Spinatrypa* Stainbrook 1951. Growth lines as in *Atrypa*. Beaks are appressed in all ontogenetic stages, and no interarea or delthyrium is exposed. The ventral umbo is expanded and rather angular. The foramen is often enlarged into the apex.

Internally, a pedicle layer or collar is characteristic, but deltidial plates are absent. A distinctive, clear-cut dental nucleus (equivalent to an embryonic lateral cavity) marks the tooth. Dorsal cardinalia tend to massivity and are thickly reinforced. The minute notothyrial pit holds a weak cardinal process, overlapping the hinge plates. Crura are straight, unfeathered. Disjunct, two-layered jugal processes contain nodules and terminate in long, sub-vertical, straight jugal plates. Spiralia with about fifteen whorls. Musculature similar to *Atrypa*.

Comparison: The genus is distinguished from *Atrypa* by its coarser, shallower, diverging ribs, strong ventral apical globosity, continually appressed beak and complete absence of deltidial plates. Internally, it is marked by its massive dorsal cardinalia, large and well-developed jugal plates and dental nuclei. In Devonian *Atrypa* the absence of dental nuclei is notable.

Atryparia n.gen. could be confused with some *Spinatrypa* which have poorly developed or broken spines, but it is still distinguished by the absence of rib rows and delthyria.

Remarks: The genus particularly favoured a substrate of clayey calcareous shale or thinly bedded argillaceous limestone showing an absence of detrital debris. Locally it is associated with horn corals and the most common accompanying atrypid is *Spinatrypa*. It only rarely outnumbers all other fauna. Frills are common on many specimens, even where the associated *Atrypa* show no development at all.

Flamand[7] illustrated a specimen, possibly related to *Atryparia*, from the Devonian near the Algeria–Morocco border in north Africa. Quenstedt[8] would seem to have been the first to figure species of the genus, calling them *Terebratula prisca* Schlotheim 1820. Collections from France and Belgium show the genus to be represented there. Its main distribution is probably limited to Western Europe and North Africa. In the Eifel region of Germany, at least three, possibly five, species, each with a

restricted vertical distribution, can be identified. A brief diagnosis of two member species follows. Details are to be published in a major paper. Descriptions are based on a small collection of about 800 specimens.

Atryparia instita n.gen., n.sp.

Derivatio nominis: instita (Latin) = a seam, border or flounce. Characteristically the species has a marked frill.

Holotypus: Specimen in Fig. 2, 1–4. *Locus typicus:* north-east flank of Mühlenberg, 800 m north-east of Niederehe, Hillesheim syncline, Eifel. *MTB Dollendorf* r5455 : h7473. *Stratum typicum:* upper layers of Müllert horizon, Ahbach beds, upper Eifelian (Fig. 1).

Associated fauna: Spinatrypa kelusiana Struve, rare *Atrypa* sp.

Diagnosis: Globose *Atryparia* with well-developed, thick, ventrally turned frills, equally convex valves except in late maturity. Ribs markedly flattened. Umbonal areas strongly convex (although not as angular as in middle Eifelian species). Internally, a cemented, elevated pedicle collar is typical.

Atryparia stabilia n.gen., n.sp.

Derivatio nominis: stabilia (Latin) = things that are stable. The ventral valve is planar, giving the shell a stable position.

Holotypus: Specimen in Fig. 2, 5–8. *Locus typicus:* Weinberg quarry, 1 km north-west of Kerpen, Hillesheim syncline, Eifel. *MTB Dollendorf* r5098 : h7572. *Stratum typicum:* Bohnert sub-horizon, Nollenbach horizon, Freilingen beds, upper Eifelian (Fig. 1). Common down

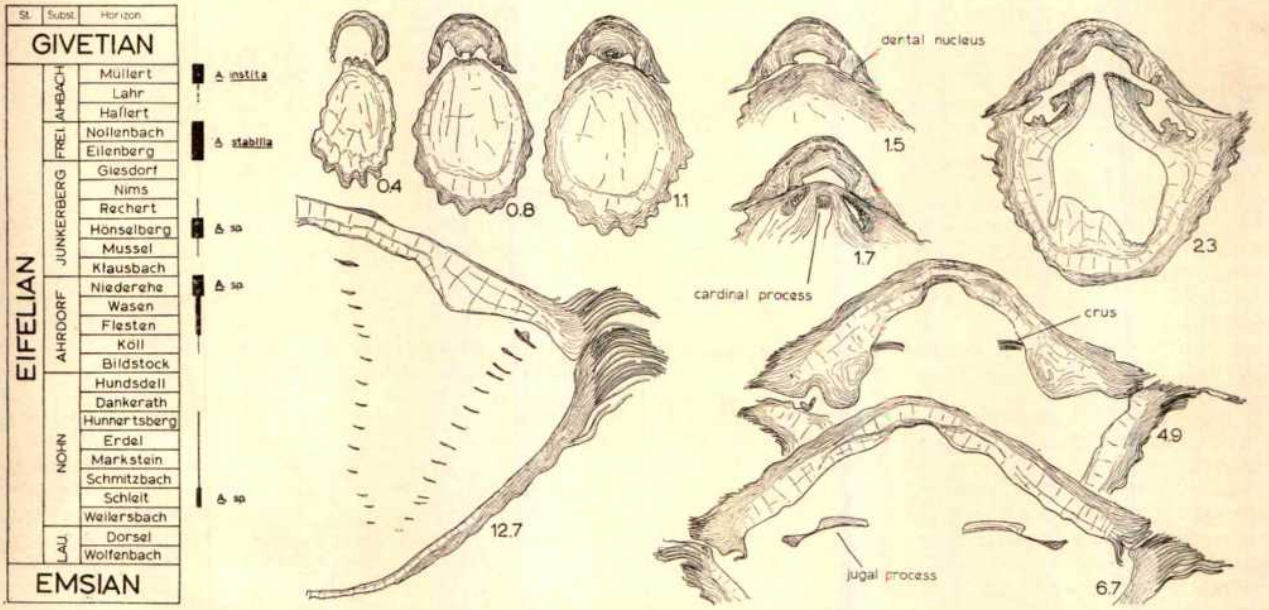


Fig. 1. Left: Stratigraphic column of the Elfel area, Germany, showing chronological distribution of *Atryparia* n.gen. Right: Transverse serial sections of *Atryparia institia* n.sp., paratype *Pc*354.*C*279*i*, Müllert horizon. Intervals in mm (× 2·5). (Abbreviations: *A.* = *Atryparia*, Lau. = Lauch, Frei. = Freilingen.)

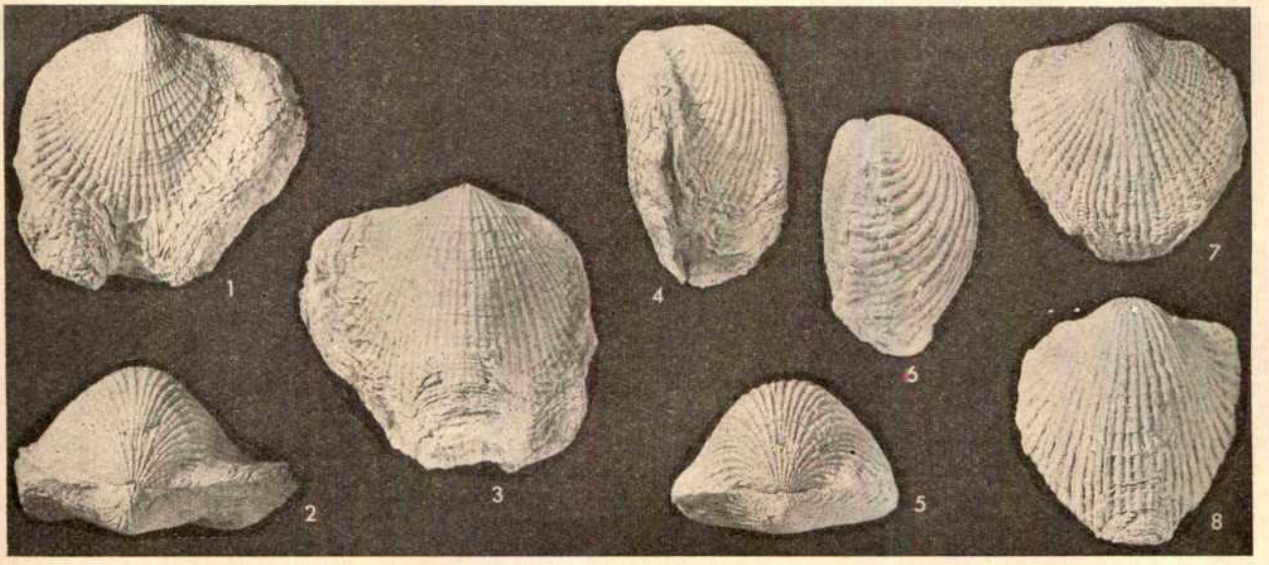


Fig. 2. 1–4, HOLOTYPUS *Atryparia institia* n.gen., n.sp., *Pc*351.*C*279*i*, Müllert horizon, *MTB Dollendorf* r5548: h7739 (× 1). 5–8, HOLOTYPUS *Atryparia stabilia* n.gen., n.sp., *Pc*355.*C*345, Nollenbach horizon, *MTB Dollendorf* r5093: h7572 (× 1)

to Eilenberg horizon, Freilingen beds. *Associated fauna: Kerpina vineta* Struve, *Carinatina plana* (Kayser), *Spinatrypa aspera* (Schlotheim), *Atrypa* aff. *squamifera* (Schnur).

Diagnosis: Flattened ventral and strongly convex dorsal valves show more prominently etched, deeply troughed ribs than *Atryparia instita* n.sp. Frills generally lacking; valves much flatter in early ontogenetic stages. Beak blunted, hinge margins straightish. Internally, a pedicle collar is lacking and the dental nuclei are very clearly defined.

Species of the new genus promise to be of considerable value in Mid-Devonian correlations of Europe, and have successfully been used in the Eifel for detailed biostratigraphy. Straight-line trends are still not fully known, although ribs appear to become progressively coarser, and there is a size increase in younger forms. It is likely also that there was little or no development of a pedicle layer in lower Eifelian species and that this structure became progressively more complex.

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NORTH MEDITERRANEAN CLIMATE DURING THE LAST WÜRM GLACIATION

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IT is generally assumed that non-glaciated regions at temperate latitudes were subjected to cool and humid climate (pluvial) during Pleistocene glacial maxima[1]. Data obtained recently in south European areas bordering with the Mediterranean seem, on the contrary, to give evidence of markedly arid climate during the last Würmian Glacial, as also pointed out by Van der Hammen *et al.*[2].

The data in question include the following:

(*a*) Pollen sequences from the sediments of Monterosi and Baccano[3], two crateric lakes of central Italy (Fig. 1) lying at an altitude of about 200 m above present sea-level. Their combined pollen diagram has been dated using carbon-14 at ten levels, and shown to extend continuously from about 25,000 years ago to the present. From 25,000 to about 15,000 yr. B.P. (that is, during the second maximum of the Würmian glaciation) the area in question was essentially treeless, and was covered by a cold steppe, with *Artemisia* as the dominant plant (Fig. 2). This steppe gave way to a grassland from about 15,000 to 12,000 yr. B.P., then to a shrubland (with *Corylus*), which preceded forestation at about 11,000 yr. B.P., that is, in correspondence with the 'Alleröd' of northern Europe. During most of the Post-Glacial period a forest (with *Quercus* dominant) was prevalent in the region. This sequence suggests cold-arid climate during the Glacial period, gradually giving way to warmer and humid conditions in the Post-Glacial.

(*b*) The presence of a Würmian steppe according to the Monterosi sediments is confirmed by a cold steppe fauna, with *Elephas primigenius*, *Equus idruntinus* and *Capra ibex*, which is recorded as having inhabited central Italy during the last Würmian peak[4].

(*c*) Pollen diagrams from lacustrine deposits at Fimon and Arqua in the eastern Po River Valley, a few metres above present sea-level[5], show an early phase where there is a paucity of arboreal vegetation and a dominance of *Artemisia*, which suggests an arid steppe environment. No carbon-14 datings have been published for these sequences, but Lona[5] attributed this portion of the diagrams to the last Glacial peak. In both diagrams *Corylus* precedes the establishment of an oak-dominated forest in the Post-Glacial, as in the Monterosi sequence.

(*d*) Preliminary pollen spectra and three carbon-14 datings of a thick peat deposit from Macedonia, Greece, have been published recently[6]. Again a steppe environment with *Artemisia* dominant prevails during the last Glacial, while a humid oak forest takes over in the Post-Glacial. Older portions of the diagram suggest that similar alternations have been present also in previous Pleistocene glacials.

(*e*) Pollen diagrams from Spain[6] reveal sequences very similar to that shown in Fig. 2. For example, carbon-14 datings have been made of a diagram from the Lagoon of Sanguijuelas in north-western Spain at seven levels; this goes from about 15,000 yr. B.P. to the present. The full Glacial is thus not represented in it; even so, during the earliest 3,000 yr. of this sequence (that is, during the Tardiglacial) the non-arboreal pollens are prevalent, and dominant among them are steppic general such as *Artemisia* and *Ephedra*, while in the Post-Glacial a forest with 'Quercetum' becomes prevalent. Other diagrams from coastal areas of southern Spain have many features in common with the diagrams from central Italy, but do not extend farther back in time than the Post-Glacial.



Fig. 1. Some of the locations where evidence of Würmian arid climate was obtained: (*a*) Monterosi and Baccano lakes; (*b*) Lazio region with cold steppe fauna in the last Glacial; (*c*) Lakes of Fimon and Arqua; (*d*) Deposit of Tenagi Philippon, Macedonia; (*e*) Lagoon of Sanguijuelas

Present results thus suggest that the main features shown in Fig. 2 are to be considered valid for the whole north Mediterranean, supporting the concept of a prevalently arid climate in this area during the last Würmian peak.

A tentative explanation for the diminished precipitation in the north Mediterranean contemporary with glaciations at higher latitudes would be as follows: much of the precipitation in the south

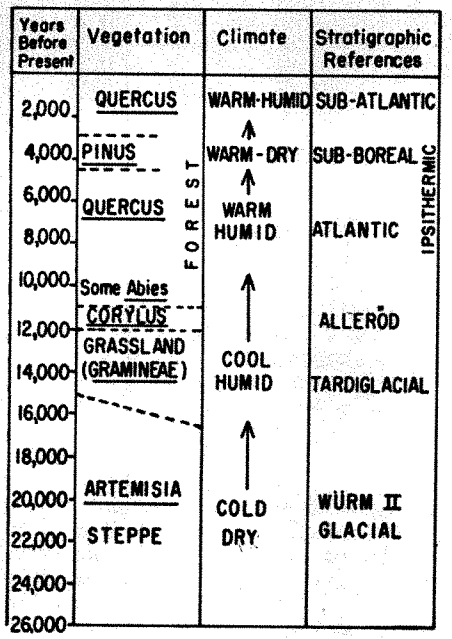


Fig. 2. Generalized scheme of the vegetation and climatic changes in central Italy at moderate altitudes from the last Glacial maximum to the present. Data derived from pollen analyses of sediment cores from Monterosi and Baccano. The time scale is based on ten carbon-14 measurement datings of the cores. The main features of this diagram appear to be generally valid for south European regions

European regions derives at present from westerly cyclonic air masses which originate over the Atlantic, where they pick up most of their moisture. During Glacial maxima the temperature of the surface water of the north Atlantic at intermediate latitudes on the average was approximately eight degrees lower than at present[7]. As a result evaporation was also lower, and drier cool winds would sweep southern European regions. At the same time, according to Emiliani and Geiss's scheme[8] of the glaciations, relatively warm water was transported into the north-east Atlantic and adjacent seas by the Gulf Stream. Such water masses would provide the moisture to feed north European ice sheets advancing southwards from the Scandinavian areas.

In addition to being drier, the aforementioned westerly air masses were probably less frequent in the Mediterranean during glacial maxima, being repelled by cold, dry winds entering the Mediterranean from the north-east (Russia). Such winds were presumably more intense and frequent than at present, due to a greater temperature gradient between glaciated north-east European areas and North Africa during glacial maxima. The increase in temperature of Atlantic water at the end of the Glacial together with the diminished importance of the north-easterly winds would cause increasing precipitations in southern Europe, and development of a humid climate, as indicated by the Post-Glacial portion of the aforementioned pollen profiles.

Evidence of aridity in southern Europe during Glacials should be detectable in Mediterranean deep-sea sediments. Indeed, cores from the western Mediterranean show Würmian sections where wind-borne minerals (mainly quartz) are prevalent; such eolian components disappear in the Post-Glacial[9]. It is also to be expected that sedimentation rates in the north Mediterranean should be highest during Late-Glacial phases due to stronger erosion on adjacent land, related to the melting of the Würmian glaciers and to the paucity of vegetation.

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BINDING OF CATIONIC DYES TO NUCLEIC ACIDS AND OTHER BIOLOGICAL POLYANIONS

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THE precipitation of polyanions by cationic dyes in its simplest form can be expressed:

$$P^{z-}zM^{+} + zR^{+} \rightleftharpoons P^{z-}zR^{+} + zM^{+}$$

where P^{z-} is a polymer P with z anionic sites, M^{+} the counterion and R^{+} the precipitating cation. Applying the law of mass action to this equation in a 'model' treatment neglecting activity coefficients, it can be shown that the ratio of the concentrations $[P^{z-}zR^{+}]/P^{z-}zM^{+}]$ changes from very low to very high values inside a narrow range of the ratio $[R^{+}]/[M^{+}]$ if z is large (for example, > 10). If the concentration $[R^{+}]$ is constant, the position of the sharp inflexion in the curve of $[P^{z-}zR^{+}]/P^{z-}zM^{+}]$ against $[R^{+}]/[M^{+}]$ can be expressed in terms of $[M^{+}]$. The region of $[M^{+}]$ in which the ratio $[P^{z-}zR^{+}]/[P^{z-}zM^{+}]$ changes markedly is defined as the 'critical electrolyte concentration' (CEC) (refs. 1, 2). The equilibrium constant determines the value of the critical electrolyte concentration, and is in turn governed by the relative affinities of R^{+} and M^{+} for the polyanionic sites[2].

'Affinity' is the sum of all the forces acting between the participants. Coulombic and van der Waals forces, hydrogen bonds and charge transfer may all be involved. An examination of a large number of polyanions, electrolytes and precipitants has shown that in those cases in which Coulombic interactions predominate, there is a distinctive pattern of critical electrolyte concentrations, and therefore of relative affinities of M^{+} and R^{+} for the polyanion[3]. The critical electrolyte concentration depends primarily on the type of anionic site on the polymer. Critical electrolyte concentrations are grouped in the order CEC ($-COO^{-}$) < CEC ($=PO_4^{-}$) < CEC ($-SO_4^{-}$) when R^{+} is an ion of low electrostatic field and polarizing power, and in the opposite sequence when R^{+} contains a chelated Al^{+++} or similar cation with a high polarizing power[4].

Molecular weight and charge density are factors affecting the critical electrolyte concentration, but except when they are low they are less important than the charge type[5].

In those cases in which the charge is not due to chelated Al^{+++}, etc., the structure of R^{+} around the cationic site can be considerably changed without altering the critical electrolyte concentration pattern[3]. The charge may be centred on one, or spread over several atoms, which may

include nitrogen, carbon or sulphur, or the cation may be polyvalent. Substituents which 'shield' the cationic charge from the anionic site lower the critical electrolyte concentration.

The establishment of a pattern of Coulombic behaviour permits the interpretation of variations from this pattern in terms of extra contributions from van der Waals forces, hydrogen bonds, etc. Thus, we find that the behaviour of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) towards a range of dyes differs from that of polyphosphates in general. This appears to be a consequence of interactions between the dyes and the purines and pyrimidines of the polynucleotides.

The critical electrolyte concentration phenomenon has been used to localize tissue polyanions with greater specificity than is possible using the dye (alcian blue) alone[2,6]. This article describes an extension of that work, using a wider range of dyes and polyanions.

The dyes used in this investigation were standard histological preparations from B.D.H. Ltd., Poole, Dorset; G. T. Gurr, Ltd., London, S.W.6; or (in the case of alcian blue and alcian yellow) gifts from Imperial Chemical Industries, Ltd., Blackley, Manchester, through the courtesy of Mrs. S. M. Neal. Polyanions, other than polynucleotides, were prepared by the method of Scott[7], and were gifts from Dr. F. E. T. Pautard and H. Zola (onuphic acid[8]), Dr. S. Gardell (keratansulphate), Dr. Cifonelli (heparan sulphate, chondroitin sulphates *A* and *B*) or were commercial samples (heparin, alginate, polyacrylate, polyphosphate). Some polynucleotides were commercial samples (thymus DNA, from Worthington Biochemical Corp., New Jersey, U.S.A.; polyadenylic and polyuridylic acid from Miles Laboratories Ltd., Elkhart, Indiana, U.S.A.; RNA core from Koch-Light Laboratories, Colnbrook, Buckinghamshire), but for the most part were made by the method of Kirby[9,10].

Polyanion solutions were spotted and dried on circles of Whatman No. 1 filter paper, which were then immersed in solutions containing dye and salt. The dyes were at 0·01 per cent w/v. All except those containing aluminium chloride were in 0·05 M sodium acetate buffer *p*H 5·7. After a minimum of 1 h at room temperature the papers were blotted and dried in air. The critical electrolyte concentration of a particular polyanion–dye complex was taken to be the highest concentration of electrolyte at which staining of the polyanion 'spot' was apparent. Binding to carbohydrate polyanions of thioflavine *T*, 9-amino acridine, acridine orange and pyronine was conveniently and sensitively assessed in ultra-violet light (Shandon 'Chromatolite'). Unbound dye fluoresced brilliantly, whereas bound dye did not. Similar observations on polynucleotides were not so easy to interpret, owing to the presence of ultra-violet absorbent material in the polyanion, and in these cases results were recorded in daylight. The results are shown in Table 1. Although similar and even more striking results were obtained in solutions of magnesium chloride, results in Table 1 are quoted in sodium chloride, since many polynucleotides are precipitable by Mg^{++}, thus rendering interpretation more equivocal.

Within the group of polyanions containing $-COO^-$ and/or $-SO_4^-$ groups and no aromatic rings, CEC ($-COO^-$) < CEC ($COO^- + SO_4^-$) < CEC ($-SO_4^-$). This sequence is the same as that observed for a large number of organic cations[3]. In general, critical electrolyte concentrations are low, and in some cases staining was observed only in the absence of added sodium chloride, that is in the presence of 0·05 M sodium acetate buffer. In the case of methyl green the buffer electrolyte was sufficient to prevent staining of most of this group of polyanions.

Critical electrolyte concentrations of those polyanions which possess aromatic rings, as purines or pyrimidines, are much higher. The difference is particularly striking in the case of pyronine. Other dyes behave similarly to those shown in Table 1, for example, methylene blue, thionine, toluidine blue, safranin, crystal violet and 'Rivanol'. In view of the wide range of chemical structures involved, the most reasonable explanation of the enhanced binding lies in extra interactions due to conjunction of aromatic systems of dyes and polyanions.

More sites should be available for interaction on single-stranded molecules with less secondary structure than on those with much double-stranded or other secondary structure. RNAs in general have higher critical electrolyte concentrations than DNAs. The lower critical electrolyte concentration of thymus DNA, as compared with rat liver DNA, is probably attributable to its greater secondary structure, due to dissimilar preparative procedures, or to native differences in the structure in the chromosomes. Up to 80 per cent of DNA of the interphase lymphocytes is in the repressed chromatin fraction[11] and DNA from the repressed chromatin has a higher T_m[12] than that from active chromatin[13], indicating a greater degree of secondary structure. Using a spectrophotometric method, Drummond *et al.*[16] have shown that there are more binding sites available to amino-acridines on thermally denatured DNA than on native DNA. Ribosomal RNA (ref. 10) which had been converted into the tetraethyl-ammonium salt, dissolved in 50 per cent ethanol and then recovered as the Na^+ salt (RNA rat liver I) showed a higher critical electrolyte concentration than ribosomal RNA which had been isolated as the Na^+

Table 1. Critical Electrolyte Concentrations (in Molarity Sodium Chloride) of Dye-Polyanion Complexes adsorbed on Filter Paper

	Alcian blue	Thioflavin *T*	Azur *A*	9-Amino acridine	Methyl green	Acridine orange	Pyronin
Hyaluronate	0·2	< 0·05	< 0·05	< 0·05	†	< 0·05	< 0·05
Alginate	0·3	< 0·05	0·1	< 0·05	†	< 0·05	< 0·05
Polyacrylate	0·3					0·2	
Chondroitin sulphate-*A*	0·8	< 0·05	0·1	< 0·05	†	0·2	< 0·05
Chondroitin sulphate-*B*	0·8	< 0·05	0·1	< 0·05	†	0·2	< 0·05
Shark chondroitin sulphuric acid	0·8	< 0·05	0·1	< 0·05	†	0·20	< 0·05
Heparan sulphate	0·5	< 0·05	0·1	0·1	†	0·1	< 0·05
Heparin	0·9	< 0·05	0·4	0·3	0·1	0·2	< 0·05
Keratansulphate	1·5		0·3	< 0·05	0·1	0·2	< 0·05
Onuphic acid (poly-glucose phosphate)	0·3	0·1	0·2			0·2	
RNA rat liver I (unfractionated)	> 1·5	> 1·2	> 1·2	> 0·3	< 0·05	1·5	> 3·0
RNA rat liver II (unfractionated)	0·5	0·5	> 1·1	0·5	< 0·05	1·0	> 3·0
RNA 18*S* rat liver	0·3	0·2	0·5		< 0·05	0·5	> 3·0
RNA 28*S* rat liver	0·5	0·5	0·7		< 0·05	1·0	> 3·0
RNA *Drosophila* (unfractionated)	0·5	0·5	> 1·1	0·4	< 0·05	1·5	> 3·0
RNA 18*S Drosophila*	0·5	0·5	> 1·1	0·4	< 0·05	1·5	> 3·0
RNA 28*S Drosophila*	0·3	0·1	0·9	0·4	< 0·05	0·8	
RNA 4*S* (high adenine)	0·3	< 0·05	0·3	0·2	< 0·05	0·2	
RNA core	0·3	0·1	0·4		< 0·05	0·1	0·1
DNA (thymus)	0·5	< 0·05	0·3	0·05	> 3·0	0·2	
DNA rat liver	0·5	> 1·2	> 1·2	> 0·3	> 1·2		
Polyadenylic acid	0·5	> 3·0			†	> 3·0	> 3·0
Polyuridylic acid	0·3	0·1			†	0·5	0·1

† Staining was not present in 0·0 M sodium chloride.

salt without the step involving solution in 50 per cent ethanol as the tetraethyl-ammonium salt (RNA rat liver II). Since this step was associated with some loss of secondary structure, the higher critical electrolyte concentration may indicate stronger dye binding due to interaction with purines and pyrimidines thus exposed. (This is especially clear in the case of alcian blue.)

Polyadenylic acid, with purine rings, offers greater potential interaction than polyuridylic acid with the smaller pyrimidines, and shows a much greater critical electrolyte concentration.

Experiments were carried out in aqueous solutions containing potassium, magnesium or lithium acetates, with 9-amino acridine or 'Rivanol'. The results were in substantial agreement with those shown in Table 1, and will be reported in detail elsewhere.

Two dyes stand out from the pattern. Methyl green is a poor precipitant for polyanions in general. DNA, however, shows a very marked and specific interaction, which is not suppressed by salt. Heat-denatured DNA shows much less affinity, implying that the double-stranded helix is necessary for this interaction. It is possible that intercalation between base pairs occurs, with alignment of the two cationic charges of the dye with ionized phosphate ester groups at opposite sides of the helix. The roughly complementary critical electrolyte concentration pattern of pyronin and methyl green is of interest histologically, and this aspect will be discussed elsewhere.

Alcian blue behaves as though it were bound only by electrostatic forces. It appears to be a better precipitant of carbohydrate polyanions than the other dyes, and its interaction is not very sensitive to suppression by electrolyte. In one instance only, with rat liver RNA (I), does the generalization not hold, and in this case the evidence shows that the RNA structure is abnormally open, with good sites available for extra binding. It seems that alcian blue can bind with forces additional to electrostatic bonds, but that it does not normally do so. In this respect it differs from all other cationic dyes tested. The geometry of the molecule is unlike that of other dyes[3]; the cationic charge is not part of the chromophore, it is polyvalent, and it is very bulky. Any one factor could upset the alignment of ring systems and charges necessary for two modes of binding. Alcian yellow, another cationic dye based on the copper phthalocyanine ring, behaves similarly to alcian blue.

Table 2. CRITICAL ELECTROLYTE CONCENTRATIONS (IN MOLARITY ALUMINIUM CHLORIDE) OF DYE–POLYANION COMPLEXES ADSORBED ON FILTER PAPER

	Alcian blue	Muci-carmine	Aluminon	Haema-toxylin
Alginate	†	0·2	0·2	0·03
Hyaluronate	†	0·06	0·4	†
Polyacrylate		> 0·5	0·1	> 1·0
Chondroitin sulphate *A*	0·3	†	0·2	†
Chondroitin sulphate *B*	0·3	†	0·2	†
Chondroitin sulphate *C*	0·4	†	0·2	†
Shark CSA	0·2	†	0·2	†
Heparan sulphate	0·1	†	0·1	†
Heparin	0·5	†	0·1	†
Keratansulphate	0·5	†	0·05	†
Polyphosphate	0·05	0·06	0·1	0·06
Polyuridylic acid	0·06	> 0·5	> 1·0	> 1·0
Polyadenylic acid	0·03	> 0·8	> 1·0	> 1·0

† Staining was not present in 0·0 M added aluminium chloride.

Similar evidence for two modes of binding was obtained by experiments with dyes which depend for their cationic charge on chelated Al^{+++} (Table 2). The pattern of critical electrolyte concentrations is CEC $(-SO_4^-)$ < CEC $(-COO^- + SO_4^-)$ < CEC $(-COO^-)$ and CEC $(-SO_4^-)$ < CEC $(=PO_4^-)$. This pattern is identical with that of 'affinities' of small highly charged inorganic cations for $-COO^-$, $=PO_4^-$ and $-SO_4^-$ (ref. 14). It seems that the character of the electrostatic interaction of mucicarmine, aluminon, and haematoxylin is determined by that of the chelated ion. The presence of purines and pyrimidines in the polyanion considerably increases the critical electrolyte concentrations, as in the non-chelate dyes, and is presumably due to similar causes, namely interactions between aromatic systems of dye and polyanion. The histological implications of this pattern in the chelate dyes will be discussed elsewhere.

The role of purines and pyrimidines in the binding of dye-like molecules to polynucleotides has been controversial since it was first suggested[15]. Measurements of metachromasia[16], fluorescent yield[17], thermal denaturation[18] and chemical reactivity of intrahelical amino groups[19] have all pointed to the existence of more than one type of binding. Intercalation provides for an extra mode of binding, apart from purely electrostatic combination with $=PO_4^-$, but a 'stacked' dye array would also provide additional affinity[20] without the necessity for intercalation. Calculations of free energies of intercalation and 'stacked dye' models[16] showed that, although both had less than DNA itself, the intercalated model had least. It is possible that intercalation and stacking can co-exist. Fluorescent quenching and metachromasia have been shown to vary according to the base composition of the polynucleotides[17,21]. Although it is difficult to interpret such findings in a way that does not involve interaction between dye and purine or pyrimidine base, the lack of a firm diagnostic method for electrostatic binding, inclusive of random and stacked arrays, in the presence of non-electrostatic binding, is conducive to confusion. The present semi-quantitative method permits an assessment of the contribution of the $=PO_4^-$ by relating it to $-COO^-$ and $-SO_4^-$ (by analogy, the properties of bromine could be related to those of chlorine and iodine). In this method a phase transition is involved, which restricts comparisons with other data, most of which have been obtained in aqueous solution. The results strongly suggest that there is an extra interaction with polynucleotide bases, which is modified by the occurrence of secondary structure in the polynucleotide, in qualitative agreement with the experimental data previously cited[16,21]. The type of interaction is not definable, but probably involves at least van der Waals and 'hydrophobic' bonds.

If it is true that some disruption of secondary structure enhances binding of aromatic compounds, it implies that small aromatic molecules may exert their maximum biological effect while secondary structure is being built up or broken down, that is, during replication and transcription.

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TRANSFORMATION OF FIBROUS TISSUE TO BONE *IN VIVO*

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NUMEROUS investigations and debates have been concerned with the source of osteogenic cells during the past century. In recent years, investigations carried out with tissue culture techniques[1,2] and with the aid of tritiated thymidine[3,4] or electron microscopy[5] have indicated that mesenchymal cells, particularly those associated with vessels as perivascular connective tissue cells, may specially act as osteoblasts. The term 'osteoprogenitor' has been applied to these cells[4]. For several years, work in these[2,6] and other laboratories[7] has indicated that it is the environment in which the cell resides that determines largely whether it will produce or destroy bone, cartilage or fibrous tissue. In view of these observations it may be presumptive to label a particular cell as an osteoprogenitor cell until it has responded to its micro-environment and begun forming bone—at which time it is an osteoblast. Generally it is agreed that the osteoblast synthesizes collagen, mucopolysaccharides and, probably, lipids[1]. These organic fractions of the extracellular osseous matrix are later mineralized in a pattern which is characteristic, at the ultrastructural level. Calcium hydroxyapatite crystals are arranged in and on the collagen fibrils, with their *c*-axes parallel to the long axes of the fibrils[8,9]. This pattern has been mimicked by collagen in tendon exposed to tissue fluids *in vitro*[10] and by turkey tendon being converted to bone[11], suggesting that pre-existing collagen can be altered so that it is mineralized.

This article reports the pattern of healing of 'Millipore'-isolated defects in the cortex of canine radii, in which additional features of the behaviour of osteogenic cells and the transformation of fibrous tissue to bone were observed. The operative technique, described previously[12], produces a chamber measuring 0·4 × 2 cm (Fig. 1) which is open to cells entering from its proximal or distal cortical ends and not directly from the marrow cavity, periosteum or overlying soft tissues. In the present investigation, groups of 3–5 adult animals were killed 8, 12, 16, 21, 24, 27, 30, 60, 180 and 320 days after operation and multiple serial histological sections were prepared from the regions of the defect. Initially the chambers were filled with a fibrin clot which was invaded by vessels and spindle-cells in a centripetal manner. This invasion occurred at different rates in different animals, depending largely on the number of cells migrating into the chamber by way of Haversian and Volkmann canals in the cortex at the ends of the chambers. After 21–31 days, most chambers were filled with a dense fibrous connective tissue (Fig. 2). This fibrous tissue seemed to be transformed directly to fibre bone and, eventually, to mature lamellar bone so that, after 320 days, cross-sections through the middle of the defects were indistinguishable from the unoperated cortex proximally and distally (Fig. 3). Newly forming bone was always anchored to pre-existing bone. No cartilage was observed in any of these 'Millipore'-ensheathed specimens. At the interface between the new bone and fibrous tissue, collagen bundles were continuous (Fig. 4). In the regions where they emerged from the woven fibre bone, these fibre bundles frequently were surrounded by plump, secretory, polygonal cells with basophilic cytoplasm and a clearly defined Golgi apparatus. These cells seemed to develop from spindle cells already present between the collagen fibres. The front of newly formed bone advanced steadily toward the centre of the chambers in a centripetal fashion, until all fibrous tissue had been converted into bone. In some animals the process was completed in 60 days, while in others small amounts of residual fibrous tissue were found in the centres of the chambers after 180 days. Although conversion of fibrous tissue began as early as 16 days, lamellar bone appeared primarily after the defects were bridged by fibre bone. At no time were significant numbers of multinucleated osteoclasts observed in the bone formed in the defects. The dead cortical bone at the ends and sides of the defects, however, was replaced ('revitalized') by the alternative waves of osteoclasis and osteogenesis, characteristic of creeping substitution.

These experimental studies suggest that mature fibrous tissue may be transformed directly to bone. The process has certain histological features in common with those reported by Johnson[13] during the ossification of turkey tendon and during the moving of teeth by orthodontic forces[14]. In the case of this and the turkey tendon study, spindle cells, apparently dormant between the bundles of

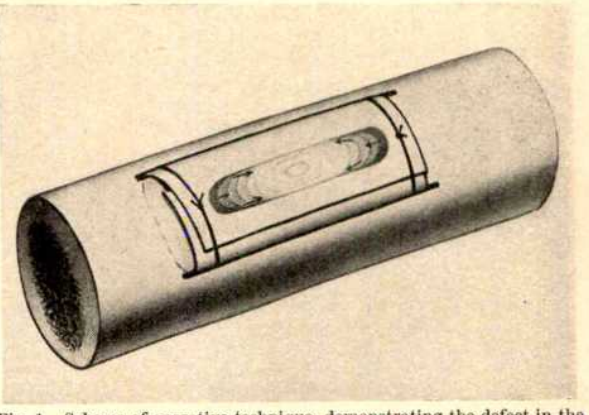


Fig. 1. Scheme of operative technique, demonstrating the defect in the canine radius, isolated from the medullary canal and overlying soft tissues by a 'Millipore' membrane. Defect heals centripetally, from either end

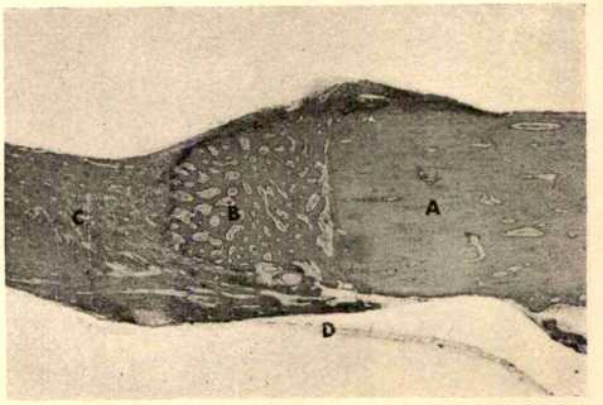


Fig. 2. 30-day longitudinal specimen from proximal one-half of defect. Haematoxylin and eosin (× *c.* 10). *A*, Cortex proximal to defect; *B*, newly formed bone; *C*, dense fibrous tissue filling centre portions of defect; *D*, 'Millipore'

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collagen fibres, became plump, secretory and resulted in the conversion of a non-mineralized connective tissue to a mineralized connective tissue. Whether these spindle cells should be referred to as osteoprogenitor cells or mesenchymal cells seems more a problem of semantics than practicality. The major point is that they altered their functional state at specific times and in specific locations. While the exact nature of the cellular activity involved in this mineralization process is not evident from this study, two possible explanations seem to exist. First, the cells may have produced materials which, when added to the collagen already present, rendered the collagen 'calcifiable'. Protein–polysaccharide complexes and/or lipids have been thought by others[13,15] to be involved in such a mechanism. On the other hand, it is possible that cellular activity may have removed agents inhibitory to calcification, such as pyrophosphate[16].

In the chambers of some animals the osteogenic response was so explosive that bridging by fibre bone was almost complete in 30 days, while in others it advanced very slowly and required 6 months to fill the gap with bone. These differences appeared to be related mainly to the rate at which cells could enter the chamber during the first 2 weeks after operation. Afterwards, the differences seemed to correlate with the rate of cell proliferation and, possibly, the amount of instability of the radius resulting from the procedure and the activity of the animals. The chronicity of the osteogenic response in some animals suggests that the stimulus for continuing bone formation may be different from that of an acute injury potential. The findings of this investigation are consistent with the view that osteogenesis requires a large number of cells with the proper genetic endowment, a nutritional environment that will support them, and a stimulus, the nature of which is still unidentified. They should encourage orthopaedic surgeons faced with treatment of fibrous union or pseudarthrosis, for they demonstrate that mesenchymal cells, residing in fibrous tissue, probably retain a capacity to form bone when properly stimulated.

Osteoblastic activity was noted where prominent, longitudinally oriented, collagen bundles emerged from bone. Frequently, these bundles seemed to be arranged along lines of tensile stress and linked the two ends and sides of the defect. Similar behaviour, that is, osteoblastic activity, is observed on the tension side of a tooth being moved by orthodontic forces[14]. Bone formation occurs between the fibres of the periodontal membrane where they emerge from alveolar bone. Furthermore, bone formation in tendon has been reported when tensile forces are excessive[17]. Although it is not known exactly how mechanical factors can stimulate this type of cell specialization, recent investigations have demonstrated that bone[18–20] and tendon[21] have piezoelectric properties and generate an electric signal in response to deformation. Furthermore, weak currents, artificially induced in bone, have been observed to affect osteogenesis[22], and it is likely that currents of similar magnitude, generated *in vivo*, may control the metabolic activity of osseous cells[23].

Finally, while the conversion of non-mineralized connective tissue to mineralized connective tissue was associated with characteristic cell activity, the conversion of fibre bone to lamellar was not. There were remarkably few multinucleated osteoclasts or resorption cavities during the reparative process. Furthermore, lamellar bone began to appear in the defect only after bridging by fibre bone was virtually complete. Is it possible that the stress patterns in the bridged chambers focused local electro-mechanical stimuli on osteocytes, thereby stimulating them to carry out cyclic, internal remodelling of the immature, weak bony structure? Such a cycle of osteolysis and osteogenesis by osteoclastic and osteoblastic osteocytes has been indicated by the recent studies of Bélanger[24].

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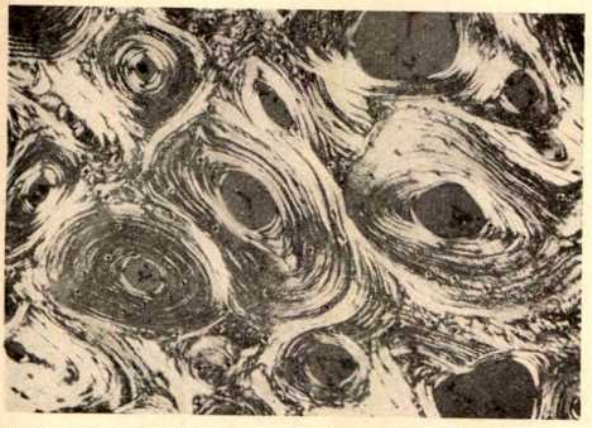


Fig. 3. 320-day specimen, cross-section from middle of defect. Haematoxylin and eosin (× *c.* 100, polarized). Birefringent lamellar pattern of osteones. This picture was indistinguishable from cross-sections taken from unoperated radial cortex proximal and distal to the defect

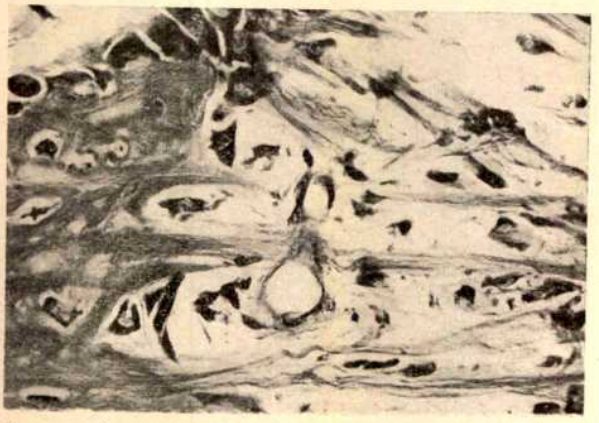


Fig. 4. 24-day specimen, longitudinal section from region at interface between new-fibre bone and fibrous tissue. Haematoxylin and eosin (× *c.* 350). Note the bundles of collagen fibres emerging from bony front and extending into the fibrous tissue. Note also osteoblastic activity along the bony front

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ORIGIN AND NATURE OF THE CUTICULA DENTIS

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THE dental cuticle first described by Gottlieb[1,2] is one of the most controversial structures in dental histology and pathology. Gottlieb described it as a 'horn layer' or 'membrane' frequently formed by the outer enamel epithelium covering the tooth crown (secondary enamel cuticle), and later, by proliferation, the root cementum. Around this structure Gottlieb[1–6] built up theories which are still largely accepted to-day concerning the eruption of the teeth, surface protection against caries, the pathology of periodontal disease, the gingival sulcus and the attachment of the gum to the tooth surface and their importance in the health and disease of the periodontium.

In histological sections stained with Ehrlich's haematoxylin and eosin Gottlieb's cuticle appears as an eosinophilic or unstained, clear, hyaline or granular membrane-like layer of varying thickness (2–15μ), lying between epithelium and the tooth surface (Fig. 2*A* and *C*), or applied to the tooth surface after loss of the epithelium. In unstained sections it appears refractile and bluish-green.

Its presence, form and general appearance are well documented[7–23]. Nothing is known of the origin of the dental cuticle and little regarding its chemical nature. Although it is almost universally accepted as being a keratinous structure, Sicher[21,24] and others have recently revived Weski's[7] and Lehner's[16] conclusions that it is not keratin formed by maturation of epithelial cells but a 'keratoid' cementing substance secreted by epithelial cells. Schüle[25,26] carried out extensive histochemical and physical investigations but gave insufficient evidence of his identification of the secondary cuticle. Wertheimer and Fullmer[22] found that the cuticle protein was not a keratin but contained a prosthetic carbohydrate of unusual composition. They interpreted their results, however, as strongly indicating an epithelial origin[27]. Almost all authors believe the dental cuticle is derived from epithelium. Turner[20] believed that it was a modification of the acquired salivary cuticle, while Waerhaug[28] suggested it could be merely a thickening of the primary cuticle.

More than 1,000 surgical and autopsy specimens of human permanent and deciduous teeth have been examined with and without their periodontium; some animal jaws were also examined, including the dog, hamster, rat and rabbit. Gottlieb's cuticle was observed irregularly distributed in 75 per cent of the teeth of the permanent dentition and considerably less in the deciduous dentition (estimated at 10 per cent). The dental cuticle was observed in some animals[30].

The cuticle was found on the exposed surfaces of the teeth but more commonly between the gingival epithelial cuff and the enamel and cementum. The cuticle on the cementum was identical in all respects with that on the enamel. It was also found in three of twelve unerupted teeth examined. In many cases in parts of its course it was composed of up to five or six layers often separated by remnants of degenerate epithelial cells or linear markings giving a laminated appearance (Fig. 1*C*). In some specimens the cuticle was present between cells of the epithelial cuff and not between the epithelium and the tooth surface. Present also were bulbous, circular and other shapes either at the termination of the cuticle or along its path which projected into the epithelium (Fig. 1*C*, 2*A*) or, not infrequently, appeared as separate 'bodies'. Investigations[30] have shown that the cuticle, 'hyaline bodies' and the multi-layered strips (noted also by Gottlieb[2] and Turner[20]) were identical with the 'hyaline bodies' present in some odontogenic cysts and elsewhere[9,27,29,31]. The similarity of the latter to the cuticle had been previously noted[9,31], but the paradontal 'hyaline bodies' have not been previously described[30].

It was clear from these and other observations that the cuticle did not arise either as a product of or a maturation

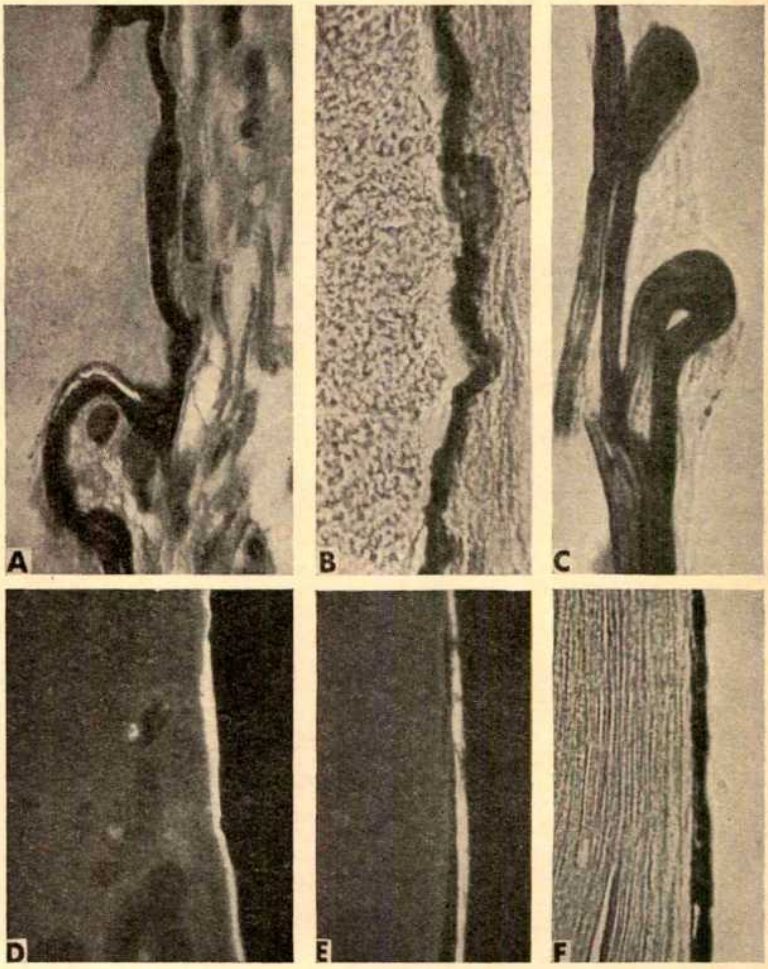


Fig. 1. *A*, 'Cuticle' between non-vital bone and squamous epithelium; stained orcein only (24 h) (× 770). *B*, 'Cuticle' between epithelium (right) and calculus mass; stained T.P.A. method, no counterstain (× 770). *C*, Multi-layered, laminated 'cuticle' with 'hyaline bodies' within and between epithelium (right) and cementum; stained Gomori's chromic acid methenamine (× 770). *D*, Autofluorescence of natural 'cuticle' on mandibular incisor between epithelium (left) and enamel surface (365 mμ) (× 770). *E*, Autofluorescence of experimental 'cuticle' of globin on an epithelial surface (left) (× 770). *F*, From same experiment as *E* showing globin layer stained by the T.P.A. method (× 770)

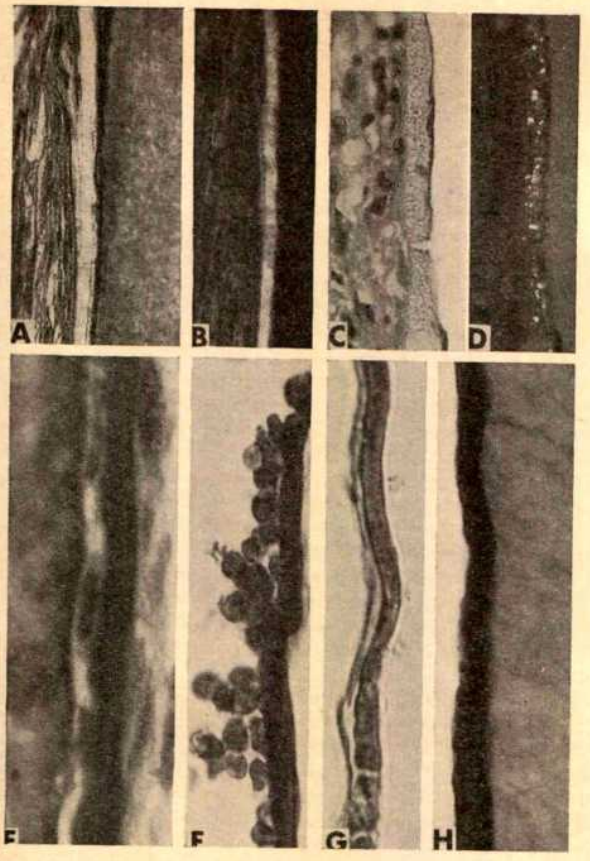


Fig. 2. *A*, Clear 'cuticle' between epithelium and cementum. Note bulbous projection and epithelial remnants between upper layer and cementum; haematoxylin and eosin (× 770). *B*, Same as *A* photographed by polarized light. *C*, Granular 'cuticle' between epithelium and enamel surface; haematoxylin and eosin (× 770). *D*, Same as *C* showing birefringent particles. *E*, Showing transitional stage of 'cuticle' from deep eosinophilia (on right, adjacent epithelial cells) to a clear layer with residual eosinophilic material (on cementum surface); haematoxylin and eosin (× 1,230). *F*, A stage of formation of experimental 'cuticle' showing conglutination of erythrocytes against wax (dissolved) to simulate enamel. Shows forming haemoglobin layer (right) and adjacent unpressed corpuscles; haematoxylin and eosin (× 770). *G*, Similar to *F*, but more complete layer. Note single erythrocytes still visible in lower part; haematoxylin and eosin (× 770). *H*, Similar experiment but, in place of wax, erythrocytes pressed and conglutinated against the surface of dentine to simulate cemental 'cuticle'. Compare with right side of layer in *E* (× 770)

of surface epithelial cells, or that it was a special structure of the enamel epithelium or paradontal epithelial cuff. This was further supported by the occurrence of similar hyaline layers in positions other than the classical sites[30], as for example on the enamel in dentigerous cysts, between hyperplastic epithelium and the cementum surface between tooth roots, between hyperplastic epithelium and the pulpal dentine within the pulp canal, between non-vital bone and encircling epithelium (Fig. 1*A*), between layers within calculus masses and between calculus and adjacent epithelium (Fig. 1*B*), between gingival surface epithelium and supra-gingival calculus deposits, and also, among other places, between cholesterol crystals where no epithelium was present. The layer was also found between epithelium and root apices in granulomatous lesions or in the process of exfoliation. Three such cases, including the case first observed by Gottlieb, appear in the literature[2,10,32].

A series of observations were made which led to a new approach to the problem of the origin and nature of the cuticle and enabled experiments to be devised to reproduce the membrane-like layer *in vitro*[30]. From the large amount of material examined, a consistent picture of the early stages of its formation was obtained. The structure appeared as a deeply eosinophilic layer of material with a slight basophilia, granular or homogeneous in consistency, varying greatly in thickness and density and developing first into a pale red, then yellowish and finally a poorly stained or unstained clear, glass-like or sometimes granular layer (Fig. 2*A* and *C*). All stages of transition from one appearance to the other were observed. Sometimes the red stage gradually or suddenly passed into the yellow and then the clear stage; in other cases the yellow or clear stage contained varying amounts of residual eosinophilic material or granules (Fig. 2*E*), which gradually diminished and disappeared as the layer became entirely clear.

Concurrently with these findings, other investigations highlighted a remarkable similarity in haematoxylin- and eosin-stained sections, between layers of keratin and membrane-like layers composed of conglutinated erythrocytes. These layers showed a number of structural features similar to those found in the cuticle. In various stages of change the cuticle showed structures which appeared to be fragmented erythrocytes.

There are no specific histochemical reactions for denatured haemoglobin, and its demonstration in tissues is difficult. Most of the general histological stains used stained the cuticle and erythrocytes in a similar manner. In some of the deeply eosinophilic cuticles an unequivocal positive reaction was obtained with the peroxidase method of Pickworth[33] for identification of haemoglobin. Reactions for iron were mostly negative. The most significant result was that obtained using the tannic acid–phosphomolybdic acid–amidoblack method for haemoglobin (which stains a deep blue) described by Puchtler and Sweat[34] and later revised by Puchtler, Rosenthal and Sweat[35] (T.P.A. method). In connexion with my studies on tissue haemorrhage, a thorough investigation of the T.P.A. method had been carried out using a wide variety of normal and pathological material, as well as various chemical and other preparations of haemoglobin and globin. The specificity of the reaction was most precise (Fig. 3), and my work supports the suggestion[34] that this stain affects the protein part of the haemoglobin molecule and reflects structural chemistry rather than amino-acid composition. The T.P.A. method showed a remarkable selectivity for the dental cuticle even at 2–3μ thick, staining it a deep blue (Figs. 1*B* and 3). The positive results with the peroxidase reaction, the specificity with the T.P.A. method, the appearances with general histological stains, and the morphological evidence in the early stages of formation showed that the dental cuticle was a haemoglobin product. It was also found that cuticle layers

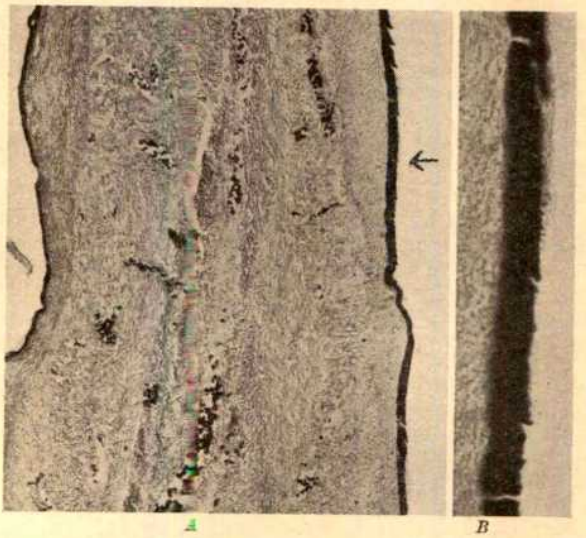


Fig. 3. *A*, Coronal section through interdental tissues of human mandibular incisors showing the newly described 'paradontal hyaline layers' between the reduced enamel epithelium and the enamel surface—note their close application to the epithelium, the specificity of the colour reaction and the similar precise staining of single erythrocytes and those in vessels (× 90). *B*, Area arrowed in *A* under higher magnification. Stained T.P.A. method, no counterstain (× 770)

exposed to the oral environment assumed a yellow or brown colour and lost their affinity for the amidoblack and many other stains. The nature of the residual eosinophilic material in the clear layers previously mentioned was investigated by staining with methyl-violet and comparing their appearances with natural and artificially produced Heinz bodies[36,37]. Their similarity suggested that they could be similar denaturation products of haemoglobin.

The elastic tissue stains were also found to be highly selective for the dental cuticle, hyaline layers and bodies except in the earliest stages of formation. Fullmer and Wertheimer[38] were the first to demonstrate the selective action of orcein on the cuticle (Fig. 1*A*), but they did not investigate other elastic tissue stains. My material showed an equally consistent selective reaction using Weigert's resorcin-fuchsin stain, Verhoeff's stain and Fullmer and Lillie's orcinol-new fuchsin[39]. With Gomori's[40] aldehyde fuschin stain for elastic tissue a positive (but variable) reaction was obtained. Using elastic tissue stains in conjunction with eosin, it was possible to trace in some specimens a transition from an unreactive part of the cuticle to a highly selective reaction. It appeared that after the layer had formed, a physical and probably a chemical change occurred in the denatured globin which apparently produced properties simulating some of those shown by elastic tissue. It is interesting to note that elastic fibres stain a pale blue with the T.P.A. method.

When examined by ultra-violet light (356 mμ) the cuticle in the light eosinophilic, yellow, or clear stages showed an intense bluish white autofluorescence (Fig. 1*D*), which was weaker or absent in the deeper eosinophilic stages. As tryptophan was found to be present it was probable that this was responsible for most of the fluorescence. The eosinophilic areas apparently contained substances, probably haem, in a state which caused a transfer of electronic energy, accounting for the reduction or quenching of the fluorescence[41]. In detail there were variations in the intensity of fluorescence which probably reflected variations in the complex nature of the degradation products of the haemoglobin[42,43]. Hileman[44] observed only a weak autofluorescence in the cuticle probably due to the examination of cuticles in the eosinophilic stages. When examined by polarized light, the layer showed a variable birefringence in a manner similar to the autofluorescence, the yellow or clear areas showing stronger birefringence than the less changed layers (Fig. 2*B*). It may be noted here that elastic tissue shows autofluorescence but not birefringence. In some of the clear cuticles highly birefringent particles were observed (Fig. 2*C* and *D*). These were possibly crystalline degradation products of haemoglobin rather than included foreign material as suggested by Turner[20].

The histochemical reactions obtained indicated the presence of sulphydryl groups, tryptophan, reducing substances, and some non-glycogen carbohydrate. Critical evaluation of many tests for lipid strongly suggested its general absence, but some residual lipid was present in a few cases; the blue sudan-black reaction was not due to lipid. What happened to the phospholipid of the erythrocyte stroma was not clear. The histochemical reactions showed variable results which might be expected in view of the chemical complexity of the denatured haemoglobin and the insensitivity of the methods available. However, the results obtained, together with the reactions of the selective and general stains, the structural features and the known properties and behaviour of squamous epithelium, indicated that a derivation from epithelium was a biological impossibility. The general and selective staining reactions and some of the histochemical reactions showed that the substance was not keratin, and no other substance with the properties described could arise from epithelial cells. The epithelial cells often showed no orientation in relation to the substance. The proximity to epithelium has in the past misled investigators into believing that it must be derived therefrom, whereas with a few exceptions, including the 'hyaline bodies', the cuticle is a structure lying between epithelium and the tooth surface. Rather than suggesting attachment of the cuff epithelium to the tooth surface, it indicates that there is a space which can be occupied by material foreign to the part.

As the cuticle was found to be composed essentially of erythrocytes deposited and conglutinated outside the normal influence of metabolic and catabolic changes associated with the vascular and reticulo-endothelial system (that is, between epithelium and the tooth surface or between epithelial cells), experiments were devised to reproduce it *in vitro* by pressing packed erythrocytes and various preparations of haemoglobin and globin[45] against hard surfaces (Fig. 2*F–H*), or between epithelial surfaces (Fig. 1*E* and *F*). Using these and other methods, membrane-like layers were produced with morphological and staining characters closely simulating those of the dental cuticle in both sectional and spread preparations. Layers as thin as 1–2μ were produced, thus explaining the very thin, amidoblack positive cuticles which could be mistaken for the so-called 'primary cuticle'. The thickness of one red cell in sections is likely to be less than 3μ. The fluorescent and birefringent properties of the experimental layers also paralleled those of the cuticle. Up to the present it has not been possible to reproduce the stage which reacts with the elastic tissue stains. Observations of membrane-like layers of keratin, parakeratin, fibrin and serum were also made, but these showed no close resemblance to the cuticle.

Summarizing, these investigations show that the dental cuticle is a fortuitous formation, foreign to the part, of haematogenous origin and composed of denatured haemoglobin. Since Gottlieb's 'hornified membrane' is neither a cuticle nor a membrane, and is, strictly speaking, a pathologically formed layer, it is suggested that it be re-named the 'haematogenous paradontal hyaline layer'. The role hitherto accorded to the 'dental cuticle' in the structure, physiology and pathology of the teeth and periodontium will, in the light of the new findings, need reconsideration. It is somewhat paradoxical that the structure considered for so long to bind the gingiva to the tooth surface should itself indicate that no such union exists.

I thank Mr. M. L. Lehman for work on the translation of the original papers by Gottlieb and other German literature. I also thank Prof. C. Lendrum for advice, Dr. B. Stevens for discussions on fluorescence and Mr. M. Rudland and Mr. A. Murdoch for technical assistance. This work was supported in part by a grant from the Research Fund of the University of Sheffield and in part by the Medical Research Council.

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SYNTHESIS OF A RIBONUCLEIC ACID VIRUS BY THE MAMMALIAN OVUM

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THE experiments described in this article are part of a research programme which has two basic aims. One is to explore the potential value of viruses as experimental tools in the study of early mammalian development. The other is to determine in greater detail the known relationship of viruses to birth defects.

The ultimate intention is to work with temperate viruses; nevertheless, initial experiments were made with a virulent virus so that any effect on egg development could be readily detected. The agent selected was Mengovirus, a picornavirus consisting of spherical particles 27–28 mμ in diameter[1]. Previously it was reported[2] that addition of the 37*A* heat-stable strain[3] of Mengovirus to cultures of two-cell mouse eggs prevented further development of the ova and ultimately caused their necrosis. These effects were clearly due to Mengovirus, since purified virus preparations were active at high dilution and were blocked by anti-viral serum. Interestingly, the zona pellucida, which was expected from other data[4] to exclude macromolecules, proved quite permeable to this virus.

Once Mengovirus was found to produce an ovo-pathological effect, the next phase of the investigation was to determine whether the egg was capable of supporting the synthesis of new virus particles. This article shows that virus synthesis does occur. However, the one-step growth curve is different from that in either mouse fibroblasts or *L* cells.

The methods used for obtaining and culturing two-cell ova have already been described[2]. The culture medium used for maintaining them *in vitro* was developed by Dr. R. L. Brinster[5]. Its essential components are sodium pyruvate (which replaces the sodium lactate used in a previous formulation) and crystalline bovine plasma albumin in a bicarbonate-buffered balanced salt solution. Zonae pellucidae were removed with 0·25 per cent pronase in phosphate-buffered saline, containing 1 per cent polyvinylpyrrolidone. This enzyme was also used to prepare the monolayers of mouse embryo cells used in the plaque assays[6].

To establish conclusively whether mouse ova could synthesize Mengovirus, the virus was diluted in culture medium to about 10,000 plaque-forming units (P.F.U.) per ml. and a series of 0·01 ml. drops were placed under mineral oil in plastic Petri dishes. Half the series of drops served as controls, while the remainder each received ten zona-free ova. The cultures were then incubated in 5 per cent carbon dioxide. After 24 and 48 h, each drop was transferred to 1 ml. of culture medium. These samples were then frozen and thawed three times to release any intra-ovular virus present. The virus content of each sample in terms of plaque-forming units was determined by plaque titration on monolayers of mouse embryo cells.

After 24 h incubation the virus content of the drops containing eggs was somewhat lower than the controls, indicating adsorption of virus to the ova. However, after 48 h half the drops with eggs were found to contain relatively large numbers of virus particles (Fig. 1), proving conclusively that the two-cell mouse ovum is capable of supporting the synthesis of this RNA virus. The inefficiency of adsorption due to the low virus concentration could have been one reason for our failure to demonstrate virus synthesis after 24-h incubation. However, another reason turned up when the one-step growth curve of Mengovirus in two-cell ova was determined in the next series of experiments.

Ova, free of their zonae pellucidae, were placed in a concentrated suspension of Mengovirus (10^9 P.F.U./ml.) for 60 min at 37° C. This treatment had previously been shown to bring about infection of all the eggs present. Unadsorbed virus was then removed by transferring the ova serially through five 0·5-ml. amounts of culture medium contained in the depressions of a glass spot-test plate. Three separate micropipettes were used during this

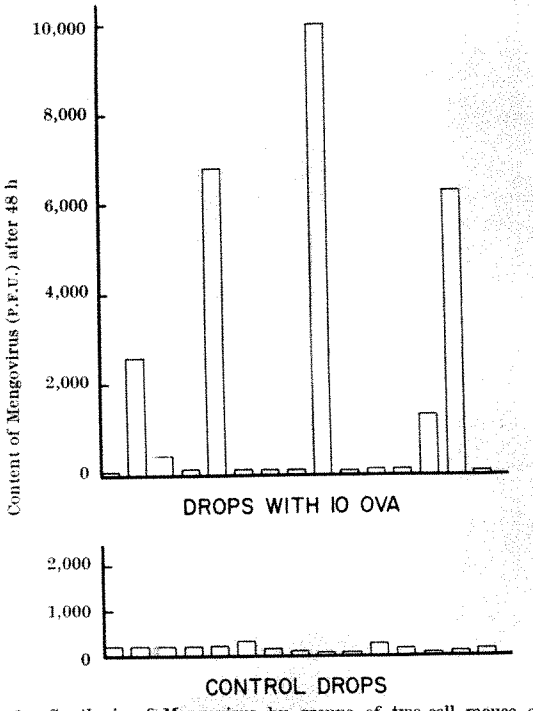


Fig. 1. Synthesis of Mengovirus by groups of two-cell mouse ova added to drops containing small numbers of virus particles. Assays were made 48 h after adding ova

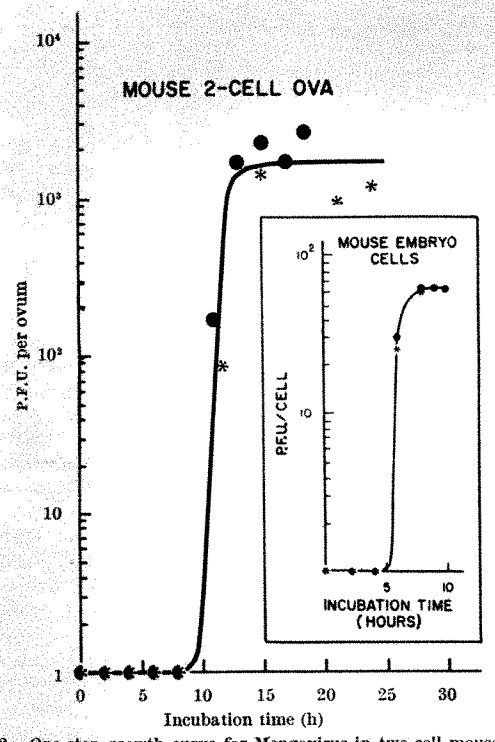


Fig. 2. One-step growth curve for Mengovirus in two-cell mouse ova. The inset shows the curve obtained with mouse embryo cells (predominantly fibroblasts)

washing process to minimize carry-over of virus. The washed ova were finally placed in a series of 0·01-ml. drops of medium under mineral oil; ten ova per drop. Culture dishes were incubated at 37° C under 5 per cent carbon dioxide and at intervals triplicate drops were sampled and titrated as in the previous experiment. For the first 8 h of incubation (Fig. 2) no virus was detected, but by 11 h about 100 P.F.U. were formed per egg. A maximum titre of about 1,700 P.F.U. per egg was reached by 13 h. At about this time necrosis was observed in some of the ova.

For comparison, a growth curve was constructed using primary mouse embryo cells, collected after one *in vitro* passage when they appeared to be predominantly fibroblasts. Because of the relatively small size of these cells compared with the ova the procedure for obtaining the growth curve was modified slightly. Sufficient virus to infect all the cells was added to a monolayer and the culture was incubated for 60 min at 37° C. The cells were then removed with pronase and washed repeatedly by low-speed centrifugation to remove unadsorbed virus. Groups of fifty cells were placed in microdrops and then processed exactly as the eggs had been. The inset in Fig. 2 shows the growth curve obtained. The latent period was about half that in the eggs. Virus synthesis in the fibroblasts was complete in about 8 h, whereas in the eggs it had not yet commenced at this time.

The mean number of infectious virus particles produced by each fibroblast was approximately 65. Brownstein and Graham[3] have reported a one-step curve for the 37*A* strain of Mengovirus in mass suspension cultures of *L* cells. Their curve displays the same characteristics as we obtained from the fibroblasts, with the exception of a greater yield (500 P.F.U. per *L* cell). If allowance is made for the larger size of the *L* cell (a mean volume of 905μ^3 compared with 140μ^3 for the average mouse fibroblast—see ref. 7), then the virus produced per unit volume is nearly the same for both cell types (0·5 P.F.U./μ^3). However, the two-cell ovum (approximate volume, 344,000μ^3—see ref. 7) yielded about 1,700 P.F.U. per ovum, or 0·005 P.F.U./μ^3. This is only 1 per cent of the virus produced by the somatic cells on a unit volume basis.

The reason for the relatively slow and inefficient viral synthesis by the ovum when compared with other cells is not known, but it may be due to an immaturity of the subcellular particles, particularly the ribosomes, involved in building the new virus units. Experiments are being planned to test this working hypothesis.

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PENICILLINASE-NEGATIVE VARIANTS OF METHICILLIN-RESISTANT *Staphylococcus aureus*

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THE contribution of penicillinase activity to the methicillin resistance of methicillin-resistant staphylococci has been controversial. Penicillinase can inactivate methicillin, although the V_{max} and the relative affinity of substrate for enzyme are only 1/30th and 1/12000th respectively of the values for benzylpenicillin[1]. Cultures of methicillin-resistant staphylococci may inactivate more methicillin than do cultures of methicillin-sensitive staphylococci[2,3]. But under comparable conditions of growth and induction, both types destroy similar amounts of the antibiotic[4,5]. The rate of such destruction is relatively low and 8–24 h is usually needed in order to reduce appreciably the concentration. Sutherland and Rolinson examined methicillin-resistant strains isolated from diverse sources and found that these strains consisted of a majority population with a comparatively slight increase in methicillin resistance and a minority population with a considerable increase in methicillin resistance[6]. In our laboratory we have confirmed that the minority population consists predominantly of mutants with increased intrinsic resistance. If penicillinase production were not a significant factor in methicillin resistance, then penicillinase-negative variants should have a degree of resistance comparable with their penicillinase-positive parents. Eriksen and Erichsen, however, reported that such variants of one strain were significantly less resistant to methicillin and concluded that resistance to methicillin was due both to penicillinase activity and to inherent resistance[7]. This article reports an experiment which I undertook to isolate and evaluate penicillinase-negative variants from five methicillin-resistant strains.

The penicillinase-negative variants were detected by Novick's test in which penicillinase-positive colonies take on a purplish colour resulting from the precipitation of an acid-insoluble *p*H indicator, *N*-phenyl-1-naphthylamine-azo-carboxy-benzene, when the colonies are exposed

to penicillin[8,9]. Loss of penicillinase activity was confirmed by a modified Got's test[10]. Evaluation of methicillin resistance was carried out by a method similar to that used by Sutherland and Rolinson[6]. Dilutions of a broth culture were pipetted on to a series of plates containing increasing amounts of methicillin up to 500 μg/ml. Three of the strains were from the same sources as those investigated by Sutherland and Rolinson. Two of these had populations with resistance patterns similar to their report, but one had a majority population with an increase in resistance to 125 μg/ml. This was strain No. 8054 which Stewart and Holt had previously reported to be of uniformly high resistance to methicillin[11]; however, even this strain contained a small population of more highly resistant mutants. Two additional methicillin-resistant strains were supplied by Prof. William M. M. Kirby. These American isolates had a composition of methicillin resistance similar to the European strains as described by Sutherland and Rolinson[6]. All five methicillin-resistant strains contained spontaneously occurring penicillinase-negative variants in a frequency of about 10^{-3} (Table 1). The penicillinase-negative variants produced a small number of highly resistant cells with the ability to form colonies on 500 μg/ml. methicillin plates. The number of such colonies, their growth rate, and their eventual size were comparable with those observed with their penicillinase-positive parents. Comparison of results on lower concentrations of methicillin indicates that the penicillinase-positive variants produced relatively more colonies on plates from 2·5 to 5 μg/ml. methicillin (Table 2). The lack of proportionality between colony count and size of inoculum found at 5 μg with the penicillinase-positive strain and at 2·5 μg with the penicillinase-negative variant is similar to that which was occasionally observed with ampicillin and *E. coli*[12]. The ability of relatively more colonies to grow with more dilute inocula is contrary to what would be predicted from an inoculum effect due to penicillinase. In some experiments the penicillinase-positive variants gave rise to comparatively more colonies than their penicillinase-negative descendants up to 50 μg/ml. with diluted inocula and up to 250 μg/ml. with undiluted inocula. If one assumes that the loss of penicillinase activity was not accompanied by the loss of any other genetic characteristics which contribute to methicillin resistance, then the ability of more penicillinase-producing colonies to grow on the lower concentrations of methicillin is at least partially a result of penicillinase activity. At 500 μg, however, growth is entirely associated with increased intrinsic resistance. With either penicillinase-positive or penicillinase-negative strains, growth in broth cultures containing 5 μg/ml. resulted in the selection of a population, many of the cells of which could grow as isolated colonies up to at least 500 μg/ml. Accordingly, the contribution of penicillinase activity to methicillin resistance is variable, depending on the test used to measure resistance. The resistance of the small number of highly resistant mutants is independent of penicillinase activity. Since the rate of inactivation of methicillin by penicillinase is low, significant inactivation would not be expected in clinical situations in which the supply of methicillin is frequently replenished, and it would appear that exposure of such methicillin-resistant strains to comparatively small concentrations of methicillin can result in the selection of highly resistant variants. The highly resistant mutants, however, grow more slowly than their parents[12a]. Consequently, their pathogenicity may also be lowered.

Genetic control of penicillinase activity appears to be associated with an extra-chromosomal element or plasmid[8]. Methicillin-resistant clinical isolates have almost invariably been penicillinase producers, although a few penicillinase-negative strains have been reported[13]. Acridine dyes have been found to cause a modest increase in the frequency of penicillinase-negative variants in some strains[14,15]. Other workers have not encountered an increased frequency[9]. With the present strains an increased frequency of penicillinase-negative variants was not demonstrated after exposure to the highest two-fold dilution of acriflavine which gave turbid growth overnight in trypticase soy broth at *p*H 7·3 (usually 12·5 μg) or *p*H 7·6 (usually 3·13 μg). Resistance to mercury salts[16] was found by Richmond and John to be co-eliminated and co-transduced with penicillinase formation[17]. They concluded that the genetic

Table 1. METHICILLIN-RESISTANT *Staphylococcus aureus*

Strain	Source	Phage type	Mercury	Tetra	STM	Erythro	Frequency of penicillinase-negative variant		
Penicillinase-positive									
No. 5982	Copenhagen[10,6]	7/77 at 1,000 RTD*	R	R	R	S	2/1835	2/1335	7/1174
No. 8054	Carshalton[11,6]	53/83A*	R	S	R	S	2/1373	1/2059	
No. 13137	Colindale[20,6]	7/47/53/54/75/77*	R	R	R	S	4/1333	3/723	
Russell	Seattle[3]	53/77[3]	R	R	R	R	2/1829		
Villaluz	Seattle[3]	53/77/83A[3]	R	S	R	S	1/584		

Penicillinase-negative variants					
Parent	No. of strains tested	Mercury	Tetra	STM	Erythro
No. 5982	14	S	R	R	—
	1	S	S	R	—
No. 8054	3	S	S	R	—
No. 13137	5	S	R	R	—
Russell	3	S	R	R	R
Villaluz	1	S	S	R	S

* Phage typing was performed by Dr. Valerie Hurst, who found that all the penicillinase-negative variants tested had the same type as their parent strains (No. 5982, six variants including the tetracycline-sensitive one; No. 8054, two variants; No. 13137, two variants). RTD, Routine test dilution.

Table 2. COLONIES OF RUSSELL STRAIN OF METHICILLIN-RESISTANT *Staphylococcus aureus* ON METHICILLIN PLATES

Methicillin (μg/ml.)	Penicillinase-positive				Penicillinase-negative			
	10^{-6}	10^{-4}	10^{-3}	Undil.	10^{-6}	10^{-4}	10^{-3}	Undil.
0	50	+	+ + −	+ + +	67	+	+ + +	+ + +
2·5	52	+	+ + − + + +	+ + +	17	43	108	+ + +
5·0	11	74	140	+ +	0	0	54	+
12·5	0	0	40	+	0	1	42	+
25	0	0	27	+	0	1	30	+
50	0	0	13	+	0	0	26	+
125	0	0	8	+	0	0	11	+
250	0	0	4	180	0	0	19	+
500	0	0	3	81	0	1	8	135

Dilutions of overnight culture streaked in 0·05 ml. amounts and incubated for 2 days. +, Too numerous to count; + +, semi-confluent; + + +, confluent

determinants of these traits were closely linked on the same plasmid. In the current strains, resistance to mercury salts was lost at the same time as penicillinase was lost in all cases (Table 1).

Tetracycline resistance was reported in one staphylococcal strain to be associated with a plasmid[18]. Of fifteen penicillinase-negative isolates from six separate experiments with strain No. 5982, only one variant had lost its resistance to tetracycline, and it would seem that tetracycline resistance in No. 5982 is not on the penicillinase plasmid. Sutherland and Rolinson[6] and Stewart and Holt[11] have reported that No. 8054 was tetracycline-resistant, but the strain supplied to us through the courtesy of Drs. B. M. Kagan and E. R. Martin was sensitive to 3 μg/ml. tetracycline—a finding which indicates loss of tetracycline resistance after serial passage in several laboratories and consistent with loss of a plasmid.

In conclusion: (1) Penicillinase-negative variants of methicillin-resistant *Staphylococcus aureus* maintain the capacity to produce mutants highly resistant to methicillin. (2) American isolates of methicillin-resistant staphylococci were similar to European strains. (3) Loss of penicillinase activity occurred spontaneously and was not increased by acriflavine. (4) Tetracycline resistance was not on the penicillinase plasmid.

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ELECTROPHORETIC AND FUNCTIONAL HETEROGENEITY OF PEPSINOGEN IN SEVERAL SPECIES

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FOR many years now, claims have been made for the existence of more than one acid-activated gastric proteolytic enzyme. Until recently such evidence depended on differences in *p*H optima, substrate specificities, and degrees of activation or inactivation by different agents. This evidence has been thoroughly reviewed and appraised by Taylor[1].

Recently a number of investigators have utilized column chromatography to isolate several pepsins from gastric juice and pepsinogens from gastric mucosa. For example, Ryle and Porter[2] separated, from crude porcine pepsin, pepsin itself and two minor components which they named parapepsin I and II. Investigation of the physico-chemical and enzymatic properties leaves little doubt, despite similarities, that these enzymes have separate identities. Chromatographic fractionation of crude pepsinogen from porcine gastric mucosa enabled Ryle[3] to isolate the zymogens corresponding to pepsin and parapepsin II. Parapepsinogen I was not found. Tang and Tang[4] described a zymogen from human gastric mucosa which, after acid activation, yields both pepsin and gastricsin. The properties of these enzymes appear identical to those of the pepsin and gastricsin previously isolated from human gastric juice[5]. Seijffers *et al.*[6] isolated three chromatographically distinct proenzymes from human fundic gastric mucosa. These were designated pepsinogens I, II and III. Extracts of pyloric and duodenal mucosa yield only a single pepsinogen which, under the conditions of investigation, is apparently identical to the pepsinogen I of fundic extracts[6]. Since then, these same investigators[7] have compared the chromatographic behaviour of pepsins with acidified enzymes which had been previously purified as pepsinogen. Such comparison indicates that pepsinogen I yields pepsin I, pepsinogen III yields pepsin III, while pepsinogen II yields a mixture of two pepsins, II*A* and II*B*. It is possible that pepsinogen II, while chromatographically pure, is a mixture of pepsinogens II*A* and II*B*. Seijffers *et al.*[8] have also identified pepsinogens II and III in human urine. The ratio of pepsinogen II and III in urine, in terms of proteolytic activity after activation, apparently reflects the ratio of pepsinogens II and III in gastric mucosa. In a total of twenty-three urines from twelve individuals, pepsinogen I was never identified.

Electrophoresis has had comparatively little use as a means of pepsinogen fractionation largely because of the lack of suitable methods. Analytical electrophoresis has, however, been utilized. Tang and Tang[4], for example, used starch-gel electrophoresis at *p*H 6·5, localized protein with amido-black stain, and showed that the zymogen isolated from human gastric mucosa was electrophoretically homogeneous. After this zymogen had been acidified, electrophoresis revealed two distinct components corresponding in position to the bands of gastricsin and pepsin obtained from human gastric juice. Post-electrophoretic analysis was achieved by proteolytic assay of unstained transverse sections of gel.

Much more extensive use of analytic electrophoresis has recently been reported by Rapp *et al.*[9] and by Kushner, Rapp and Burtin[10]. These investigators adopted the technique of Uriel[11] for the demonstration of proteolytic activity after electrophoresis in agar. This procedure consists of post-electrophoretic immersion of agar plates in a solution of albumin, buffered at acid *p*H, followed by fixation and staining with amido black. The intact albumin fixed by agar is stained by the dye, whereas digested albumin in zones of proteolytic activity remains unstained. By this means four proteases are detectable in gastric mucosal extracts after electrophoresis in agar at *p*H 8·2. These proteases (P) are designated I, II, III and IV in order of decreasing mobility[8]. Immunoelectrophoretic investigations indicate that II, III and IV are

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antigenically and immunologically different. Subsequent correlative column chromatography, electrophoresis and immunochemical analysis[10] established that these zymogens possess the properties of pepsinogen. Moreover, at least five electrophoretically distinct enzymes are detected after acid activation. Immunological correspondence could be shown between the acidified and non-acidified forms of pepsinogens II, III and IV as well as between human pepsinogen II and purified porcine pepsinogen.

The objective of our investigations has been to delineate the origin and functional significance of the multiple pepsinogens and their derivative proteolytic enzymes. The immediate purposes of this report are, first, to describe a simple, reliable method for analytic and preparative starch-gel electrophoresis of pepsinogen and, secondly, to indicate that the several pepsinogen components defined by this method differ not only in electrophoretic mobility but also, when activated, in *p*H optima and proteolytic specificity. Comparison of the electrophoretic mobilities of multiple pepsinogens from several species makes possible the provisional suggestion that the several pepsinogens of a species are probably the product of a single gene. If this view is correct, then the differences between several, if not all, pepsinogens of a species presumably arise after the protein chain has been synthesized.

Materials. Whole stomachs were obtained within 10 min of slaughter from two pigs, two dogs and one rabbit. All animals were healthy. The dogs and the rabbit were killed with intravenous pentobarbital, the pigs at the abattoir. Human gastric mucosa was obtained, at surgery, from an individual with a pre-pyloric ulcer. Mucosa removed from the most proximal and distal portions of this specimen corresponded histologically to fundic and pyloric mucosa respectively.

Random urine specimens were collected from four pigs, three cows and three dogs. Initial morning urine specimens were obtained from two healthy humans. Specimens were promptly brought to *p*H 7·5 by addition of EBT buffer[12] (*vide infra*). After they had first been centrifuged to remove solid matter all urine specimens were maintained at 3° C until used.

Crystalline porcine stomach pepsinogen, *B* grade, lot number 108363, was purchased from Calbiochem, Los Angeles, California.

Preparation of gastric mucosal extracts. Stomachs were opened immediately after collection, washed with chilled isotonic saline, and placed on ice. Within 1 h of collection inside a 4° C room, strips of gastric mucosa were removed from the fundic and pyloric ends of each stomach and thereafter stored separately at −20° C. Extracts were prepared by homogenization of 2·5-g wet mucosa and 2 ml. of distilled water in a chilled Servall homogenizer at 50,000 r.p.m. for 3 min. The homogenate was washed into a tube with 2 ml. of water and centrifuged at 40,000*g* for 20 min at 3° C. The supernatant solution was removed, again centrifuged, and stored at 3° C.

Treatment of urine samples. Seventy-five ml. volumes were concentrated at 3° C by ultrafiltration in collodion bags manufactured by Membranfiltergesellschaft, Göttingen. Small amounts of precipitate were removed from the concentrates by centrifugation and the clear supernatant fluid stored at 3° C.

Assay of peptic activity. The method of Sievers and Fischer[13], as modified by Hanley[14], was used. The reactants for assay consisted of 2·5 ml. of 2 per cent bovine haemoglobin (Armour) in 0·01 N hydrochloric acid, 2 ml. buffer solution (*vide infra*), and 0·5 ml. of solution to be assayed. The last was suitably diluted with water until within the range of method sensitivity. Two 2-ml. aliquots were removed immediately after mixing the reactants. Two ml. of 10 per cent (w/v) trichloroacetic acid (TCA) were added to one aliquot which then served as the blank. The second aliquot was sealed, incubated at 37° C for 24 h, and thereafter 2 ml. of 10 per cent TCA were added. The remaining millilitre of digestion mixture was assayed for *p*H. A final reactant mixture *p*H of 1·5–4·3 was obtained by reciprocal alteration of two buffer solution eluants, namely, 0·2 N hydrochloric acid and a solution which contained 0·2 M DL-glycine and 0·1 N sodium hydroxide. Tyrosine-like material in 1 ml. of TCA supernatant solutions was determined after incubation with 2·5 ml. 4 per cent (w/v) sodium carbonate in 0·1 N sodium hydroxide and 0·5 ml. Folin–Ciocalteau reagent (diluted 1 : 3 with water) for 30 min at 37° C. Absorbance was measured at 700 mμ. The difference in absorbance between blank and test aliquots served as a measure of proteolytic activity.

Starch-gel electrophoresis. Vertical starch-gel electrophoresis was usually performed using a *p*H 8·5 buffer containing EDTA, boric acid and *tris*-(hydroxymethyl) aminomethane (EBT) buffer[12]. A few analyses were performed with Smithies borate buffer[15] and with Poulik's discontinuous buffer[16]. Gels contained seven sample slots and each slot had a capacity of approximately 0·08 ml. After being subjected to electrophoresis at 3° C for 11 h at 5 V/cm a horizontal slice of the gel was soaked in 0·06 N hydrochloric acid for 2 h, thereby lowering the *p*H to approximately 2 and converting pepsinogen to pepsin. One section was then developed with amido-schwarz dye[15]. Gels stained with this dye develop a diffuse light blue background due to the residual protein present in hydrolysed starch[17]. This blue background is locally lost, and replaced by visible non-staining white zones wherever there are proteolytic enzymes present, such as activated pepsinogen. The proteolytic activity of such non-staining zones is verified by a method in which bovine fibrin (Armour), previously dyed with indigo carmine[18], is dusted on to the cut gel surface of the remaining slice of the original acid-soaked gel. The gel is then covered with a wad of filter paper moistened with 0·06 N hydrochloric acid, and incubated for several hours. The gel surface is stained blue by liberated dye. A blueprint of such reaction can be obtained by subsequent drying of the filter paper to which zones of blue dye have adhered.

Estimation of molecular sizes. The relative molecular sizes of several components were estimated, first, by the method of Smithies[19] utilizing the principle that electrophoretic migration in starch gels is a function of starch concentration and, secondly, by the filtration method using 'Sephadex *G*-75' (Pharmacia).

Electrophoretic fractionation of pepsinogen. After starch-gel electrophoresis of 0·5 ml. of pepsinogen, the edges of the gel were marked at intervals, and thereafter a thin horizontal slice removed for staining with amido-schwarz. The balance of the gel was kept frozen in order to obviate diffusion. After the zones of proteolytic activity had been identified by staining with amido-schwarz the positions of these zones were referred to the remainder of gel. The gel was then divided into parallel 1/8-in. wide strips. Pepsinogen was eluted from each section by means of concentric electrodialysis[20] in Visking 'Nojax' cellulose casing at 3° C. Fractions were recovered in approximately 7 ml. EBT starch-gel buffer. Elution was essentially complete within 3 h. Serial eluates were then assayed for proteolytic activity at *p*H 2·0 and *p*H 3·5. Recovery was excellent and in many instances considerable dilution was necessary to bring eluate activity within range of the assay method. The amount of pepsinogen present as protein in such fractions was crudely estimated by comparison of proteolytic activity of acid-activated fractions with t[illegible] derived from known concentrations of unf[illegible]orcine crystalline pepsinogen. Such estima[illegible]quivalent milligrams of pepsinogen [illegible] that this expression is an e[illegible] take purifica-

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Table 1. Composition of Insulin–Pepsin Incubants

Insulin *B* chain (mg)	Solvent	Equivalent μg of pepsinogen*	Source of pepsinogen fractions
1	5% formic acid, *p*H 1·9	1	Pig and man
1	5% acetic acid, *p*H 2·9	1	Pig
3	5% acetic acid, *p*H 2·9	5	Pig

* See section *Electrophoretic fractionation of pepsinogen*.

Digestion of insulin B chain by acid-activated pepsinogen fractions. Insulin *B* chain was prepared from crystalline bovine insulin (Lilly) according to the method of Sanger[21] or by the unpublished method of Dintzis and Dintzis. Samples of *B* chain were digested with pepsin obtained by acid activation of electrophoretically prepared fractions of pepsinogen. Fractions examined in this manner were derived from porcine crystalline pepsinogen as well as from the pepsinogen of human gastric pyloric mucosa. Relevant differences between the composition of the various digestions are shown in Table 1. All digestions were incubated for 18 h at 37° C. Thereafter, samples were dried *in vacuo* and subjected to paper electrophoresis at *p*H 6·4 in pyridine–acetic acid–water buffer (10 : 0·4 : 89·6 v/v) for 3·5 h at 35 V/cm under cooled 'Varsol'. Guide strips were cut from the dried completed ionograms and stained with 0·2 per cent ninhydrin in acetone followed by development with chlorine[22]. Bands located in this fashion were eluted with 6 N hydrochloric acid from the unstained remainder of the paper into 10 × 75 mm 'Pyrex' test-tubes, sealed under nitrogen, and hydrolysed for 18 h at 100° C. Hydrolysates were dried *in vacuo* and subjected to electrophoresis in formic acid buffer *p*H 1·85 (ref. 23) for 2 h at 35 V/cm under 'Varsol'. Dried ionograms were developed with 0·2 per cent ninhydrin in acetone and the amino-acid composition of the hydrolysate thereby determined.

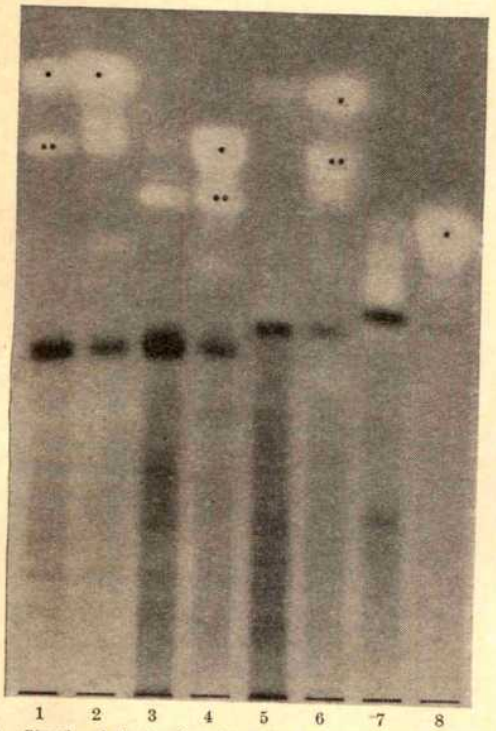


Fig. 1. Starch-gel electrophoresis of gastric pepsinogens from several species. In this and succeeding similar figures gel buffer is *p*H 8·5 EBT (see text), origin is at bottom and anode at top. Following electrophoresis, gels were made acid and thereafter devel[illegible]mido-schwarz stain. The same means of development wer[illegible]similar succeeding figures except Fig. 2. Pepsinogen[illegible] pepsins, appear as white, non-staining area[illegible]staining background of the gel. The [illegible]epsinogen terminology. One dot in[illegible] PG II. The ratio of PG II : [illegible] the pig, 0·90 in man a[illegible] not indicated by [illegible] 0·73, 0·77 [illegible] (4) hum[illegible] black dots against the [illegible] used in all [illegible] PG I migration distan[illegible] and 0·73. (1) Pig [illegible]

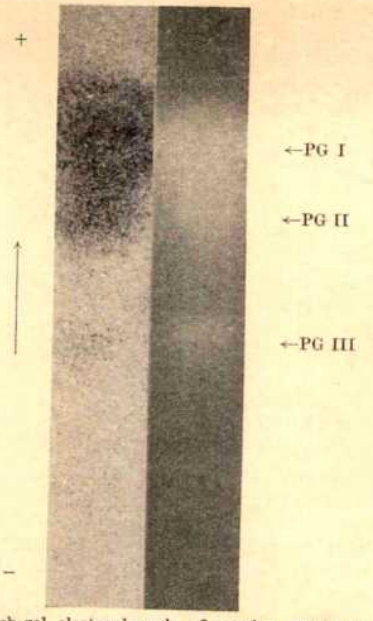


Fig. 2. Starch-gel electrophoresis of porcine crystalline pepsinogen developed, on left, by the method of proteolysis of dyed fibrin and, on the right, by negative staining with amido-schwarz

Starch-gel electrophoretic heterogeneity of pepsinogens present in pyloric and fundic mucosal extracts from single stomach of pig, human, rabbit and dog is shown in Fig. 1.

The general pattern of the result was similar with a discontinuous buffer[16], with a borate buffer[15], and with EBT buffer. The last was used for most examinations. The gel shown in Fig. 1 was developed with amido-schwarz stain. The activated zones of pepsinogen appear as white unstained areas on the pale-blue background of the gel. When present in large concentrations, such as are present in undiluted fundic extracts, pepsinogen appears as a narrow, positively staining, band surrounded by a white halo. Contaminating, non-proteolytic, proteins appear as darkly stained areas of which the most intense has a mobility equivalent to that of albumin. Use of the dyed fibrin method on mirror images of such gels (Fig. 2) readily confirms the fact that only the white areas contain detectable proteolytic activity.

The various pepsinogens defined in this way are provisionally designated PG (pepsinogen) I, II and III in order of decreasing mobility toward the anode at *p*H 8·5. Porcine crystalline pepsinogen exhibits three bands of which the slowest, PG III, is also the weakest in terms of proteolytic activity (Fig. 9); indeed, it does not always reproduce photographically (Fig. 3, slots 1 and 7). PG III of the pig can, however, be clearly seen in the extract of fundic mucosa (Fig. 1). Pyloric mucosal extracts from pig, human, rabbit and dog give appreciably weaker reactions than fundic preparations of similar wet weight (Fig. 1). In addition to such apparently quantitative differences in activity there are other differences. For example, PG I and II of pig pylorus exhibit approximately equal intensity of development whereas PG I from pig fundus is considerably more intense than PG II. In pyloric extracts of human stomachs the intensity of PG II considerably exceeds that of PG I, whereas in human fundic extracts, as in the pig fundic extracts, PG I is more intense than PG II. In the rabbit pyloric extract, relative intensity of the pepsinogens is such that only PG I was clearly distinguishable whereas in fundic extracts PG I and II have similar intensity. Although dog pepsinogen does not give discrete banding there is an evident difference between pyloric and fundic extracts (Fig. 1). However, subsequent work still in progress is revealing that human gastric mucosa taken from an area confined to within a few centimetres of the pylorus contains [illegible]y a single pepsinogen corresponding in mobility to

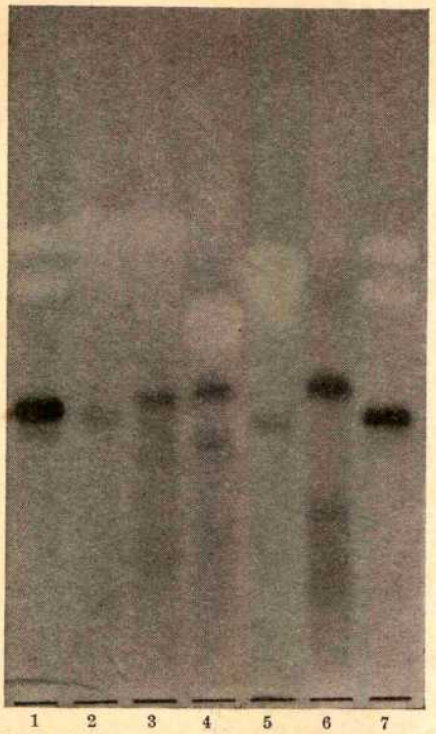


Fig. 3. Starch-gel electrophoresis of urinary pepsinogens from several species. Activated pepsinogens were developed with amido-schwarz stain. Samples shown are (1) and (7) crystalline porcine pepsinogen standard (PG I and PG II are visible); (2) pig urine; (3) cow urine; (4) dog urine; (5) human urine; (6) urine from gastrectomized dog

PG II. Study of distal pyloric mucosa of the other species is pending. Our provisional designation of three principal pepsinogens in man, pig and rabbit must be circumspect since, for example, PG II and PG III of fundic extracts are, in fact, composed of double bands. This phenomenon was consistently reproducible. PG II, derived from either pig or human stomach, showed a greater tendency to be solitary in pyloric extracts than it did in fundic extracts. This was true even when increased concentrations of zymogen were examined. The principal pyloric PG II component consistently corresponded, in migration, with the slower component of fundic PG II.

Although considerable differences in electrophoretic mobility of pepsinogens occur between the species examined, there exists in all species an approximate constancy in the ratio of PG II : PG I migration distances from origin. In fundic extracts the ratio of PG II : PG I migration distances is 0·89 in the pig, 0·90 in man and 0·89 in the rabbit. The respective PG III : PG I ratios for these species are 0·73, 0·77 and 0·73.

Fig. 3 shows the results of starch-gel electrophoresis of concentrated urine from pig, cow, dog and human. Only the pig urine demonstrates two proteolytic components; these correspond exactly to PG I and PG II of the crystalline pepsinogen standard. These two components in pig urine were consistently identified in different preparations of the same urine specimen and in urine from three other pigs. In different urine specimens obtained from one human subject, bands corresponding in position to both PG I and PG II were occasionally seen in both amido-schwarz and dyed fibrin methods of analysis. In other specimens obtained from this same subject only one rather broad band was seen. The leading edge always corresponded in mobility to the leading edge of human PG I obtained from mucosal extracts. The reason for this inconsistency in the demonstration of these two components in human urine is unknown. Dialysis, salting-out procedures with ammonium sulphate, and precipitation of proteins with acetone had no material effect on such results. When the urine of dogs was examined, one animal, although considered healthy, was found to have no demonstrable pepsinogen. A second dog had had a total gastrectomy and demonstrated no pepsinogen bands. Results for urine from a third dog are shown in Fig. 3.

In the species examined there were no differences which could be attributed to the sex of the subject. On no occasion was a third band, corresponding to PG III of the gastric extracts, observed in urine. The proteolytic activity and the electrophoretic pattern of pepsinogen, whether from urine or mucosal extracts, were not influenced either by freezing at −20° C for 3 weeks or by storage at 3° C for 6 weeks.

Characterization of PG I and PG II from Man and Pig

(1) *Relative molecular size.* The relative electrophoretic migration of PG I and PG II derived from porcine crystalline pepsinogen remained constant in gels of varying starch concentration[19], thereby suggesting similarity in the molecular size and shape of these two zymogens. Such a result excluded a monomer–dimer relationship between PG I and PG II. The fact that both pepsinogens are recoverable only from the internal volume of 'Sephadex *G*-75' (Pharmacia) (developed in dilute EBT buffer) corroborates this view. Dimers of pepsinogen would have a molecular weight of twice 45,000 and thus be excluded from the internal volume of 'Sephadex *G*-75'.

(2) *Electrophoretic isolation and purification of individual pepsinogens.* A representative proteolytic assay of acid-activated fractions recovered by starch-gel electrophoresis is shown in Fig. 4. Unactivated fractions of PG I and PG II, obtained in this way, were concentrated fifty-fold by ultrafiltration and re-examined electrophoretically. In each case, only a single sharply defined band of pepsinogen could be identified. Moreover, re-run preparations were free of extraneous positively staining material such as is apparent in both crude extracts and crystalline pepsinogen, thereby suggesting at least a degree of purification. In this manner electrophoretically homogeneous, partially purified PG I and PG II were prepared from porcine crystalline pepsinogen and from human pyloric mucosa. Human pyloric extract rather than fundal extract was chosen for starting material since pyloric PG II tends to be single rather than double banded as in the case of the corresponding fundal zymogens. Initial attempts to isolate useful amounts of the weakly active PG III from porcine material were unsuccessful.

(3) *Proteolytic activity of activated pepsinogens at various hydrogen ion concentrations.* Variation of proteolytic activity with *p*H was determined for activated PG I and PG II electrophoretically isolated from porcine crystalline pepsinogen (Fig. 5) as well as for activated PG I and PG II isolated from human pyloric mucosa (Fig. 6). In both species activity of PG I is maximal at approximately *p*H 2·0 whereas activity of PG II is maximal at approximately *p*H 3·0. The *p*H activity maxima of human activated pepsinogens are somewhat broader than those of porcine pepsinogen, but otherwise similar. No conclusions should be drawn from the varying degree of proteolysis at the *p*H maxima since dilution was not

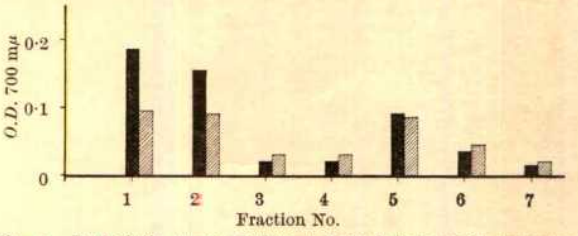


Fig. 4. Proteolytic assay of eluates derived from serial transverse sections of starch gel containing electrophoretically fractionated porcine crystalline pepsinogen. After separation and elution at an alkaline *p*H aliquots of fractions were examined for proteolytic activity (see text) at approximately *p*H 1·9 (black bars) and at *p*H 3·5 (cross-hatched bars). Fractions are numbered 1 to 7 in order of decreasing migration toward the anode. Fractions 1 and 2 correspond to PG I while fractions 5 and 6 correspond to PG II

always comparable and extraction from gel not always complete.

(4) *Digestion of the B chain of insulin by activated pepsinogens.* Ionograms of peptides derived from peptic hydrolysis of insulin *B* chain are shown diagrammatically in Fig. 7. The amino-acid composition and inferred sequence (given the known sequence of *B* chain) of these peptides are shown in Table 2. A comparison of the sites of *B*-chain hydrolysis by pepsin derived from electrophoretically purified preparations of human PG I and PG II and from porcine PG I and PG II appears in Fig. 8. As can be seen, the specificity of PG I and PG II is uninfluenced by species source. However, in both species PG I and PG II produce different patterns of hydrolysis. In either species the principal difference between PG I and PG II appears to be due to a rapid split between the leucine and valine at positions 11 and 12 by activated PG I, but not by activated PG II. Other differences were the selective cleavage between the phenylalanines at positions 24 and 25 by activated PG II and between phenylalanine and tyrosine at positions 25 and 26 by activated PG I. Such differences between PG I and PG II were not influenced by the *p*H at which hydrolysis was performed (Table 1). All our observations are, with a few minor exceptions, corroborative of those described

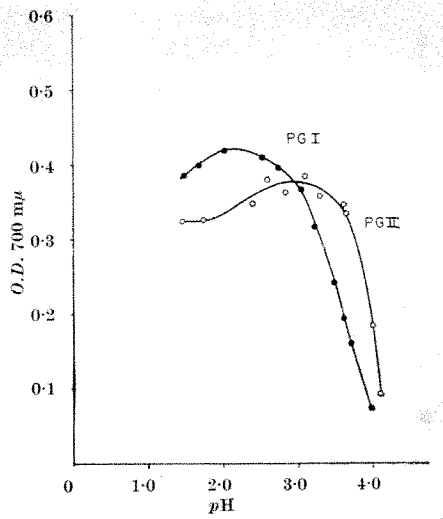


Fig. 6. Influence of *p*H on proteolytic activity (see text) of electrophoretically fractionated pepsinogens obtained from human pyloric mucosal extracts. Closed circles indicate PG I and open circles PG II. PG I aliquots were diluted 1 : 2 before assay of proteolytic activity and the TCA supernatant a further 1 : 4 prior to measurement of absorbance. PG II aliquots were diluted 1 : 8 before assay and thereafter TCA supernatant was diluted 1 : 2

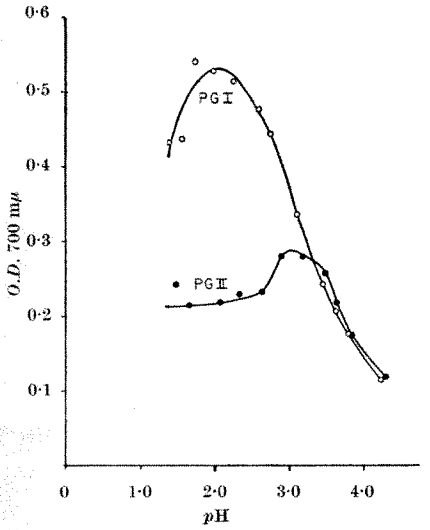


Fig. 5. Influence of *p*H on proteolytic activity (see text) of electrophoretically fractionated pepsinogens obtained from porcine crystalline pepsinogen. Open circles indicate PG I and closed circles PG II. PG I was derived from fractions 1–2 shown in Fig. 4 and PG II from fraction 5

by Ryle and Porter[2] for the digestion of *B* chain by porcine pepsins. The pepsin derived from PG I apparently corresponds to their parapepsin II and the pepsin from our PG II to their parapepsin I.

(5) *Effect of temperature.* Fig. 9 shows the effect of heating on the electrophoretic pattern of porcine crystalline pepsinogen (2 mg/ml. of EBT buffer, *p*H 8·6). Aliquots were heated at 37°, 46°, 55°, 64°, 73° or 82° C for 30 min and thereafter cooled by being plunged into iced water. PG III appears to be the most thermally labile of the three pepsinogens. Its intensity is diminished at 46° C and destroyed at 55° C. At 64° C all three pepsinogens, except for a very small amount of PG I activity, appear to be destroyed. At still higher temperatures, PG I, or something which has the mobility of PG I, survives the treatment at increased temperatures with little or no loss of proteolytic activity. At these higher temperatures PG II and III are replaced by a smear of moderate intensity. This smeared area lies somewhat nearer to the origin than the normal position of PG III. Results such as are shown in Fig. 9 were obtained repeatedly in separate experiments.

(6) *Effect of alkali.* Preparations of porcine crystalline pepsinogen, 2 mg/ml., were brought to *p*H 7·5, 8·3, 9·3 and

Table 2

Peptide No.	Peptide sequence identified from its amino-acid composition	Remarks	Source of enzyme
1	Tyr Thr Pro Lys Ala	High yield	Pig pepsinogen I
3	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu	High yield	
4	Ala Leu + free amino-acids	Low yield	
6	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe Phe	High yield	
7	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe	High yield	
9	Val Glu Ala Leu	High yield	
10	Val Glu Ala	Low yield	
2	Phe Tyr Thr Pro Lys Ala	High yield	Pig pepsinogen II
4	Free amino-acids	Very low yield	
5	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu Val Glu Ala Leu	High yield	
7	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe	High yield	
8	Leu Val $CySO_3H$ Gly Glu Arg Gly Phe	Low yield	
1	Tyr Thr Pro Lys Ala	High yield	Human pepsinogen I
3	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu	High yield	
4*B*	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu Val Glu Ala Leu Tyr	Low yield	
4*A*	Ala Leu + Tyr		
6	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe Phe	High yield	
7	Leu Val $CySO_3H$ Gly Glu Arg Gly Phe Phe	High yield	
9	Val Glu Ala Leu	High yield	
10	Val Glu Ala	Low yield	
2	Phe Tyr Thr Pro Lys Ala	High yield	Human pepsinogen II
4*B*	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala	High yield	
4*A*	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu Val Glu Ala Leu	High yield	
5	Phe Val Asn Gln His Leu $CySO_3H$ Gly Ser His Leu Val Glu Ala Leu Tyr Leu Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe	Low yield	
7	Tyr Leu Val $CySO_3H$ Gly Glu Arg Gly Phe	High yield	

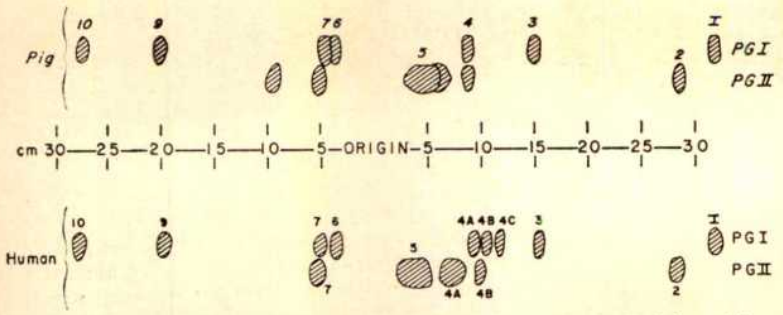


Fig. 7. Composite diagram of paper electrophoresis at *p*H 6·5 of insulin *B*-chain peptides derived by digestion with PG I and PG II from both man and pig. Derivative peptides are given the same arbitrary numbers here and in Table 2. Central line of figures refers to migration distances from origin

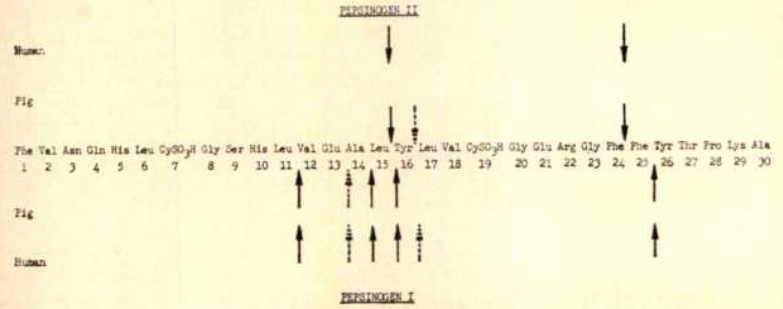


Fig. 8. Sites of digestion of insulin *B* chain by acid activated human and porcine PG I and PG II. Solid arrows refer to principal hydrolytic activities and dotted arrows to minor activities

10·1 with a glycine–sodium hydroxide buffer. After standing at 3° C for 16 h, the solutions were subjected to electrophoresis. From Fig. 10 it is evident that increasing alkalinity results in the destruction of PG III and PG I. PG II, however, persists at *p*H 10·1 although with some diminution of proteolytic activity.

Origin of Zymogen Heterogeneity

The sum of precedent investigations and our own leaves little doubt that more than one gastric pepsinogen exists. Explanations for the origin of such heterogeneity may, for the sake of discussion, be resolved into two extreme alternatives: either each pepsinogen represents the product of an individual gene or all pepsinogens are produced by a single gene and diversity thereafter created by modification of a single gene product. Precedent for the first of these alternatives is provided by the human haemoglobins. In this instance, four different genetic loci each produce similar but distinctive polypeptide chains[24]. Precedent for the second alternative derives from vertebrate myoglobin where, in each individual, a multiplicity of electrophoretically distinguishable components arise through modification of a single gene product[25].

We favour the second alternative as the origin of pepsinogen diversity and believe that evidence for this view is provided by the approximate constancy, in three species, of the migration ratios PG II: PG I and PG III: PG I. Before interpreting such concordance it should first be recalled that both PG I and PG II are functionally distinctive both in *p*H optima (Figs. 5 and 6) and proteolytic activity (Fig. 8). It is possible, therefore, to identify PG I and PG II in both man and pig by criteria other than electrophoretic mobility and thus eliminate, for example, the possibility that human PG I corresponds to porcine PG II and human PG II to porcine PG I. Accordingly, it appears that the principal difference between the corresponding pepsinogens of man and pig lies in differences in electrophoretic mobility at *p*H 8·5. This difference presumably reflects change in one or more amino-acid residues such that, at an alkaline *p*H, the net negative charge of human PG I is less than that of porcine PG I.

In the event that there are separate genes for PG I, PG II and PG III in both man and pig, then there must have been concordant mutations in all three pepsinogen genes in at least one species so as to allow approximate constancy of the migration ratios PG II : PG I and PG III : PG I. Such concordant mutation demands a good deal of coincidence and leads us to favour the alternative explanation whereby post-synthetic modification of the product of a single gene is the source of zymogen heterogeneity. If correct, the latter explanation provides a source of individual variation beyond that directly specified by a structural gene. Such variation might have biological utility in that it is associated with a more diverse set of functional properties than is evident with only one kind of zymogen.

Although the nature of the postulated modification is unknown it is clear from mobility in starch gels of varying porosity and from 'Sephadex' passage that heterogeneity is not the result of whole pepsinogen polymerization. This conclusion is in accordance with that of Ryle[3], who found the molecular weight of parapepsinogen II to be similar to that of pepsinogen.

It is possible that electrophoretic and functional distinctions between pepsinogens arise in the association of the zymogen molecule with phosphate. The pepsinogen molecule contains one phosphate radical in a diester linkage[26], and it is plausible to believe that this phosphate might, as it does in pepsin, exist as the diester or as the monoester or be absent. In connexion with this possibility it is of interest that three electrophoretically distinctive ovalbumins exist and contain, in order of decreasing mobility, 2, 1 and 0 atoms of phosphorus[26]. It is particularly relevant to the present discussion that incorporation of phosphorus into ovalbumin apparently occurs after polypeptide synthesis[27]. Our preliminary attempts to alter the electrophoretic mobility of porcine crystalline

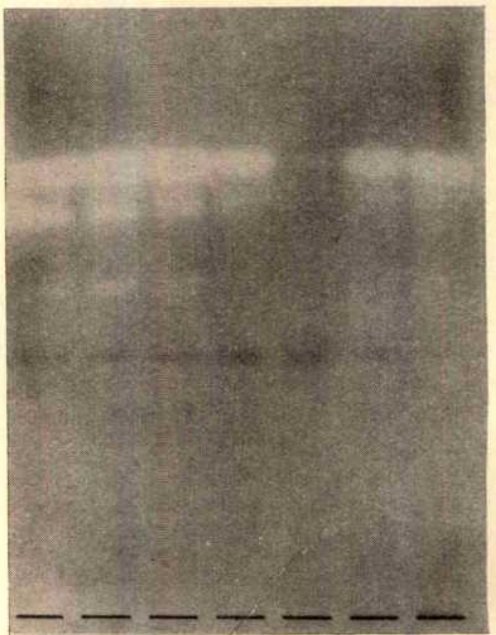


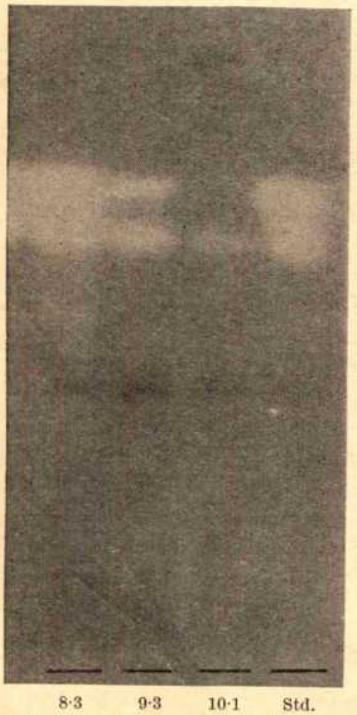
Fig. 9. Starch-gel electrophoresis and subsequent amido-schwarz development of aliquots of porcine crystalline pepsinogen (2 mg/ml. EBT buffer) treated for 30 min at varying temperatures prior to electrophoresis. Figures refer to degrees centigrade

pepsinogen by incubation with partially purified calf intestinal alkaline phosphatase and potato phosphatase were unsuccessful. Success might have depended on contamination of these phosphate monoesterases with small amounts of diesterase and further attempts with other sources of phosphatase might be profitable. Even if not the cause of pepsinogen heterogeneity, it is conceivable that the double-banding of certain pepsinogen fractions (Fig. 1) might be related to the phosphate content.

Irrespective of the origin of such pepsinogen heterogeneity, its functional aspect, as manifest by differential proteolysis of insulin *B* chain, may be useful to investigators of protein primary structure. Although evidence is limited to that from electrophoretic analysis it seems probable that a preparation containing largely the proteolytic characteristics of PG I can be obtained by treatment of crude pepsinogen at 73° C (Fig. 9), while a preparation containing only PG II can be obtained by treatment of crude pepsinogen at *p*H 10 (Fig. 10).

Differences in Zymogen Heterogeneity in Tissues and Fluids

A further dimension of pepsinogen heterogeneity exists between tissues and fluids of an individual. Taylor[28] concluded from a comparison of *p*H activity curves that fundic and pyloric proteases were different. Seijffers, Segal and Miller[6] found only one pepsinogen after chromatography of pyloric extracts whereas three were present in fundic extract. The present investigation also indicates differences between fundic and pyloric mucosa. Our initial results seem to indicate that pyloric mucosa contains two of the three pepsinogen components of fundic mucosa and not one as reported by Seijffers *et al.*[7]. However, subsequent work, still in progress, with human partial gastrectomy specimens is revealing that gastric mucosa from a narrow area adjacent to the pylorus does in fact possess only one pepsinogen corresponding electrophoretically to PG II. It is likely that the pyloric mucosal extracts shown in Fig. 1 contained some fundic type peptic cells. Of the three pepsinogen components of these cells, only PG I shows up—it being the 'strongest'.



8·3 9·3 10·1 Std.

Fig. 10. Starch-gel electrophoresis at *p*H 8·5 and subsequent amidoschwarz development of porcine crystalline pepsinogen which had been subjected to varying alkalinity, for 16 h, prior to electrophoresis. Figures refer to *p*H of treatment

Similarly, although there is no doubt that PG I and PG II both may appear in urine, other investigators[7] find only one. Clearly it is now necessary to utilize several methods, including those of protein composition, to compare the various pepsinogen fractions obtained in several laboratories.

Summary

Pepsinogen, derived from each of several species, may be easily resolved into multiple components by means of alkaline starch-gel electrophoresis. The technique involves acid activation of pepsinogen after electrophoresis and subsequent identification either by digestion of dyed fibrin or more simply by digestion of residual protein present in starch. In the latter method, gels are developed with a general protein stain and peptic activity appears as white non-staining areas on a blue background.

Two principal pepsinogens, PG I and PG II, have been obtained from porcine and from human material by means of preparative starch-gel electrophoresis. Comparison of the configuration of *p*H-activity curves and patterns of insulin *B*-chain hydrolysis obtained after acid activation of these pepsinogens indicates that: (1) PG I and PG II are substantially different functionally as well as electrophoretically; (2) despite inter-species electrophoretic differences there is substantial functional correspondence between PG I of man and pig and between PG II of man and pig.

Comparison of individual porcine pepsinogens establishes that the observed electrophoretic differences are not polymeric by nature. A clue to the origin of the multiple zymogens is provided by a comparison of the mobility of human, porcine and rabbit pepsinogens which indicates that interspecies differences are accompanied, in each species, by approximately parallel changes in the mobility of all principal pepsinogens. Such concordance suggests that the diverse pepsinogens originate, in each individual, through the modification of a single gene product rather than as the products of several genes. If correct, this explanation provides an instance in which functional diversity is extended beyond that which is genetically determined.

A further diverse aspect exists, as there are differences in the number of pepsinogens found in fundic and pyloric extracts, and also in urine.

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IMMUNOELECTROPHORETIC PROPERTIES OF LOW-DENSITY HUMAN SERUM LIPOPROTEINS

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THERE is some controversy concerning the immunochemical properties of human serum or plasma lipoproteins. Evidence for immunochemical heterogeneity has been obtained from quantitative precipitin analyses and from immunodiffusion experiments. By means of quantitative precipitin analyses, ultracentrifugally isolated S_f6 and S_f13 lipoproteins were shown to cross-react with anti-S_f6 and anti-S_f13 rabbit antisera, but not to be immunochemically identical[1]. By immunodiffusion in tubes[1] and in plates[2] five or more lipid staining zones of precipitations were obtained. The immunodiffusion data were interpreted to indicate heterogeneity. Gitlin[3] has also reported heterogeneity of lipoproteins (see refs. 1 and 4 for discussion); more recently Lawrence and Shean[5] have reported two and three zones of precipitation by immunoelectrophoresis. Levine *et al.*[6] have used quantitative precipitin analyses and immunodiffusion to study low-density lipoproteins. While these authors interpret the results of precipitin analyses as evidence for homogeneity of lipoproteins, their results do appear to suggest that immunochemical differences exist between the different lipoprotein classes (see ref. 1 for discussion). Evidence for immunochemical homogeneity is based primarily on the finding of only one zone of precipitation by immunodiffusion[4,6], by immunoelectrophoresis[7–9], or by both[10,11].

In view of the continuing reports that only one zone of precipitation forms between low-density human lipoproteins and antiserums[8–11], this article presents the results of immunoelectrophoretic experiments which clearly show multiple zones of precipitation and which also reveal the existence in the S_f17+ fraction of a component with the immunoelectrophoretic mobility of albumin. The status of the immunochemistry of lipoproteins at present is briefly discussed. The available evidence strongly suggests immunochemical heterogeneity of low-density human lipoproteins.

Low-density human serum lipoproteins S_f6, S_f13 and S_f17+, and lipoprotein free serum fractions were prepared and characterized as previously reported[1]. The starting material was pooled fresh serum.

Antisera were produced in rabbits and monkeys. All animals were kept for 6 weeks and bled three or four times during this period to obtain pre-immunization serum. Thirty rabbits and thirteen monkeys (five *Macacus* and eight *Cercopithecus*) were divided into three groups. The first group was immunized with S_f6, the second group with S_f13, and the third group with S_f17+. About 10 mg lipoprotein fraction contained in 0·5 ml. was emulsified with an equal volume of Freund's complete adjuvant. A mixture of freshly prepared lipoprotein and adjuvant was given to each animal twice weekly for 10 weeks. Commencing 3 weeks after the last injection, animals were bled three times at intervals of about 5 days, and were then allowed to rest for 3 weeks. Further courses of injection were then given. Each course consisted of two injections, 5 days apart, of newly prepared lipoprotein fractions plus adjuvant, in quantities indicated here, waiting 2·5–3 weeks and then three bleedings, again at 5-day intervals, and a rest period of 3 weeks. Quantitative precipitin analyses were done by the usual procedure[12].

Immunoelectrophoresis was performed essentially by the technique of Grabar and Williams. The dimensions of the immunoelectrophoretic plates were 10 × 20 × 0·5 cm. Ion agar No. 2 (Consolidated Laboratories, Chicago, Illinois) was used, concentration 0·8 per cent in barbital buffer, $\mu = 0{\cdot}05$, $p\mathrm{H} = 8{\cdot}1$, 0·9 per cent sodium chloride. Rectangular antigen wells 1·5 × 0·3 × 0·5 cm were filled with 0·25 ml. lipoprotein fractions containing 20–30 mg lipoprotein/ml. and 0·8 per cent agar. After electrophoresis, antibody wells were filled with unfractionated antiserums, which contained not less than 1 mg precipitable antibody/ml. (tested with S_f6). Diffusion was allowed to proceed for 28 days. Immunoelectrophoresis plates were stained with sudan black *B*. Immunoelectrophoretic analyses of whole human serum served as immunoelectrophoretic and staining controls.

Ultracentrifugally prepared non-lipoprotein-containing serum fractions were tested by precipitin reaction with the three types of anti-lipoprotein antisera. In no instance was precipitate observed. This finding confirms and extends our previous observations that lipoproteins are antigenically distinct from non-lipoprotein-containing serum proteins[1,13] and served to ascertain that the antisera used were specific for low-density lipoproteins.

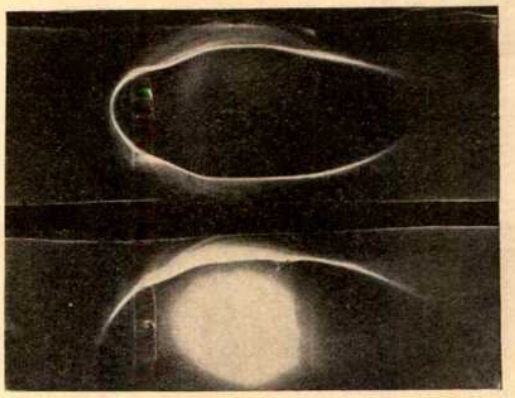


Fig. 1. Immunoelectrophoretic analysis of S_f6 lipoproteins. Upper antigen well: 20 mg/ml.; lower antigen well: 80 mg/ml. Top antibody well, rabbit anti-human serum; centre antibody well, rabbit anti-S_f17+; bottom well, normal rabbit serum

Fig. 1 shows the immunoelectrophoretic results with two concentrations of S_f6 lipoproteins and antisera. Six to eight zones of precipitation can be readily seen, all staining with sudan black *B*. This experiment shows that ultracentrifugally isolated S_f6 lipoproteins are electrophoretically reasonably homogeneous but that several zones of precipitation form which coalesce at the cathodic end. Multiple lipid staining zones of precipitation were similarly obtained with isolated S_f13 lipoproteins and anti-low-density lipoprotein antisera.

The results of immmunoelectrophoretic analyses of S_f17+ lipoproteins and of whole human serum, using several antisera, are shown in Figs. 2–4. Similarly prepared antigen and antiserum preparations were used in these experiments. It can be seen that some of the S_f17+ lipoproteins move with mobility of albumin, some with intermediate mobility, and some, probably chylomicrons, remain at the origin. With the monkey antisera an additional zone of precipitation is observed with certain S_f17+ preparations.

Among the questions which are raised by these results are: (*a*) What is the reason that after more than a dozen reports on immunodiffusion and immunoelectrophoresis of lipoproteins there is not even agreement whether only one zone or several zones of precipitation form between low-density lipoproteins and anti-lipoprotein sera? (*b*) How should the finding of multiple zones of precipitation in Fig. 1 be interpreted? (*c*) What is the significance of the finding that a component in the S_f17+ fraction moves with the mobility of albumin?

The answer to (*a*) may well be found by considering the differences in the experimental procedures in the various investigations. Since we wish to ascertain whether immunochemical heterogeneity exists between or within the different lipoprotein classes, we work with adequately high lipoprotein concentrations and with antisera or antiserum fractions which contain not less than 1 mg

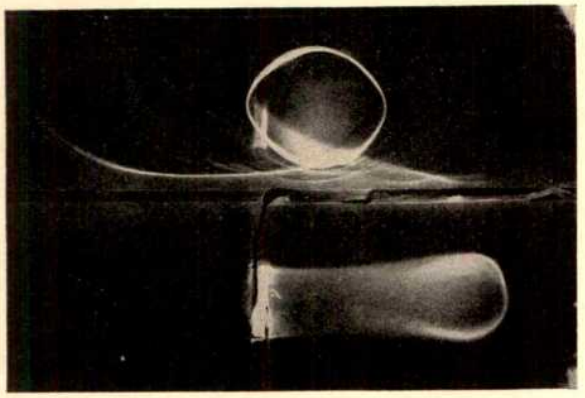


Fig. 2. Immunoelectrophoretic analysis of S_f17+ and serum control. Upper antigen well: normal human serum; lower antigen well: 40 mg/ml. S_f17+. Top antibody well, rabbit anti-S_f17+; centre antibody well, rabbit anti-human serum; bottom antibody well, rabbit anti-S_f17+ (same as in top well)

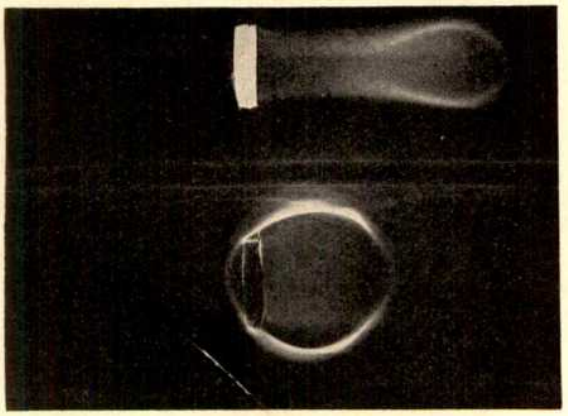


Fig. 3. Immunoelectrophoretic analysis of S_f17+ and serum control. Upper antigen well: 40 mg/ml. S_f17+; lower antigen well: normal human serum. Top antibody well, rabbit anti-S_f17+; centre antibody well, rabbit anti-human serum; bottom antibody well, rabbit anti-S_f17+ (same as in top well)

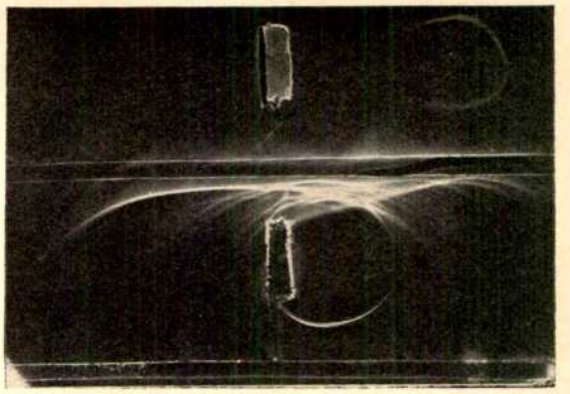


Fig. 4. Immunoelectrophoretic analysis of S_f17+ and serum control. Upper antigen well, 10 mg/ml. S_f17+; lower antigen well, normal human serum. Top antibody well, monkey anti-S_f17+; centre antibody well, monkey anti-normal human serum; bottom, monkey anti-S_f17+ antiserum

antibody/ml., often considerably more. In all the investigations reported in the literature in which only one zone of precipitation was observed, the lipoprotein concentrations were low, approximately those found in normal human serum or less; antibody content is not usually determined. It has been our experience that only one zone of precipitation can be readily obtained if the lipoprotein and/or antibody concentrations are sufficiently low. While we have not rigorously analysed the conditions (concentrations, geometry, etc.) which yield one or multiple zones of precipitation, it appears reasonable to conclude that the discrepancies in the literature are due to differences in the reactant concentrations used in the different investigations.

How can the results in Fig. 1 be interpreted to yield information about homogeneity or heterogeneity of low-density lipoproteins? In recent years a considerable amount of dogma has developed to aid the interpretation of immunodiffusion and immunoelectrophoresis results. A fundamental tenet of this dogma is that multiple zones of precipitation should be independent to be interpretable as evidence for the presence of independent antigen–antibody systems. While this is intuitively reasonable, direct evidence is lacking. The zones of precipitation in Fig. 1 are not independent; the multiple zones fuse into one zone at the cathodic end. Fusion of zones of precipitation has been observed frequently and has usually been interpreted to indicate antigenic similarity or 'partial identity'[14]. Other interpretations have been suggested. Kaminski has observed this type of precipitation (ref. 15, Fig. 6) and has suggested that it may be due either to the same mechanism as that which gives rise to Liesegang rings or to heterogeneity of antibodies. We have observed this type of precipitation regularly only with two types of serum proteins: with albumin and with low-density lipoproteins. We have occasionally (with certain rabbit or monkey antisera) observed it with γ-globulins. On the basis of the work of Kaminski[15] and others[16,17] it has been suggested that "When either antigen or antibody is in great excess the band may widen and even divide into several lines, although the antigen and antibody are pure"[18]. Evidence to substantiate this suggestion is also lacking at the present time; it is not likely that ovalbumin[15], serum albumin[16] or tetanus and diphtheria toxoids[17] can qualify as 'pure'. Twice-recrystallized human mercaptalbumin, for example, has been shown by high-voltage starch-block electrophoresis to contain at least nine components[19]. The Liesegang ring phenomenon is the *bête noire* of immunodiffusion and has sometimes been used to explain away results which cannot at present be explained. So far as I know the Liesegang ring phenomenon is not understood; it is questionable whether it occurs during immunodiffusion. Heterogeneity of antibodies also does not

seem to be a likely explanation, since heterogeneity would also be expected to exist in antibody populations against other antigens and multiple zones of precipitation should then be the usual finding. From these considerations it may be concluded that the most reasonable explanation is that the multiple zones of precipitation indicate heterogeneity of antigen, and that fusion of zones at one end suggests antigenic similarity or 'partial identity'[19]. It may be anticipated that the development of a rigorous analysis of immunodiffusion will eventually clarify such questions, but at the present time we are still attempting to understand the processes which give rise to the initial zones of precipitation[20].

There has been general agreement that low-density lipoproteins move in agar with the mobility of β-globulins while high-density lipoproteins move with the mobility of α-1 globulins. The report by Heide *et al.*[7] suggests that S_f13 lipoproteins have slightly greater mobilities than S_f6 lipoproteins. Walton[8] has recently investigated this problem; he found only very slight differences between mobilities of S_f3–9 and S_f20–400 lipoproteins and attributed these to preferential interaction of S_f3–9 lipoproteins with agar. He noted that the mobility of low-density lipoproteins in agar is somewhat concentration-dependent; a progressive displacement of precipitation zones toward the anode was observed at low lipoprotein concentrations. Our finding that S_f17+ lipoproteins contain a fraction which moves with mobility of albumin was unexpected. S_f17+ lipoproteins certainly contain no material of hydrated density greater than 1·015 g/ml. S_f17+ reacts with antibody against S_f6, S_f13 and S_f17+, but not with antibody against albumin nor with antibody against the lipoprotein-free serum fraction. It is difficult to estimate the concentration of the albumin-mobility material; it would appear to constitute 10–20 per cent of S_f17+. Here again the reason why this fast-moving material has not been previously observed and why it is not observed in Figs. 2–4 in whole serum is almost certainly simply a matter of initial antigen concentration. Not enough S_f17+ containing the albumin-mobility fraction is present in undiluted serum to allow formation of a zone of precipitation.

We are left, then, with the following experimental observations which appertain to heterogeneity of low-density human plasma or serum lipoproteins: (*a*) the finding that antibodies against low-density lipoprotein can be completely absorbed by either S_f6, S_f13 or S_f17+ lipoproteins but that, none the less, S_f6 and S_f13 lipoproteins react differently by quantitative precipitin analysis[1]; (*b*) the multiple zones in Fig. 1 and the immunodiffusion results already cited; (*c*) electrophoretic mobility differences in agar between S_f6 and a component of S_f17+ lipoproteins (Figs. 2–4); (*d*) data from amino-acid end group analyses which show that low-density lipoproteins contain more than one N-terminal amino-acid[21,22]; (*e*) a recent report in abstract form which notes that delipidized low-density lipoproteins contain three protein components by ultracentrifugal analysis and two components by immunoelectrophoresis[23]. These observations, taken together, lend weight to the hypothesis that low-density lipoproteins are immunochemically heterogeneous.

On the basis of immunochemical data, we were previously led to suggest the following antigenic composition of lipoproteins: "Lipoproteins of highest hydrated density, *HDL*-3, are antigenically distinct from all other lipoproteins. *HDL*-2 contain several antigenic components, at least one peculiar to *HDL*-2, others in common with low-density lipoproteins. Continuing the hydrated density scale of lipoproteins towards lower densities, new antigenic components become apparent. Low-density lipoproteins S_f6 and S_f13 contain at least 6 or 8 components. It might be expected that in lipoproteins of still lower hydrated density additional antigenic components may be present, but that some of those associated with S_f6 or S_f13 might be absent. This type of distribution might be thought to exist for lipoproteins up to the chylomicron range"[1]. While, at the present time, the data are still fragmentary, it would appear that the available evidence points in the direction of our early suggestion.

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ESTABLISHMENT OF AN EXPERIMENTAL CAT BREEDING COLONY

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THE Departmental Committee on Experiments on Animals under the chairmanship of Sir Sydney Littlewood[1] recommends that the Secretary of State should be empowered, subject to consultation with the interests concerned, to prohibit the use of laboratory animals (other than monkeys and animals of farm species) not bred for the purpose in registered laboratories or breeding units outside laboratories.

It is more than likely, therefore, that these recommendations will induce other laboratories to consider the breeding of cats, and the recent increased demand for visiting our experimental and breeding colonies supports this assumption. We feel that our experience in establishing an experimental cat-breeding colony should be of interest to other scientists and laboratories who are using animals for experimentation under the Cruelty to Animals Act, 1876.

An experimental cat-breeding colony was contemplated in Sandwich in 1958 for a number of reasons.

It is a common experience that the supply of laboratory animals, especially cats, is extremely erratic and unreliable. This is based on seasonal breeding peaks and also on the high demand for cats by university departments during term-time. A proper planning of experiments is thus made quite impossible. The great demand and low supply lead in some cases to quite excessive charges by animal suppliers. The condition of cats supplied for experimental purposes is generally extremely poor and it is not uncommon to lose more than 50 per cent of the intake within 1 week before the start of experimentation since most laboratories will quarantine animals supplied from outside sources. This leads obviously to a considerable wastage of already very scarce and expensive laboratory animals. The main cause of death in quarantined cats is respiratory and gastro-intestinal disease.

The poor condition and low resistance of these animals prejudice the performance of lengthy and elaborate experiments under anaesthesia and reduce considerably the success rate. On account of the condition of the animals, experiments have to be repeated unnecessarily, thus again leading to wastage of valuable animals. The scientific value, if any, of experiments carried out on such animals must consequently be very limited. Apart from this wastage of animals, the scientist undertaking the experiment also loses still more of the already limited time available for experimentation. Experiments on animals in poor general condition are thus a highly uneconomic procedure from the point of view not only of the cost of the animals but also of the high cost of the time wasted.

There is, of course, always the risk that, under present conditions when dealers offer or supply large numbers of cats for experimental purposes, the laboratory may be receiving stolen property with all its subsequent legal implications.

We are particularly interested in neurophysiological experiments using *inter alia* stereotactic techniques, and it was thus most desirable to work on a uniform strain of animal which would increase the success rate of accurate electrode placing. A great number of such experiments involves recovery from the anaesthetic, and a good state of health is obviously highly desirable.

For these organizational, economic, scientific and legal reasons it was decided to start at first a small holding colony in Sandwich in the Department of Pharmacology in the summer of 1958. At this time the cats, usually at the kitten stage, were obtained locally, with open knowledge of the reason for their purchase, our object being to keep the animals until they were of a sufficient size and in good physical health for experimental use. Any new intake was inoculated against feline enteritis and was housed indoors within wire-mesh walled rooms some 12–15 ft. sq. in a separate wing of the laboratory building. The cats were fed on a diet suggested by Short[2] consisting of a proprietary food mixed with 'Diet 41' meal with a supplement of cooked fish and reconstituted dried milk for drink.

We experienced the usual trouble associated with a mixed intake-infection—usually respiratory. To overcome and prevent droplet spread a suitable barrier was erected between the rooms instead of the original wire-mesh partitions. A controlled ventilation system was installed connecting to each of the four rooms in which the animals were housed. Many kittens died and those that survived made little headway and were largely unsatisfactory. It was discovered that the haemoglobin content of the animals generally had become very low, in some cases less than 50 per cent of normal values. In an effort to overcome the anaemia, fresh butchers' liver was added to the diet; however, this produced a diarrhoea, but the situation was eased by pre-cooking the liver.

On review, we came to the conclusion that the diet recommended was insufficient to maintain cats, let alone meet the requirements of young growing kittens or support any future breeding, and that an indoor environment was not ideal. Apart from a more adequate diet the animals would most probably improve if they had access to the open air with a natural source of ultra-violet light, as an indoor installation had proved ineffective in controlling airborne germs—or in increasing the animals' resistance to infection.

Late in 1959 the cats were moved outside. Their night quarters consisted of a walled-in area at the base of a water storage tower. Shelves were provided for sleeping accommodation and a trap kept them penned in at night. They were let out daily into a wire-mesh surrounding pen, inside of which were several small alder trees which provided shade and some old tree boughs for claw exercise. The cats were now being fed with liver, fish, tinned cat food, and some potato, and received 'Minadex' in their milk feed. Within a short period an improvement was noticeable.

In the light of our experience and findings plans were going ahead for the establishment of suitable buildings to house breeding colonies of cats and dogs[3] in another area on the Company's site. These were finally completed in the summer of 1961.

The new cat unit is of cavity-walled structure with a screeded concrete floor on 2-in. hollow clay pots, some 120 ft. long by 30 ft. wide with laterally extending external grass runs. Adjoining the building at one end is an annexe housing a preparation room and a toilet. Inside, the floor slopes inwards along the length, to a grilled gulley. A corridor runs along this central line and connects to eight cat rooms on either side. The walls of these cat rooms consist of wire-netting fixed to wood studding and continue upwards to the roof ridge. On either side of each room are open mesh shelves some 3 ft. from the floor with a wooden shelf above. To supply warmth during the cold months a steam-heated pipe runs under the lower shelves. Along the roof ridge four large fans exhaust the inner air to the outside and these are thermostatically controlled to cut out when the inner temperature falls below 58° F.

Each cat room has on the outer wall a draught-proof hinged-exit fitment to allow the cats free access to the outside grass run. This run, 15 ft. wide and 50 ft. long, terminates with a 3-ft.-wide sand pit covering the width of the run which the cats invariably use as a defaecating area (gross material being removed daily by raking). The terminal fence is fitted with a padlocked exit gate. These cat pen runs are constructed of 2-in. tubular metal framework with 1-in. woven-mesh wire 7 ft. high; to prevent escape the whole is covered above with 1-in. chicken mesh wire, supported on wire lacing.

Adjacent to the end of this building are four stud pens, constructed of wood posts with wire-mesh and sited in a row with a communal corridor. Each pen has a sturdy wooden kennel standing 1 ft. high on centrally placed paving squares in a 10-ft. × 8-ft. turfed area incorporating a small sand bed.

As has already been stated, we were buying young cats, mainly weaned kittens, occasionally a complete litter with the mother. The cats were of all colours and fur length but mainly of the tabby variety. Our intention was to select from the short-haired tabby types the nucleus on which to found our breeding stock.

Every new cat was inoculated against feline enteritis and received a booster dose after a year in the colony. It was also assumed that all cats would harbour some form of endo- or exo-parasites and these were routinely dealt with by the customary proprietary treatments and became satisfactorily controlled. In 1962 the unit became a closed colony and since then these parasites have virtually disappeared. However, most, if not all, still harboured respiratory disease agents and by cross-infection became enzootic within the colony with death

resulting in quite a number of animals. With attention to hygiene, antibiotic treatment and general nursing care the immune survivors became the heterogeneous nucleus from which the colony has developed. For breeding we chose only the short-haired tabby types with a well-developed skull and culled for experimental use the long-haired, coloured and narrow-skulled cats.

Originally, for mating purposes some thirty queens were kept together with from two to four toms until the females were obviously pregnant: these queens were then removed to another room. We were hoping to keep manual labour and costs to a minimum by utilizing a communal form of littering down using large nesting boxes instead of small individual boxes or cages. This was not successful as some mothers apparently abandoned their young to a more generous 'mothering' queen for suckling who often supplied sustenance to some fifteen to twenty kittens. Because of several days' difference in the age of the young, the smaller kittens were often pushed out and trampled on and under this local overcrowded environment respiratory virus could and did easily gain a foothold. An isolation technique was adopted by suitably placing six to eight large cat cages within a room. In each cage was placed a cardboard carton with some straw for the pregnant queen to litter down. The queens were allowed out separately for exercise.

As parturition time approaches the queen prepares her nest and shows little interest in leaving the cage. She is disturbed as little as possible by the animal staff, litters occurring naturally with the queens being left to carry on with their instinctive nursing care. Apart from two premature litters occurring in young primipara queens there have been no complications during pregnancy.

Cardboard litter boxes—though sanitarily ideal from a disposal point of view—do involve considerable time in assembly. Often, too, destruction by the queen or the young kittens during the confined period causes the need for frequent replacement: this with large numbers of litters is costly both in labour and materials. Recently we have replaced the cardboard boxes with a chipboard industrial container; these have sufficient weight so as not to topple, stand up well to washing and sodium hypochlorite disinfection (thus allowing repeated use) and stack neatly when not required. It is hoped to try a plastic container—as soon as a suitable box becomes available.

By this isolation technique at littering time one is able to keep infection at a minimum, observe and record individual or litter progress and keep a firm check on their parentage and identity. The litter is kept together for 12–13 weeks (the mother being removed at weaning, 6 weeks); during this confinement the kittens are tattooed by four digits—two in each ear—coded as in a like manner to our dog colony[3]. For example, looking at the cat's face from the front and reading from left to right on the ears the four-digit code $4FH5$ represents 1964 (4): June (F): the eighth litter (H) occurring in that month and the fifth kitten (5) of that litter. This coding allows any member of the departmental staff to identify age, individual and litter mate directly without recourse to any records.

At 5 and 8 weeks the kittens are given an inoculation against feline enteritis, at 10 weeks they are wormed and at 13 weeks are removed from the litter cages, sex segregated into rooms so that the young are approximately of the same age and size, where they stay until they are removed for experimental or other purposes, usually after they are at least 6 months old.

Our cats are extremely tame, and come running for attention when a room is entered, thus making capture easy. This is undoubtedly due to the frequent handling soon after birth by the animal technicians. Apart from the frequent physical inspection, the kittens are weighed weekly for 8 weeks, collectively for the first four and individually thereafter and monthly up to 24 weeks. Weights, together with tattoo coding and other relevant data, are recorded on a breeding record sheet and eventually filed.

Our requirements for experimental purposes are cats of a reasonable size still possessing the vigorous recuperative powers of the young—to be produced, for economic reasons, in the least possible time and weighing 2 kg. The weight curve plotted from the collected data shows that this weight occurs when the animal is between 5 and 6 months. The curve is shown in Fig. 1 and for comparison the weight curves found in cats from two other sources[4,5] are included.

The queen after weaning is wormed and returned to stud, usually with a different tom cat. In this way it is possible to obtain three litters within the year. For example, two queens have produced seven litters, one within 23 months and the other in 25 months.

Between January and December in 1963 and 1964 there were 350 litters, 175 in each year. Fig. 2 shows graphically how these litters occurred month by month with three distinct overlapping peaks of kitten production for each year. This is linked in the lower graph with the average litter size per month, indicating that for this colony there is a tendency for larger litters within the second and third production peaks which occur in the latter half of the calendar year.

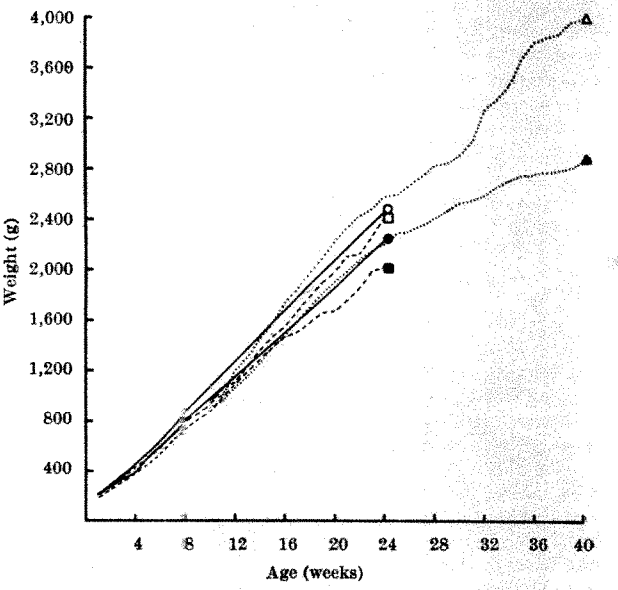


Fig. 1. The graph shows the mean growth curves for male cats (open symbols) and female cats (closed symbols) obtained from three sources. △ and ▲, Short, 200 cats (ref. 4); ○ and ●, Reinert and Smith, 597 cats; □ and ■, Dickinson and Scott, 81 cats (ref. 5)

Normally we expect to take our yield from the first five litters before discarding the queen. By comparing suitable animals with their record it is possible to select the cats most likely to produce good, healthy litters regularly. By using some eighty queens with ten different toms we hope to maintain our requirement and at the same time outbreed as much as is possible within a closed colony.

Breeding losses have been confined to the young, those occurring within the first 3 days and the rest (mainly after weaning) up to about 15 weeks. The kittens up to and included in this period were grouped together after weaning. From the beginning of 1965 the litter has now been confined together for up to 13 weeks before sex grouping and this procedure has so far minimized the losses.

Results for 1963 and 1964 have been tabulated in Table 1 and show the number of litters occurring each month according to the litter size. The combined totals show a normal distribution with a slight shift to the left ranging

Table 1. Numbers of Kittens and Litters born in 1963 and 1964

	Litter totals											Kittens			
	Number of kittens born in any litter										Per month	Mean per litter	Total		
	1	2	3	4	5	6	7	8	9	10			Born	Weaned	Used
January															
February			13	13	10	1			1		38	4·105	156	134	77
March	1	7	13	33	13	1	4		1		73	4·027	294	234	183
April			6	13	10	4	3	1			37	4·675	173	133	101
May			5	2	1	1	1				10	4·1	41	22	17
June			3	9	12	5	5				34	5·0	170	134	94
July		3	7	10	24	10	2	2		1	59	4·864	287	250	160
August		3	7	11	9	9	2	1			42	4·571	192	151	110
September	1		3	2	4	2					12	4·16	50	40	33
October		1	4	8	9	7	3	1			33	4·909	162	113	73
November			1	2	5	2					10	4·8	48	47	20
December				2							2	4·0	8	8	7
Total 1963		7	38	52	41	26	8	1	1	1	175	4·46	781	656	431
1964	2	7	24	53	56	16	12	4	1		175	4·57	800	600	444
1963–64	2	14	62	105	97	42	20	5	2	1	350	4·52	1,581	1,256	875

over from one to ten kittens occurring in a single litter with the mean at 4·52. Losses occurring at birth and during the growing stage lower this mean to 2·5 cats per litter available for experimental purposes—that is, a total of 875 young adult cats from 1,581 kittens born over 2 years, roughly a production of five cats from each breeding female a year. Breeding losses are just more than 40 per cent and it is to be hoped that these can be substantially reduced—although losses almost as high have been found in dog colonies[3] where greater prophylactic measures against infectious disease can be given.

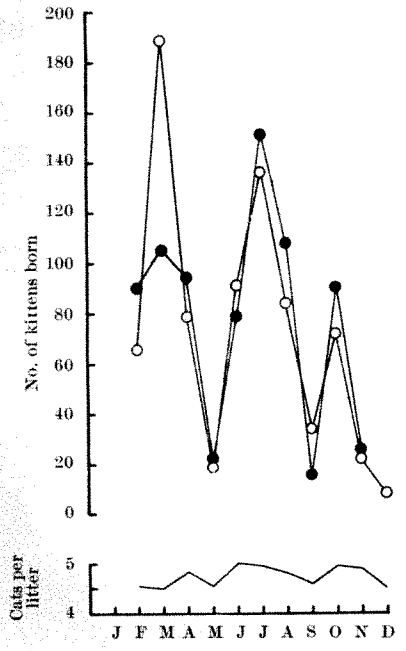


Fig. 2. The upper graphs show the number of kittens born in 1963 (●) and 1964 (○). The lower graph illustrates the average litter size occurring in each month for the combined totals

The colony has been reared on a diet consisting of: 83 parts cooked horsemeat (6 parts offal); 5 parts whole cooked potato; 12 parts 'Terrier' meal; 0·3 parts bone meal, plus salt and dried yeast. All cats have two feeds daily, the main portion being fed in the afternoon.

If the weight curve up to 24 weeks of 597 cats is compared with the findings of Short[4] (who used 200 cats) and of Dickinson and Scott[5] (who used 81 cats) it is found that it occupies a medial position (Fig. 1). The male cats were consistently heavier from the age of about 3 weeks.

Dickinson and Scott showed slightly less kittens born per litter over 5 years, averaging 4·03 kittens for 180 litters producing 727 cats. We have obtained a mean of 4·52 for 1,581 cats; this seems to indicate that our present cooked diet supports reproduction without any lowering of litter size since we are now in our third and fourth generation. We confirm the findings of these workers that a peak production occurs in March and only small numbers in May; however, contrary to their findings, our results reveal a distinct third reproductive peak in the autumn months, which is linked to a third peak in litter size.

Information on losses is remarkably scanty—although it is well known that the cat has a very high incidence of disease, especially of respiratory origin—as many have found to their cost. Until satisfactory vaccinal or other protection is easily and cheaply available, little can be done to change this state of affairs apart from good husbandry and adequate diet. Our 40 per cent loss, in rearing the cats to 6 months of age when they are of practical experimental size, emphasizes the high cost involved in breeding and maintaining these animals, and must reflect the unlikelihood of obtaining at any time well-nourished, parasite-free and reasonably healthy cats in quantity from any source other than from recognized colonies.

Ideally it would, perhaps, be best to start a colony with Caesarian-derived animals, suitably quarantined, to ensure that no infectious agents have passed the placental barrier; and afterwards to breed from these. This is long-term, costly and does not meet the immediate urgent demand; however, this procedure does not prevent the possibility of infection at a later stage, introduced by birds or stray animals when housing cats under open-air conditions.

Animal technicians and other personnel should use rubber footwear to pass through barrier foot-baths and be supplied with adequate clean protective clothing. There should also be provision for hand-washing facilities.

With the introduction of new breeding adults there should be vigorous observance of quarantine, such as separate accommodation remote from the existing colony. It is also desirable that a proof litter should be reared in the same quarters before the adult is finally introduced into the main colony.

Our final advice, based on bitter experience, and in spite of a genuine desire to help those interested in starting cat colonies for themselves, is rigidly to exclude visitors, solely to prevent the disastrous and decimating effects of a fresh introduction of infection.

[1] *Rep. Dep. Comm. on Experiments on Animals* (H.M.S.O., London, 1965).
[2] Short, D. J. (personal communication, 1958).
[3] Reinert, H., and Smith, G. K. A., *J. Anim. Tech. Assoc.*, **14**, No. 2 (1963).
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[5] Dickinson, C. D., and Scott, P. P., *Brit. J. Nutr.*, **10**, 304 (1956).

CYTOGENETIC VARIATIONS INDUCED WITH A MAGNETIC PROBE

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EXPERIMENTAL evidence concerning the possible influence of magnetic forces on cytological development in living organisms is, at the present time, in a somewhat controversial state. Several investigators[1–3] have reported a retardation or growth-arresting effect of magnetic fields both at the cellular level as, for example, in carcinoma tissue as well as in whole organisms such as mice. Recently, however, Halpern and Greene[4] found that a 1,200-gauss magnetic field had no significant influence on the growth of human cancer cells (HeLa) in tissue cultures. In most of these reported investigations, the experimental arrangements have been such that the organisms were tested in essentially a homogeneous magnetic field. One finds very few discussions either of the influence of field gradients or of direction and polarity effects.

In proliferating tissue as well as in resting cells, changes in local ion concentration are known to have pronounced effects on general cell morphology[5] and cellular functions. Within a uniform magnetic field the force on an ion is simply charge times the vector product of velocity and field strength. Ideally, under uniform conditions the path described by the ion is circular and motion in a transverse direction is at a minimum. With increasing gradient of flux a lateral ionic drift mobility occurs and the direction of ion flow, although a more complex motion, could, as a result of this additional degree of movement, increase the probability of the field influencing intercellular bonding sites. It is thus conceivable that one might gain additional knowledge concerning the physiological action of magnetic fields on cellular organization by using high localized field gradients rather than striving for homogeneous test regions. This would be especially true if ion-drift mobilities were intimately related to the basic cellular functions.

Based on these considerations, a magnetic probe device was designed to provide an intense, non-homogeneous field in specific regions. This article presents the initial results of subjecting living systems to the probe. In the majority of experiments, the approach has been to apply the magnetic probe to the gonadal organs in *Drosophila melanogaster* at various stages of metamorphosis. The results of the probe treatments are examined in terms of reproductive response effects such as sex ratios, progeny yields and rate of development. Although in the course of these experiments phenotypic variations were observed, extensive crossing tests have not been conducted to determine mutation rates. The main emphasis in this research was placed on examining the question of magnetic fields affecting development and fecundity.

The initial results from the probe tests demonstrate that the application of high field gradients to the developing reproductive organs in *Drosophila melanogaster* has a pronounced influence on development time and on the yield of progeny. A growth retardation effect in an interval of development, including the embryonic and post-embryonic stages, was found to be genetically transferred to succeeding generations even though only pupae producing the first or F_1 generation were exposed to the probe. Viability in the first few inbred generations, as measured by progeny yields, is shown to decrease exponentially with increasing development time; a subsequent increase in sex ratio is suggested. We shall present exploratory data which show variations in the probe influence as a function of pupal age, and we shall also discuss a proposed mechanism based on enzymatic inhibition induced by the magnetic field.

The probe was designed to produce an intense localized field over a region comparable with the diameter of the ovaries and testes in *Drosophila* (approximately 100μ). A coil (5·8 cm long) of No. 34 insulated copper wire was wound uniformly around a 6-mm diameter rod (of low remnant field) with a final resistance of 1,700 ohms (26,770 turns). At one end of this rod a 1-in. section was prepared with a conical taper down to a final diameter of 125μ with the end machined flat. Two parallel connected 22·5 V batteries were used to power the coil and this gave a current of approximately 12 m.amp.

The magnetic field characteristics of this probe were mapped using a model 110 Bell gauss-meter. The effective area on the meter sensor was approximately 1 × 2 mm. The field strength was determined as a function of distance from the probe tip. During calibration, the field contribution from the shoulders of the probe was reduced by extending the tip through a sheet of magnetic shielding metal; 1 mm of the probe was allowed to protrude. Using the ratio of the area on the flat probe tip to the much larger area of the test sensor on the gauss-meter, the effective field strength at the probe surface was determined to be 21·8 ± 1·0 kG. The field gradient within the first 2 mm from the probe tip was approximately 9 kG/mm.

The probe was mounted in a vertical position over a table with a movable stand (glass microscope slide). With this apparatus the specimen could be brought within proximity of the magnetic probe tip, thus allowing treatment of selected portions on larvae, pupae and adult flies. In Fig. 1, a pupa is shown set in position below the probe tip. In the case of eggs, the single egg within the chorion (~ 0·1 mm long) was exposed to the magnetic field.

Using this particular probe design and current load, no significant heating effects were observed for exposure periods of 30 min or more (less than 3 per cent current variation). The current was monitored during each exposure test and the polarity of the probe tip (N. or S. pole) was designated.

Although *Drosophila* were exposed to the magnetic probe in the egg, larval, pupal, and adult forms, the majority of these exploratory data were obtained in the two last-mentioned stages of development. The following general test procedure was used in the probe treatments: (1) With the organism on the movable stage, the glass platform was raised until a 0·5-mm air gap remained between the probe and the test specimen. (2) The field

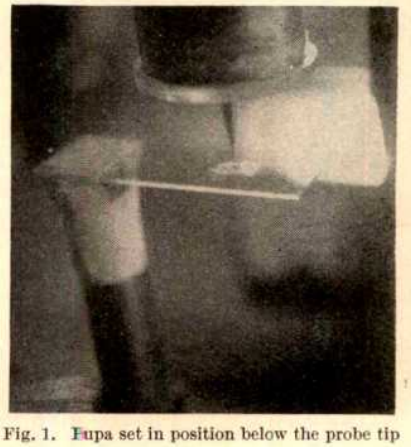


Fig. 1. Pupa set in position below the probe tip

was then switched on for the designated length of time. After the test interval the stage was quickly lowered several centimetres and the test specimen removed from the area before the current was switched off. This was done to prevent transient exposure to a field of reversed polarity. (3) In the case of pupae, the probe was positioned at the dorsal location of the developing reproductive organs. After each exposure the treated pupa was placed with a small segment of the paper on which it had formed into a culture bottle containing fresh medium. (4) Adult flies were anaesthetized with ether and the reproductive organs subjected to the probe treatment. In this case, the probe tests were of short enough duration so that the flies remained anaesthetized and immobile under the probe. The exposures were of 5 min duration or less.

The medium, a cornmeal–molasses type with propionic acid mould inhibitor, was placed in the bottom of 4-oz. culture bottles to a depth of about 2·5 cm. The laboratory temperature was 22° ± 2° C, and both barometric pressure and relative humidity were monitored. Parents were left 10 days in the bottles. Countings were made over an 8-day interval starting with the day the new generation of adult flies were seen.

In most of the test series, three or four generations of flies were examined following the probe treatment; six pairs of flies were randomly selected as the parent stock for each new generation. In some cases where the effects were pronounced, the repeated filial generation crosses were continued for a number of generations. Wherever applicable the usual statistical analyses were used in analysing the data. Control cultures were prepared in the same manner as probe-treated specimens except that a brass 'dummy probe' was applied. The dummy probe was machined to the exact dimensions of the test probe. The exposures to the dummy probe were usually of 10 min duration.

The total period of embryonic and post-embryonic development was taken as the number of days from the start of a new culture until eclosion, that is, when the new generation of adult flies first appeared. Throughout the following sections this interval of metamorphosis will be referred to as the 'development time'. Over the several months during which the probe tests were conducted a total of 52 control (Ore-*R*) cultures were recorded; the mean development time was 13·60 days with a standard error of ±1·12 days (the extreme values were 11 and 16 days).

A mean development time of 13·6 days is somewhat longer than is usually reported in the literature (~ 10 days); however, this reported value does not include the pre-egg-laying period when the flies are introduced into the new culture. Miller[7] recently demonstrated that larval competition in high-density cultures may increase the period of development. This factor was examined by placing single, fertile eggs in fresh cultures. It was noted that imagos appeared after 10–12 days of development; however, a factor of 24–48 h is to be added to this since the usual pre-egg-laying period in the new culture may be several hours (recovery from etherization, etc.) and the eggs may have gone through a maximum of 22 h development before laying. Applying a 1–2 day factor brings the single egg development time within the 11–16 day range of the regular control cultures. It appears, therefore, that overcrowding and larval competition are not factors and the 13·6 day value is a characteristic of this strain (Oregon-*R*). Different strains apparently disclose some variations in development time[7]. The investigations discussed here were all conducted with the Ore-*R* strain.

(1) *Influence on development time.* The probe influence on development time was followed in detail through a number of generation series which were started with treated pupae. In general the development time alteration is inheritable and is not readily selected out of the population. The typical example shown in Fig. 2 was initiated with 110-h-old pupae (six in group) exposed 30 min to the N.-oriented probe. The mean for the previously mentioned 52 control cultures is shown by the heavy horizontal line in Fig. 2 and the shaded area designates the standard error of the mean. Although the individual generation points disclose considerable variability the points lie well outside the statistical values formed by the control group. With succeeding generations there appears to be a general levelling-off effect in the development time; however, it is apparent that the development retardation is not rapidly selected out of the test culture. In the F_7 of this series reciprocal crosses were made with untreated flies from a stock culture. Both test cross-cultures were examined for two generations each, and it was observed that the development time in every case was within the normal range (11–16 days). From these test crosses it may be concluded that the alteration in development time shown in Fig. 2 does not involve a dominant mutant effect.

In some cases the adult flies from the probe-treated pupae produced only one or two flies in the F_1 with development times of more than 20 days. Many pupae formed but did not hatch. This effect was particularly noticeable when treating pupae less than 20 h old. In one case the six adults from 20-h-old pupae, treated 10 min with the S.-oriented probe, produced one F_1 female after a 32-day development time. The remaining pupae in the culture died before hatching.

Even with a brief exposure of only 2 min, an effect on progeny yield was noted, most pronounced from pupae treated in the early stages of growth. The results for two generations are shown in Table 1 for 20-h and 72-h-old pupae tests. Treatment of the 20-h-old pupae increased the development time in the F_1 and in the F_2 larvae formed, but they did not pupate; death occurred after going through the larval stage. Treatment of the 72-h-old pupae did not produce the lethal effect in the second generation, and the development times were within the range of the controls although at the upper limit.

(2) *Response of individual pupa to probe exposure.* To obtain some idea of the male and female response to the probe treatment, a series of single pupa tests were made. The treated pupae were about 37 h old and were given a 30-min exposure. Each treated pupa was placed in a separate culture bottle and, after hatching, was mated with several untreated flies. The probe-treated males were crossed with virgin females from a stock culture. The results summarized in Table 2 demonstrate that only in the case of the male progenitors were the development times altered; however, the influence was not the same in every case. Perhaps it is necessary to treat flies at a definite stage of development to obtain the same results. In these series there could have been a 3 or 4 h variation

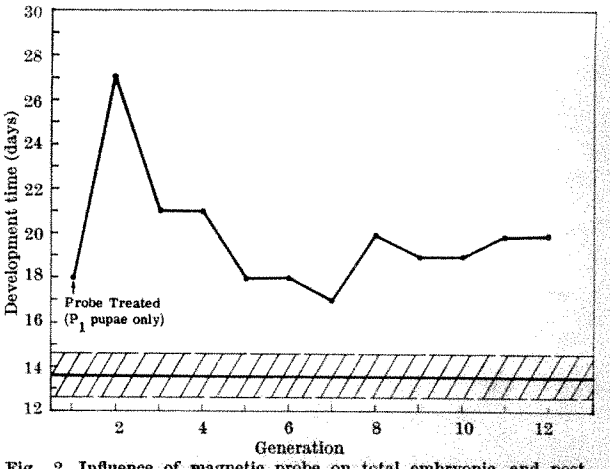


Fig. 2. Influence of magnetic probe on total embryonic and post-embryonic development time (110-h-old pupae–N. probe, 30 min). Mean and standard error for control cultures indicated by heavy horizontal line and cross-hatched area

Table 1. Six Pupae Exposed 2 min with N.-oriented Probe

Generation	20-h-old pupae Total No. progeny	20-h-old pupae Development time (days)	72-h-old pupae Total No. progeny	72-h-old pupae Development time (days)
F_1	85	21	123	16
F_2	0	—	106	16
F_3	—	—	190	16

Table 2. Single Pupa—36-h-old Exposed 30 min to Probe
Mean values from 4 generation series

♂ Series Probe orientation	♂ Series Progeny	♂ Series Development time (days)	♀ Series Probe orientation	♀ Series Progeny	♀ Series Development time (days)
S.	151·5	12·2	N.	145·7	12·5
S.	119·1	17·2	N.	134·0	12·8
N.	39·0	24·0	S.	91·0	11·7

Table 3. Fertilized Flies Exposed 2 min—N.-orientation

Generation	Probe treated Total progeny	Probe treated Development time (days)	Controls Total progeny	Controls Development time (days)
F_1	86	13	131	14
F_2	115	15	114	16
F_3	0	—	148	14

in the pupal ages and this may account for the different response effects in male pupae given the same treatment. The influence of pupal age is discussed in greater detail in a later section.

(3) *Treatment of adult females.* Three adult female flies were treated 2 min with the magnetic probe after they had been fertilized. After exposure to the probe they were placed in a fresh culture without the presence of males. This ensured that the eggs deposited would be only those treated *in vivo*. The results of this test together with a temporally corresponding control series are given in Table 3. The development times did not appear to be influenced by the probe. In the third generation of the probe-treated series, eggs were deposited but no larvae formed. Many flies in the second generation showed wing abnormalities.

From the 2-min results it was thought that a longer exposure would produce a more drastic effect on the reproductive responses; however, such was not the case. Two groups consisting of three fertilized females in each group were exposed 5 min to the probe using an N. and S. orientation. The groups were examined through four generations and in these series the third generation sterility effect did not appear, and there was no apparent effect on development time.

(4) *Development time alteration through male progenitors.* In one test series, six 21-h-old pupae were treated 10 min with the S.-oriented probe and only five males were hatched in the first generation with a development time of 28 days. These five male flies were crossed with normal untreated females and the development times noted for ten generations. The results are plotted in Fig. 3 and it can be seen that there is a significant increase in the development times. In the third generation the development time was within the control level; however, in the F_4 there was a very sudden increase in the development

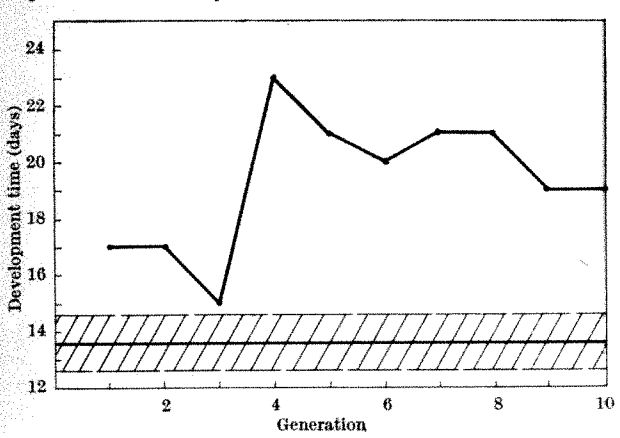


Fig. 3. First generation males from probe-treated pupae crossed with normal females (21-h-old pupae—S. probe, 10 min). Mean and standard error for control cultures indicated by heavy horizontal line and cross-hatched area

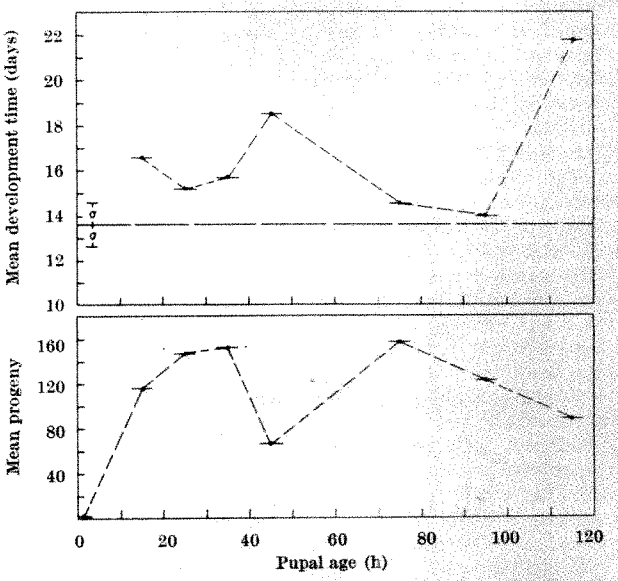


Fig. 4. Variations in development time and progeny with age of treated pupae (points are mean values from four or more generations)

time showing that the effect was not selected out of the culture in the previous generation. This significant increase in development time as carried over by these five males is in contrast with the previous test crossing in the seventh generation of the 110-h pupae (Fig. 2). In this case two generations of reciprocal crosses did not disclose alterations in the development times. From these initial test crosses it appears that the probe effect on development does not follow a simple Mendelian expression.

(5) *Exposure in head region.* More than fifty pupae of various ages were exposed in the head region (10 min treatment—N. orientation). The purpose was to find out if the probe exposure affected an area containing major imaginal disks. This treatment produced a high pupal mortality; more than 50 per cent of the pupae exposed in the head region did not reach the imago stage. Between 5 and 10 per cent of the imagos from head-treated pupae showed severe wing abnormalities and body distortions. Generally the abnormal flies did not live for more than an hour after hatching and crossing experiments were not possible. Possibly the probe influenced the release of growth and development hormones from the brain and corpora allata located near the brain.

(6) *Development, fecundity and age of treated pupae.* Although no attempt was made to investigate explicitly the relationship between pupal age and the probe effect, enough experiments were made at different pupal ages to warrant at least a cursory survey of the results. Data from a number of probe-treated generations were grouped according to the age-level at which they were originally exposed. A 10-h interval was arbitrarily chosen for the test period and both the progeny and development times were tabulated within each age interval.

The mean values for both progeny and development times are plotted in Fig. 4 as a function of pupal age. Each point in Fig. 4 represents from 4 to 20 generations of data and the horizontal lines indicate the age range included. The zero progeny point at the 1-h age-level in the lower curve in Fig. 4 represents a lethal effect. Pupae treated at this early age generally died before hatching.

It is interesting to note that there is an approximate inverse correspondence between development time and progeny. It should be pointed out that all data shown in Fig. 4 represent either singly treated male pupa or the treatment of both male and female pupae. The results of treating a series of female pupae then crossing with unexposed males disclosed no significant change in development time and the fecundity was essentially

unaltered. These results are in accord with the male progenitor effect previously discussed. It appears from Fig. 4 that there are certain stages in the pupal phase of morphogenesis when the organism is more sensitive to the probe treatment than at other stages of development.

In the following paragraphs we shall advance an hypothesis concerning the mechanism of action. We shall show that the ideas suggested, pertaining to the influence of the probe field on living organisms, agree qualitatively and to a limited extent quantitatively with the experimental results in the preceding sections. These concepts are presented with the realization that a demonstration of agreement between experiment and theory does not necessarily rule out other possible explanations for this phenomenon. It is anticipated that as more experimental data are accumulated any hypothetical foundation which might be advanced at this early stage will experience alterations.

Before proposing concepts relating to the probe findings, it is worthwhile to examine the comments of others who have been involved with biomagnetic effects. One of the first positive effects of a magnetic field on enzyme activity was reported by Smith and Cook[7]. The activity of trypsin *in vitro* was found to increase when exposed to a magnetic field. These authors postulated that this effect suggested an increase in the state of organizational order in the enzyme. The energetics of magnetic field–enzyme interactions has also been recently considered by Gross[8]; an observed decrease in antibody formation in a magnetic field was thought to be related to the possible orientation of large protein molecules. Gross pointed out that even though the energy for a flipped electron spin in a 5-k.-oersted field is approximately 1/500 kT at 25° C, this is still of sufficient magnitude to distort the bonding angle of an unpaired electron and it is in the large molecular aggregates that such magnetic effects are possible. Jonnard[9] emphasized that the velocity of a chemical reaction is slowed down if paramagnetic reaction products are formed.

From the foregoing, it does not seem unreasonable to postulate that the magnetic probe increases the free-energy, ΔF, in an enzyme-substrate system. This change in free energy would alter the equilibrium constant, K, of the chemical reaction according to the relation:

$$K = \exp(-\Delta F/RT) \qquad (1)$$

where R is the gas constant and T the absolute temperature. An examination of the data obtained in the probe tests suggests that an alteration in ΔF may have changed the temporal sequence of gene activation during the cyto-differentiation stages (Fig. 4). A determination of these thermodynamic alterations would not, however, reveal anything about the actual reaction mechanisms. For a clearer understanding of probe-induced variations in reaction rates it would be advantageous to examine the chemical kinetics of the effected enzyme systems. It has been pointed out by Wagner and Mitchell[10] that a change in the velocity of an enzymatic reaction of only a few per cent can make a difference in coat colour in the fur of a mammal, or even between life and death of the organism.

If the developmental reactions in *Drosophila* have been effected to the depth suggested in the preceding discussion, then the question remains of what to look for in terms of changes in the reaction kinetics. When examining this point, one turns to the parameters that were observed to be altered by the probe treatment, namely, reproductive yields and development times. As data were accumulated from various series of probe-treated pupae, it became apparent that variations in progeny yields were related to the alterations in development time; this is certainly indicated by the reciprocal nature of the curves shown in Fig. 4.

Conceptually, progeny yields were considered to be an expression of probe-induced variations in enzyme activity within the magnetic field-enzyme-substrate system. The magnetic field-induced inhibition may be expressed by the general equilibrium equation:

$$E + I_m \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_m \qquad (2)$$

where E is the original enzyme concentration, I_m the magnetic field inhibition, E_m the concentration of the altered enzyme, k_1 the reaction rate of the probe-induced alteration and k_2 the rate of return of the altered enzyme to the original activity.

It appears, however, that this inhibition may be considered irreversible ($k_2 \rightarrow 0$) since the altered enzyme activity E_m is transferred to succeeding generations of flies. At least restoration to the original enzyme activity ($E_m \rightarrow E$) is not significant over the interval commensurate with the period of morphogenesis and k_2 may be taken as zero.

Irreversible inhibition, as pointed out by Webb[11], is expressed by first-order kinetics. The rate of reduction of normal enzyme activity can be written as:

$$-\mathrm{d}E/\mathrm{d}t = k_1(I_m)E \qquad (3)$$

This may then be expressed in the integral form:

$$\int_{E_0}^{E_m} (1/E)\mathrm{d}E = k_1(I_m)\int_0^t \mathrm{d}t \qquad (4)$$

and after performing the integration the following relationship is obtained between the altered enzyme concentration and reaction time:

$$E_m = E_0 \exp(-k_1(I_m)t) \qquad (5)$$

where E_0 is the initial concentration. This initial concentration of enzyme E_0 represents the concentration when the probe is applied to the organism. The value of E_0 is not the same for each probe-treated series but depends on the stage of post-embryonic development (Fig. 4). The change in this initial concentration brought about by the probe treatment induces an alteration in the development rate.

From equation (5), the validity of the foregoing assumptions can be examined by plotting the logarithm of the progeny yields against the development time. The production of offspring reflects the degree of success of various enzymatic reactions, particularly developmental enzymes involved in morphogenesis. It does not seem unreasonable, therefore, to postulate that progeny yields in the probe-treated series are an expression of the altered enzyme E_m and that development time is directly related to k_1, the reaction rate. In Fig. 5 the mean values for a number of four generation series indicate experimental agreement with the equation (5) relationship. The solid square at the left of the curve gives the mean and standard deviations for the 52 control cultures. The scatter of the data

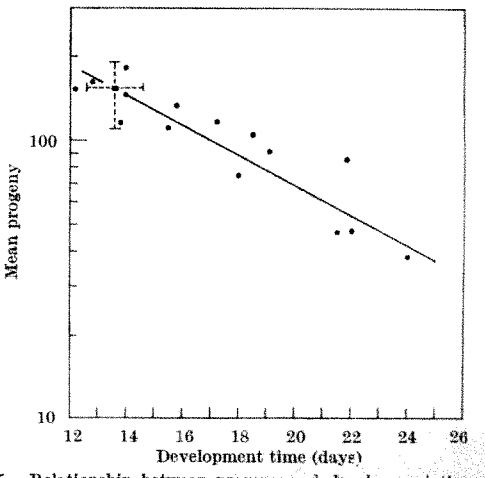


Fig. 5. Relationship between progeny and development time; points represent means of four-generation series treated with magnetic probe in pupal stage of first generation (solid square and dashed lines represent mean and standard deviations of 52 control cultures)

points in Fig. 5 may be attributed primarily to fluctuating environmental conditions. Although local factors such as diet, laboratory temperature, etc., were maintained constant, it has been demonstrated by Levengood and Shinkle[12] that variations produced by 'external' effects such as solar flares may influence *Drosophila* fecundity. These external environmental parameters were not constant over the several months during which the data in Fig. 5 were accumulated.

From the data in Fig. 5 the supposition is made that the ratio of altered to unaltered reactant species (E_m/E_0) is a controlling factor in determining the final progeny yield. An increase in the development time suggests that in terms of relative enzyme activity $E_m < E_0$ following the probe treatment; the active fraction may be expressed by:

$$i = 1 - \exp(-k_1(I_m)t) \quad (6)$$

From the relationship postulated between progeny yields and this fractional activity, the specific rate of inhibition from the Fig. 5 data is:

$$k_1 = 0{\cdot}146 \times 10^{-5}\ \text{sec}^{-1} \quad (7)$$

If introduced at the sensitive periods of metamorphosis, the inhibition seems to have a pronounced effect on the development cycle. Such an inhibition, once established, becomes an inheritable factor, traceable through an increase in the total period of embryonic and post-embryonic cytodifferentiation (Figs. 1 and 2).

A further relationship between alterations in development time and fecundity of the organism was shown in an examination of sex ratios. The mean values for a number of four-generation series are shown in Fig. 6 with the probe orientations designated. There is considerable scatter in the data and the dashed line was sketched in merely to show the trend. This indicated increase in sex ratio may also be related to the altered enzyme reaction kinetics. In this as well as in the previous series there appears to be no marked influence of probe orientation. From the previous considerations of free-energy changes resulting from molecular orientation, it might be expected that the N. and S. orientations on the probe would produce similar effects. One would anticipate polarity differences only in the case of large anisotropies in the molecular dipoles. If such anisotropy exists, the present data indicate that the effect is not pronounced.

At this stage of the experimentation one can only make speculations concerning the type of genetic inheritance primarily involved in the alterations of development time and fecundity. Tentatively, from the manner in which development time alteration is transmitted to succeeding generations and results from a limited number of test crosses, the presence of a cytoplasmic inheritance is indicated. The concept has been advanced[10] that cytoplasmically inherited characteristics can be induced by changes in reaction rates, and this would be in accordance with the hypothesis of variations in the reaction kinetics. Cytoplasmic changes appear to be instigated more readily than gene alterations. In one respect the data

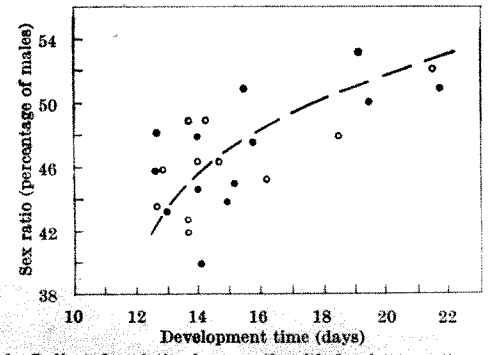


Fig. 6. Indicated variation in sex ratio with development time. Points are means from four generation series (probe treated in pupal stage of first generation. ●, N.-oriented probe; ○, S.-oriented probe

presented in the preceding sections are in disagreement with present-day knowledge concerning mechanisms of cytoplasmic inheritance. Generally, in non-Mendelian inheritance it is the female which carries the effect[10]. A comparison of single-probe-exposed pupae (Table 2) demonstrated, however, that the alteration in development time was initiated through male and not through female pupae. Male adults from treated pupae (Fig. 3) also transferred the increased development time when crossed with normal females, whereas females have not demonstrated any transference when crossed with normal males (occasionally treated females are sterile). The previously reported production of normal genotypes by males has been attributed to the fact that gametes contain very little cytoplasm. The greater volume of cytoplasm in the female egg theoretically increases the probability of cytoplasmically induced mutations. If, as suggested in the mechanism proposed here, the magnetic field through orientation of large molecules reduces the free energy of a small percentage of the enzyme reactants, a small volume of cytoplasm in the sperm could assume greater importance than the larger volume in the female egg. In the sperm we will assume that the percentage of the reactant species per unit volume of cytoplasm is about the same as in the egg cytoplasm. One explanation for the greater effectiveness of the male gamete is the possibility that cytoplasmic bodies in the sperm are in greater proximity with the nuclear material and induced orientation effects could increase the probability of interactions between mitochondria and the nuclear membrane. This in general might explain the greater sensitivity expressed by male-treated pupae.

Perhaps in the 'cramped quarters' of the sperm the magnetic field is operating on fixed-charge systems as described by Ling[13]. In such a fixed-charge system only one species of ion is markedly restricted in motion and presumably in this case the restriction would be on the protein with its associated cations. The anions such as OH^- groups possess a greater degree of freedom in the matrix. The application of the non-uniform magnetic field alters the ionic flow pattern and as a consequence may effect the bonding energy between the protein and its counter ion. One possible site for such an energy alteration is within the interstices of the mitochondria. An induced orientation within the mitochondria may influence the nature of the polypeptide chains and afterwards alter the internal energy control mechanisms. It was suggested by Bourne[14] that the movement of mitochondria may be due to ion pumping and under such conditions the probe could effectively alter ion paths. Such speculations will, however, depend on further experimentation for confirmation or denial.

These initial investigations suggest that the magnetic probe may be important as a technique for producing modifications in cytodifferentiation. This method of locally applying intense field may also be of prime significance in the control or alteration of cell development in carcinoma tissue.

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LETTERS TO THE EDITOR

RADIOPHYSICS

Pre-absorption Events at 230 Mc/s

The Type IV radio event of February 5, 1965, was observed with the University of Michigan 28-ft. paraboloid at frequencies of 230 and 345 Mc/s. Following 1847 U.T. many short-duration bursts similar to the 'spike' bursts described by de Groot[1] were observed, superimposed on the continuum at these two frequencies. This communication describes four bursts at 230 Mc/s which were preceded by brief decreases in the background radiation intensity. We interpret these decreases as absorption of the type IV continuum. The four events occurred between 1849 and 1851 U.T. Although numerous bursts occurred at 345 Mc/s, no similar pre-absorptions were observed at this frequency. No instances of post-absorption events were evident on either frequency. Three of the pre-absorption events are shown in Fig. 1. The characteristics of the events are given in Table 1. The flare with which the radio events were associated started at 1750 U.T. and reached a maximum near 1810 U.T. Type IV radiation was observed at metre wave-lengths at Fort Davis from 1800 until 1940 U.T. and at decametre wave-lengths at Boulder from 1810 until 1905 U.T.

Table 1 (see Fig. 2)

Time U.T. (Peak)	t_1 (sec)	t_2 (sec)	t_3 (sec)	t_4 (sec)
(*a*) 18h 49m 13s	0·40	0·10	0·08	0·25
(*b*) 18h 50m 47s	0·45	0·11	0·09	0·25
(*c*) 18h 51m 02s	0·20	0·10	0·06	0·10
(*d*) 18h 51m 04s	0·33	0·07	0·08	0·30

Suspected absorption of solar radio waves has been reported on several occasions[2-4]. The most certain cases of such absorption[2,3] appeared to be associated with active dark filaments crossing the flare regions. Because of the nature of the four events we observed, it seems unlikely

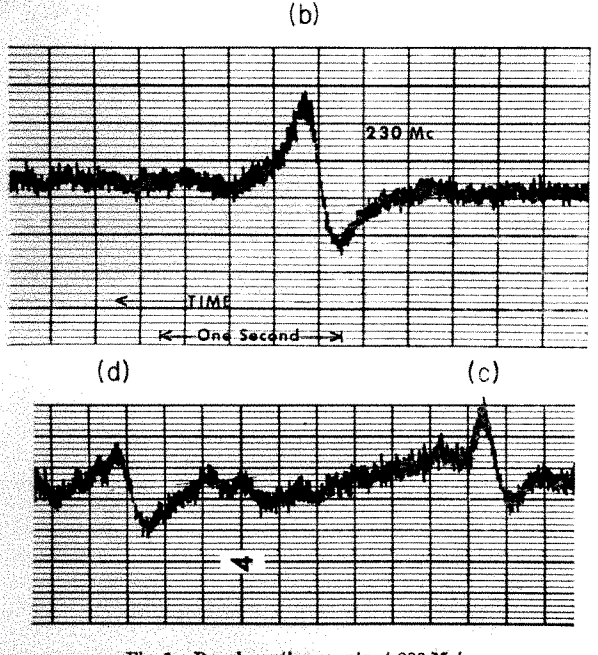


Fig. 1. Pre-absorption events at 230 Mc/s

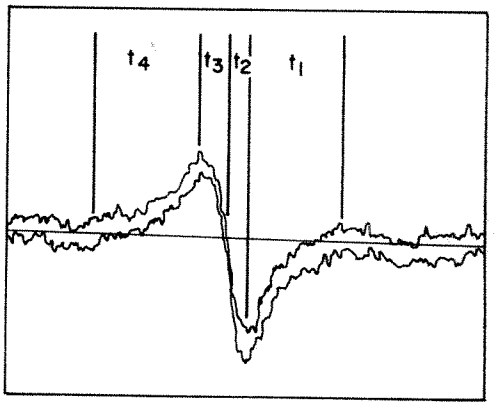


Fig. 2. Event (*b*)

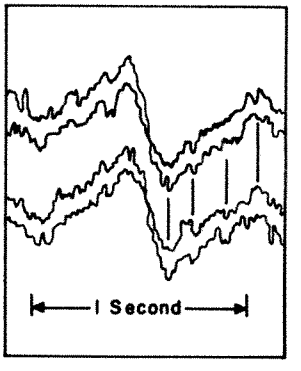


Fig. 3. Event (*d*) shown with its reflexion

to us that the absorptions were due to the drifting of material across the emitting region. The four events exhibit considerable symmetry; the total energy absorbed is comparable with that emitted. Each event is nearly symmetrical about the cross-over point, that is, $t_1 = t_4$, $t_2 = t_3$ (Fig. 2). Moreover, inspection of event (*d*) reveals a curious reflexion of small details about the cross-over. In Fig. 3 the event is compared with its reflexion. It will be noted that three features separated by 0s.13 are found in the falling portions of both the emission and absorption features. The symmetry of these events and lack of post-absorption events tend to rule out scintillations as the source of these phenomena. We are left with the likelihood that true absorption, storage, and re-emission of the radio-frequency energy occurred during these four events.

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PHYSICS

Dual- and Multiple-beam Interferometry by Wavefront Reconstruction

THE formation of optical images by wave-front reconstruction as originated by Gabor[1] and more recently extended by Leith and Upatnieks[2] has made it possible to perform interferometry with light reflected or scattered from an irregular three-dimensional object[3]. Some of the possibilities of this method have been discussed in a previous publication[4], and more recently Stetson and Powell[5], Collier, Doherty and Pennington[6] and Brooks, Heflinger and Wuerker[7] have demonstrated several potential applications. This communication reports the successful achievement of multiple-beam as well as two-beam interferometry from the surfaces of standard engineering components. It would seem that many problems involving dilatation of a structure may be investigated in this way.

The principle of the method consists of recording the hologram of an object using coherent laser light, and afterwards replacing the processed hologram plate in its original position in the same apparatus. One then sees the reconstructed image of the object superimposed on the object itself. Any slight change in the surface contours produced, for example, by mechanical strain or thermal expansion will give rise to interference effects between corresponding points on the object and its holographic image, and fringes will be seen to cross the surface.

Fig. 1 shows the experimental arrangement used to produce holographic recording of a piece of steel channel-section girder, and to demonstrate interference effects on distorting it. Coherent light from a helium–neon gas laser is directed through a microscope objective, *O*, which diverges the beam through a suitable angle so that the object is illuminated obliquely. The photographic plate, *H*, now receives an undisturbed spherical wave, together with light scattered from the rough surface of the girder, *G*. The resultant hologram contains sufficient information to reconstruct a three-dimensional image of the girder in exactly the same position as the original.

Fig. 2 shows the appearance, when viewed through the replaced hologram, of the girder after it has been clamped across its webs. Two-beam interference fringes are formed over the surface, their contrast being governed by the relative brightness of the object and reconstructed image, and their number and position on the degree of distortion. The angles of illumination and viewing must also be taken into account for a quantitative assessment.

For a permanent fringe record it is unnecessary to form 'live' fringes between the object and its recorded image. Instead, a double hologram may be made by exposing the photographic plate twice, once with the girder unclamped, and a second time with it clamped. The fringes are now 'frozen' into the photographic recording, and the object, together with the fringes covering it, may be reconstructed without special re-positioning in the apparatus. In fact, it is no longer necessary to use highly coherent light for the purpose, a divergent beam of 'monochromatic' light, for example, from a filtered mercury lamp being sufficient.

An extension of the method of 'frozen' fringes makes it possible to observe multiple-beam interference. Here a multiple-exposure hologram is made with equal increments of strain between exposures. Fig. 3 shows the reconstruction from a five-exposure hologram of the girder. In this experiment the strain was obtained by welding vertical steel bars to the sides of the girder and connecting their upper ends together with a long threaded bolt equipped with a wing-nut and self-aligning washers. Equal turns of the nut then resulted in equal increments of strain in all parts of the structure. The fringe pattern obtained is now a sharpened version of the two-beam interference pattern which would have resulted from a single-strain increment. Three subsidiary interference

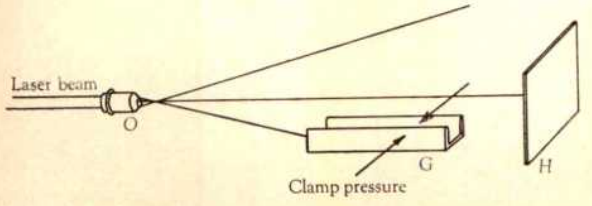


Fig. 1. Experimental arrangement for producing hologram of channel-section girder

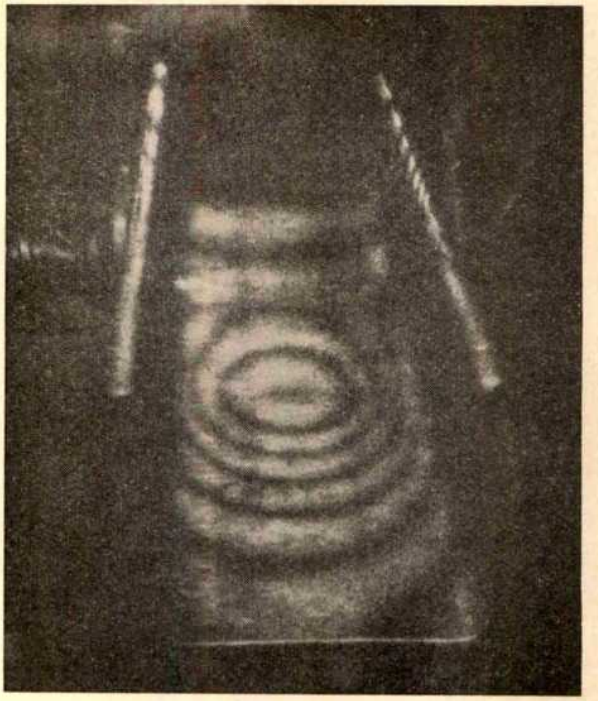


Fig. 2. Two-beam interference pattern on girder due to deformation

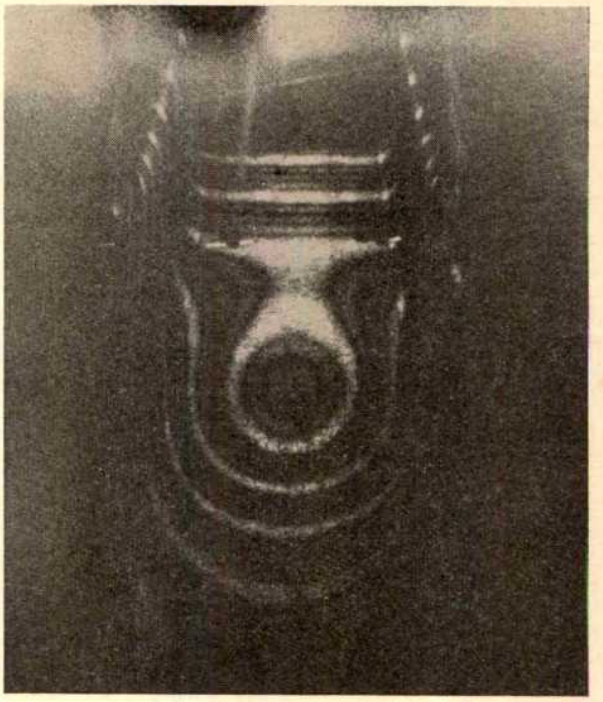


Fig. 3. Five-beam interference fringes from multiple-exposure hologram

maxima are also present between each sharp fringe. With further exposures the main fringes sharpen still further and the subsidiary maxima become weaker. It is also possible to suppress these maxima by an apodization procedure involving only the duration of each exposure.

Sharp multiple-beam effects are obtained only when each individual point on the surface of the object is displaced by equal amounts between exposures. To produce Fig. 3 it was assumed that the structure behaved perfectly elastically, which was borne out in the result. However, if due to overstress, for example, Hooke's law had not been obeyed, the fringes would no longer have been sharp. It is therefore possible that this method could be used to detect weak spots in an engineering component.

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CRYSTALLOGRAPHY

Molecular Dimensions and Crystal Structure of Dimethyl Sulphoxide at −60° C

Dimethyl sulphoxide (DMSO) has been much discussed and investigated in recent years because of its remarkable properties in medicine and as a solvent. We have now determined its molecular dimensions and crystal structure, at −60° C, from an analysis of three-dimensional X-ray diffraction data. The sample, being a liquid at room temperature, was enclosed and sealed inside a Lindemann glass capillary of 0·3 mm diameter. It was crystallized, *in situ*, inside a low-temperature Weissenberg camera[1] by slow freezing, melting and refreezing with a jet of cold gas obtained by boiling liquid oxygen[2]. The to-and-fro horizontal movement of the crystal goniometer shaft, involved in this process, was made sufficiently slow and mechanical by coupling the shaft to the spindle of the camera motor by suitable reduction gears. Crossed polaroids mounted on the camera base were used to ensure the quality of the crystal, before single-crystal X-ray pictures were taken. Using copper $K\alpha$ radiation, rotation and hkL (L = 0,1,2,3) equi-inclination intensity data (on multiple films) were collected.

DMSO crystallizes in the monoclinic system, the cell dimensions, at −60 ± 5° C, being a, 11·496 Å, b, 6·742 Å, c, 5·234 Å and β, 95·37° (measured from $hk1$ and $hk2$ photographs by the offset method of Buerger[3]). Absent spectra: $h0l$ when h is odd, $0k0$ when k is odd. The space group is therefore $P2_1/a$. The calculated density, at −60° C, for four molecules in the unit cell is 1·285 g/c.c. and compares well with the liquid density of 1·101 g/c.c. at 20° C.

The x, y co-ordinates were first determined from an M_4 minimum map[4] derived from a sulphur-sharpened Patterson function. They were later obtained independently, together with the z co-ordinates, from a three-dimensional Patterson synthesis. Refinement of the structure was carried out first by $hk0$ difference Fourier maps and then by eight cycles of least squares, with thermal anisotropy

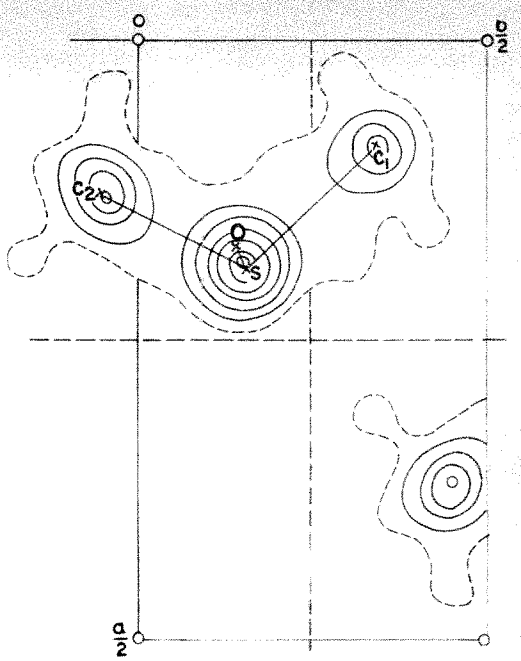


Fig. 1

taken into account in the last four cycles. The final R-factor for all the observed hkL (L = 0,1,2,3) reflexions is 0·129 (R_{hk_0} = 0·104). The final atomic co-ordinates (in fractions of cell edges) with their estimated standard deviations (inside brackets), obtained from Cruickshank's formula, are listed in Table 1.

Table 1

Atom (referred to in Fig. 1)	x	y	z
S	0·1912 (0·0002)	0·1539 (0·0006)	0·6869 (0·0010)
O	0·1711 (0·0006)	0·1365 (0·0014)	0·4060 (0·0024)
C_1	0·0885 (0·0012)	0·3362 (0·0026)	0·7855 (0·0042)
C_2	0·1280 (0·0012)	− 0·0615 (0·0020)	0·8230 (0·0042)

The DMSO molecule has a pyramidal shape with the following dimensions (standard deviations are given inside brackets): S-O = 1·471 Å (0·0080), S-C_1 = 1·812 Å (0·0140), S-C_2 = 1·801 Å (0·0096), C_1-S-O = 107·04° (0·57°), C_2-S-O = 107·43° (0·56°) and C_1-S-C_2 = 97·86° (0·54°). In the case of DMSO vapour, it is found[5] that two molecular models fit the electron diffraction pattern equally well. The present dimensions obtained from the crystal are very close to the averages[5] of the dimensions of these two models. The S-C single bond length in DMSO (1·81 Å) is significantly longer than that found in diphenyl sulphoxide[6] (1·77 Å). This difference is only to be expected as the carbon atom in the latter compound is in the sp^2 state unlike the sp^3 carbon in DMSO.

In the crystal structure the molecules are very closely packed with all the molecular approaches corresponding to those expected for van der Waals interactions. The nearest intermolecular C-C contact is between the centrosymmetrically related C_2 atoms (3·72 Å). Analysis of thermal vibration amplitudes of atoms as well as the location of hydrogen atoms is being worked out.

All the crystallographic computations were carried out on the National Elliott 803*B* computer at H.A.L., Bangalore, using programmes written earlier by one of us (K. K. K.).

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CHEMISTRY

Electron Spin Resonance Technique for investigating Reactions of Free Radicals with Compounds of Biological Interest: Nucleic Acid Constituents

Earlier, Ingalls and Wall[1,2] reported the use of electron-spin resonance spectroscopy to investigate the reactions of hydrogen and other gaseous free radicals with polymers in a finely divided fluff state. Such fluffs are highly accessible to gaseous free radicals, and react readily to produce polymer radicals which are stabilized by their immobility and separation from one another. Consequently, during exposure to a stream of gaseous atoms, easily observable numbers of polymer radicals rapidly accumulate. A limitation of this technique is that only high-molecular weight materials can be prepared as fluffs. This communication describes a general method of radical stabilization which permits investigation of materials of low molecular weight, and discusses spectra illustrating their application.

The essential feature of the method is the use of quartz wool as a supporting medium. A solution of the material to be investigated is added to a tube containing quartz wool, quickly frozen, and freeze-dried. During the drying process the solute is trapped between the quartz strands to produce a highly dispersed sample. This sample is placed in the electron spin resonance cavity and a gas stream containing atoms is passed over it. Investigations of radical reactions can then be carried out just as with fluffs. The quartz wool has negligible effect on the reactions or the electron spin resonance spectra of the radicals: polyuridylic acid preparations exposed to hydrogen atoms as self-supported fluffs or supported on quartz wool give rise to comparable spectra.

This technique has been used to examine the reactions of hydrogen and deuterium atoms with several constituents of nucleic acids at room temperature[3]. These reactions are of interest because radicals formed by the action of ionizing radiation on thymidine[4,5], thymine[5,6], and DNA[6] all seem to have a hydrogen atom added to the C_6 position of the pyrimidine ring of the thymine part of the molecule. If this interpretation is correct, the same electron spin resonance signal might be expected when hydrogen atoms produced by an electric discharge in hydrogen gas react with these substances.

Such is found to be the case with thymine[7]. The electron spin resonance spectrum of the thymine–hydrogen atom reaction product consists of eight lines (Fig. 1*a*) and is similar to that obtained on γ-irradiation of crystalline thymine[8]. The spectrum of the deuterium atom product (Fig. 1*b*) differs in a way which is consistent with addition of an atom to the molecule. A more complex result is obtained with thymidine[7]. When it is first exposed to hydrogen atoms, the spectrum is similar to that of the thymine product, but with a small singlet component. On continued exposure, however, the spectrum becomes

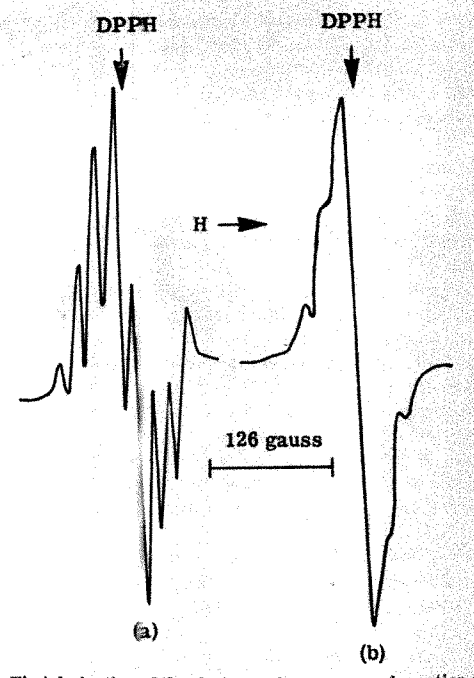


Fig. 1. First derivative of the electron spin resonance absorption spectra of thymine after reaction with (*a*) hydrogen atoms; (*b*) deuterium atoms

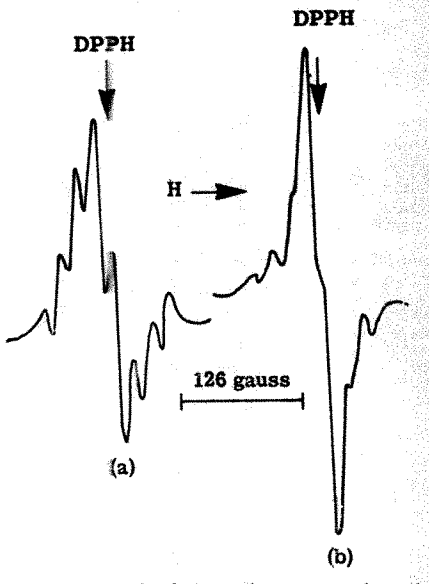


Fig. 2. First derivative of the electron spin resonance absorption spectra of thymidine after reaction with hydrogen atoms. (*a*) Early spectrum; (*b*) late spectrum

similar to the singlet obtained on exposure of deoxyribose to hydrogen atoms, but with a small thymine component (Fig. 2).

Parallel experiments have been done with the uracil family of pyrimidine compounds. Exposure of uracil to hydrogen atoms gives a product with a spectrum similar to that of the thymine product except that the two end lines are absent. Uridine and polyuridylic acid, both of which have a ribose component, behave in the same manner as thymidine. This agrees with the finding that hydrogen atom reactions with ribose and deoxyribose give products with similar spectra. The same late spectra are produced by deuterium atom exposure, suggesting that abstraction from the sugar with the formation of the same radical is occurring.

The details of the sequential change in the spectra of the sugar-containing compounds are not completely understood. It is similar, however, to changes observed during

excited-rare-gas bombardment of polystyrene–polymethyl methacrylate mixtures and co-polymers. In these, the polystyrene product spectrum appears first, but on continued reaction the polymethyl methacrylate product spectrum dominates[9].

The radical build-up in all systems investigated reaches a steady state during the first few minutes of exposure. After exposure the signal intensity remains essentially constant for an indefinite period in a vacuum or an inert atmosphere. However, on admission of dry air it decreases immediately to one-half to one-third the steady-state value, usually without a change in spectral shape; and in ordinary moist room air it is, in most cases, immediately destroyed.

Our present research is directed toward continuing investigations of nucleic acids and their components. This technique is by no means limited to these compounds, however. It should be possible to use it with almost any compound, organic or inorganic, which has a sufficiently low vapour pressure to remain in the cavity during the experiment. With simple modification, such as by using an adsorbing material as a support, it may also be possible to extend the method to include volatile substances as well.

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Graphite–Carbide Materials prepared by Hot-working with the Carbide Phase in the Liquid State

Although processes of hot-working are attractive as a means of reducing the porosity and permeability of graphite, the results of measurements of some of the other physical properties are disappointing[1,2]. For example, bodies hot-worked in compression show some increase in compressive strength parallel to the direction of hot-working, but this gain is offset by a decrease in strength in the perpendicular direction. Although appreciable reductions in the porosity and permeability have been attained, attempts to reduce these properties to negligible values by hot-working to high strain-levels have not been successful.

The work described here is concerned with a new process wherein graphite bodies containing dispersed carbide particles are hot-worked at temperatures such that the carbide phase is in the liquid state. Two carbides have been used, zirconium carbide and molybdenum carbide, with carbide-graphite eutectic temperatures at 2,850° (ref. 3) and 2,584° C (ref. 4) respectively.

The carbide-bearing bodies were prepared by hot-pressing (at approximately 800° C) a dispersion of fine carbide particles in a graphite matrix composed of 80 per cent graphitized petroleum coke and 20 per cent pitch binder; the nominal carbide content was 10 vol. per cent. Using a hot-working device, which is described in ref. 2, these bodies were deformed in compression to strain-levels as high as 60 per cent true strain.

Photomicrographs of graphite bodies containing molybdenum carbide particles are shown in Fig. 1. After hot-working, the graphite matrix is very dense, and the carbide particles are uniformly dispersed with a particle size generally less than 5μ. The carbide content decreases with increasing hot-working strain, the excess liquid carbide appearing as droplets on the surface of the specimen.

Some results for the compressive strengths are given in Fig. 2. These tests were made on cylinders 0·25 in. in diameter by 0·375 in. in height cut with the cylinder axis either parallel or perpendicular to the direction of hot-working. Hot-working of the carbide-bearing bodies results in large increases in strength parallel to the direction of hot-working and modest increases in the perpendicular direction. These effects are in sharp contrast to the results for a similar graphite matrix hot-worked without the carbide additives.

The effective strengthening of the graphite caused by the presence of the liquid carbide phase during hot-working is also shown by the measurements of flexural strength shown in Fig. 3. It should be pointed out that this strengthening is not found when the carbide-graphite body is hot-worked at temperatures such that the carbide phase is solid[2]. The strengthening mechanism appears to be more complex than a simple reinforcement of the

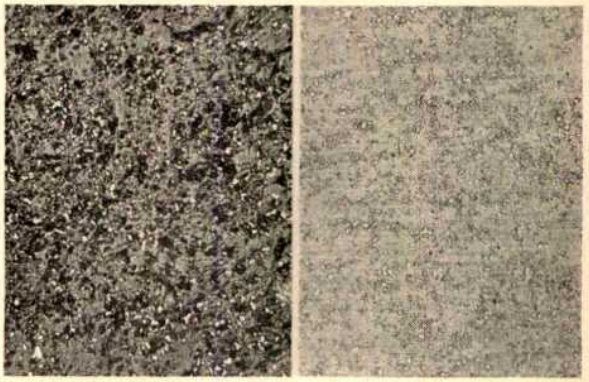


Fig. 1. Microstructures of graphite bodies containing molybdenum carbide particles; left, after annealing at 2,700° C; right, after hot-working to 56 per cent strain at 2,700° C. (× *c.* 120)

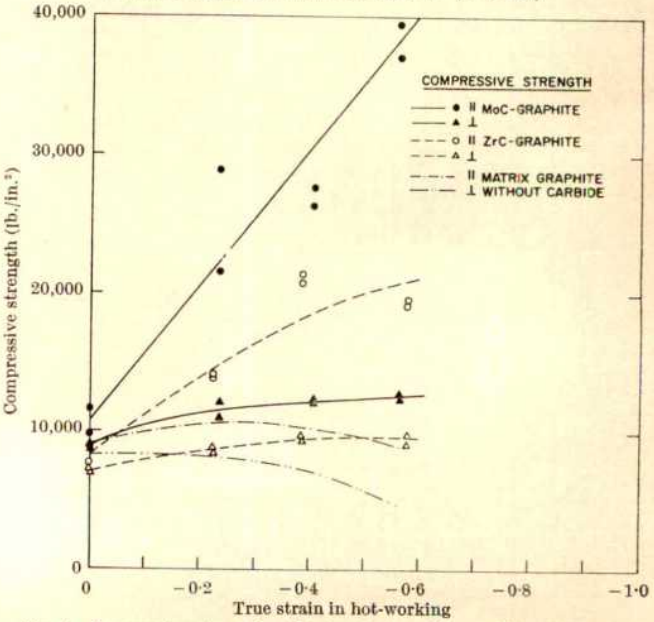


Fig. 2. Compressive strengths of hot-worked matrix graphite with and without a dispersion of carbide particles

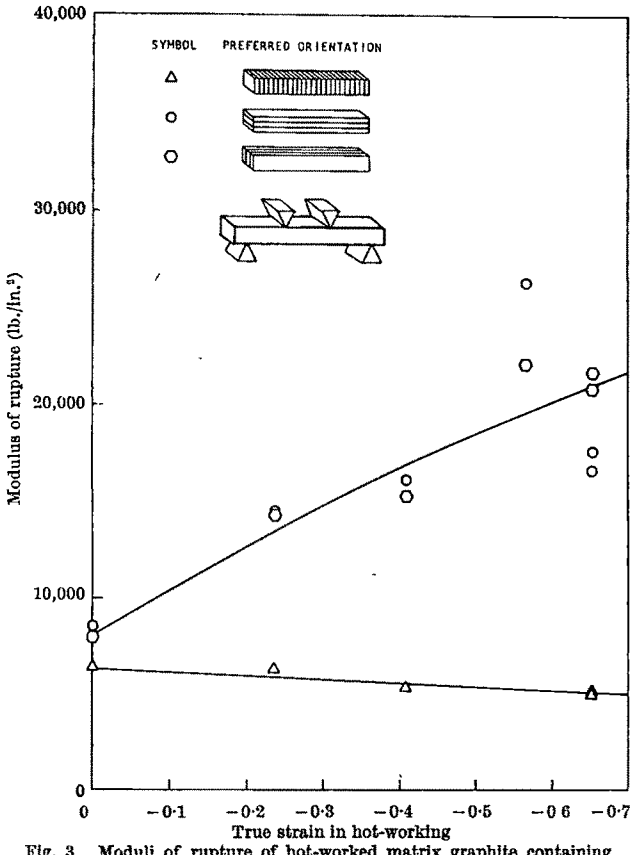


Fig. 3. Moduli of rupture of hot-worked matrix graphite containing molybdenum carbide particles

graphite matrix because the higher strengths are observed for specimens with decreasing amounts of the carbide phase.

Preliminary measurements of the helium permeability of the carbide-bearing bodies hot-worked above the carbon–graphite eutectic temperature indicate that the liquid carbide is effective in blocking pores. The permeability of the starting material is of the order of 2×10^{-4} cm²/sec; when hot-worked, even to modest strain-levels, the permeability is decreased to less than 10^{-6} cm²/sec.

Hot-working with a dispersed liquid phase appears to be a logical development of the concept of liquid-phase sintering[5]. The compressive deformation acts to drive the liquid phase into intimate contact with the porous matrix as well as to reduce the diffusion distances for the sintering process. In contrast to hot-pressing with a conventional piston and cylinder device, the hot-working process affords an opportunity for the excess liquid to escape through a free surface so that the resulting graphitic materials contain only that amount of carbide necessary to plug up the pore structure.

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Anharmonic Oscillations in Electrical Potential at Probe Electrodes in Membrane Transport Cells

Forgacs[1] has described experiments which led to the conclusion that steady-state flow may not occur during ion transport through ion-selective membranes. His observations may be summarized as follows. Constant direct current (from rectified power supply or accumulators) was passed through the cell:

$$Ag(s)/AgNO_3\ (0{\cdot}01\ M)/M/AgNO_3\ (0{\cdot}01\ M)/Ag(s)$$

where M is a homogeneous cation or anion exchange membrane. The solutions were stirred at 1,100 r.p.m. Saturated calomel probe electrodes connected through 0·01 M potassium chloride/0·01 M potassium nitrate salt bridges to an oscilloscope were used to measure potential differences across the membrane. It was found that: (1) the observed potential underwent anharmonic oscillation (frequency not stated) about a constant average value; (2) the peak-to-peak amplitude of the oscillations increased with increasing current density; (3) similar oscillations of smaller amplitude were observed when the membrane was removed; (4) similar oscillations of much greater amplitude were observed when the solutions were replaced with 0·01 M silver nitrate in an agar gel.

Observations (1), (2) and (3) have been confirmed in this laboratory, using homogeneous cation exchange membranes between 0·01 M potassium chloride solutions and reversible Ag/AgCl electrodes. It has not been possible to confirm observation (4), however, nor has it been possible to accept the conclusion concerning the absence of steady-state flow conditions. The correct explanation of the observed effects is important, since the use of probe electrodes in membrane experiments is widespread.

Fig. 1 is a sketch of the top half of cell 1. The bottom half was a mirror image of the top, and the cell was operated with the membrane surfaces horizontal. The probe electrodes were chloridized silver wires, and were located in planes distant 2 mm from the membrane surfaces. Cell 2 was simply a 10-mm glass tube provided with four joints which carried current electrodes and probe electrodes (saturated calomel connected through 0·01 M potassium chloride salt bridges). Open tubes opposite the probes permitted the introduction of special stirrers.

In cell 1 it was found that rotation of the drive magnets without the stirrers produced no oscillations in potential at the probes. No oscillations could be detected across a resistor in series with the current electrodes, even when the stirrers were in operation. The frequency of the oscillations was twice the frequency of rotation of one

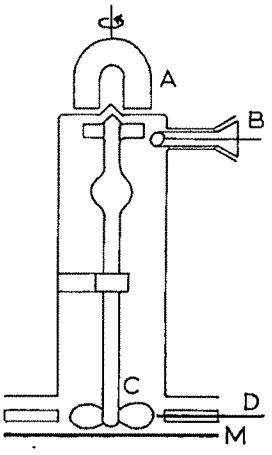


Fig. 1. Membrane transport cell. *A*, Drive magnet; *B*, current electrode; *C*, two-bladed glass stirrer with enclosed magnet; *D*, probe electrode mounted on 'Plexiglass' flange; *M*, membrane

stirrer (180–275 r.p.m.). If two stirrers were used, there were four oscillation peaks per stirrer rotation, unless the stirrer blades were adjusted to pass the respective probe electrodes at the same time. The peak-to-peak amplitude of the oscillations was proportional to current density, and had the value 6·1 ± 0·4 mV/m.amp/cm² of membrane surface between current densities of 0·2–3·0 m.amp/cm². These effects are due, evidently, to an interaction between stirrers and probe electrodes.

Two hypotheses concerning this interaction were tested. The first assumed that there was a large enough potential drop across the probes to establish local concentration gradients. These gradients could then be perturbed by the stirrers, and a perturbation in potential would result. Experiments were conducted (cell 1) in which all but the flattened ends of the Ag/AgCl probes were insulated, and the probes were bent so that their end faces were perpendicular to the direction of current flow. Oscillating potentials of the same magnitude were found, and the hypothesis was therefore rejected.

The second hypothesis was that the stirrer blades distorted the potential distribution near the probe electrodes. A number of experiments supported this hypothesis. (1) When the probes were lengthened and bent so that the uninsulated tips were at least 2 cm from the stirrer paddles, the amplitude of the oscillations was reduced to zero. (2) When the probes were bent so that their tips were within 1 mm of the membrane surfaces, the amplitude was about one-fifteenth of its value with the probes near the stirrer blades. (3) When a stirrer blade was close to a probe and stationary, an extra, constant potential arose between the probes, and disappeared when the blade was moved away. (4) Both insulating and conducting stirrer blades caused fluctuations in probe potential (cell 2).

More direct support for this hypothesis was obtained from equipotential maps constructed to be two-dimensional electrical analogues of the cell. These maps were made on 'Teledeltos' electrically conducting paper, used with copper strip electrodes and a Servomex type *FP*92 field plotter. The maps for two different orientations of insulated stirrer blades are shown in Fig. 2. The change in potential distribution as the stirrer goes from the 'horizontal' position to the 'perpendicular' position is evident, and is sufficient to cause a change in potential at the probe. Similar distortions of the field were obtained using conducting stirrer blades in the maps.

The amplitude of oscillation will be greater with a membrane present simply because of the higher resistance of the membrane and the greater potential drop across it than across an equal thickness of solution. The percentage distortion of the field by the stirrers is constant. Hence, the total amplitude should be proportional to current density. Although this is the case in the experiments reported here, it is only roughly true in Forgacs's experiments. Forgacs used current densities up to 30 m.amp/cm²,

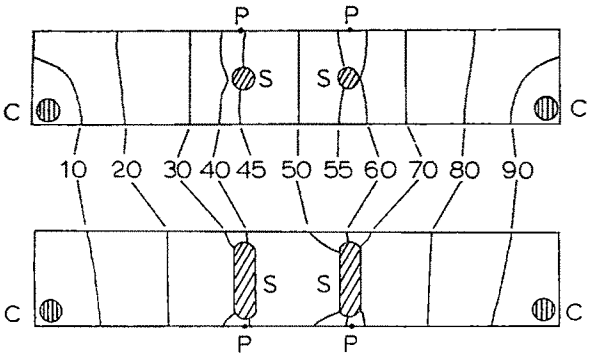


Fig. 2. Two-dimensional potential distributions. *C*, Copper electrodes; *P*, probe positions; *S*, 'stirrers' (holes in paper). Stirrer in 'horizontal' position (below), 'perpendicular' position (above). Numbers are percentage of applied voltage

and it is possible that additional effects due to concentration gradients near the membrane surfaces were encountered by him.

Small, irregular oscillations were observed in cell 2 when it was filled with agar gel, and the current electrodes disintegrated rapidly. For this reason, no relevant conclusions can be drawn from such experiments.

The foregoing experiments demonstrate clearly that unless careful attention is given to the design of stirred membrane transport cells, probe electrode measurements can be misleading. While it is possible to produce small oscillations in potential at the membrane surface, this effect is due to the stirrers, and not to any departure from steady-state conditions inherent in the membrane.

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It appears to me that the explanation given by Prof. Lorimer and Brydges is a reasonable interpretation of the results which they obtained with their experimental system. They show that a system incorporating ion-exchange membranes may be subject to oscillations for reasons not associated with the membrane itself. Their reasoning, however, cannot be related to general systems of this type, and certainly not the experimental set-up which I used in my experiments, the results of which were reported in my communication[1].

The differences between the two experimental systems are as follows: (1) The stirrers in my experiments were placed behind the top of the probe electrodes and consequently outside of the potential field between the electrodes and the membrane. In Lorimer and Brydges's experiments the stirrers were placed in such a position that the rotation of the blades was able to affect the potential field between probe electrodes and membrane. (2) Lorimer and Brydges found a correlation between the frequency of potential oscillations and the frequency of the stirrer(s) rotation. Such a correlation would be expected from the experimental arrangement. In my experiment, the oscillations were anharmonic, with randomly changing frequency, and did not appear to be influenced by the condition and rate of stirring. (3) Lorimer and Brydges found a direct proportionality between amplitude of oscillations and current density within the range stated, whereas the results reported by me show no such proportionality at this range. The oscillations become significant only at current densities higher than those which they used.

The fact that the electrical supply system is not described by Lorimer and Brydges makes it difficult for me to evaluate fully their experimental results.

It is regrettable that in the short note I submitted, I did not include some of the details of the experimental procedure given here; however, this was in the nature of a preliminary communication of work which was and is being continued. I did not state or imply that steady-state conditions do not exist in all circumstances, but that they may not necessarily be present. Any theory of ion transport through membranes which assumes *a priori* steady-state conditions may over-simplify the problem.

This work was, and continues to be, part of an effort to elucidate the phenomena of permselective membrane polarization which are not fully explained by existing membrane models. This is being done by investigating unusual aspects of membrane behaviour, finding an explanation for such behaviour and thereby increasing the range of applicability of the model. Several publica-

tions during the previous few years have dealt with such phenomena[2–5]. It was thought, at the time that my first note was published, that the oscillating effect might be a good starting-point for further development. Unfortunately, these effects have presented so complex a picture that I have not yet derived a conclusion worthy of publication, except for an additional short note[6] on the influence of membrane/solution boundary conditions on such oscillations.

Nevertheless, I should like to use this occasion to present a number of generalizations which have been based on observations made during the past few years. (1) The described anharmonic oscillations need not necessarily occur with all types of membranes under all conditions. (2) Generally, such oscillations increase more than linearly with current density. (3) They generally increase in amplitude with time of direct current transfer through the membrane, or may appear after some time of current passage. No case has been observed where the oscillations disappear during current transfer.

I hope that with the continuation of the work, results will be obtained which will provide a wider understanding of the problem, in which event they will, of course, be submitted for publication.

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Electronic Structure of the Pt_6Cl_{12} Molecule

IT has recently been found that in addition to the crystal form which consists of ribbons of $PtCl_2$ units, each chlorine being shared by two platinum atoms[1], $PtCl_2$ crystallizes in a form[2] containing Pt_6Cl_{12} molecules which are isostructural with the $Ta_6Cl_{12}^{2+}$ cation[3]. However, these isostructural species are not isoelectronic, the cation effectively having forty valence electrons[4] and the platinum compound seventy-two. In both cases each chloride anion is assumed to contribute two 'σ' electrons only; the four 'π' electrons may, in general, be neglected and assumed non-bonding[5]. The bonding within the $Ta_6Cl_{12}^{2+}$ cation has recently been described in terms of a topological-equivalent-orbital approach[4] and it is the purpose of this communication to extend the treatment to the isostructural platinum compound.

When three precisely equivalent orbitals interact they do so to form a single and a degenerate pair of molecular orbitals. In the present example there are eight sets of three orbitals of this type, one set for each of the faces of the octahedron[4]. These orbitals are a_1b_2e hybrids located on each platinum atom (here we classify the hybrid orbitals by the symmetry species which they span in the C_{4v} local symmetry at each platinum atom; in this way we give more flexibility to the mixing coefficients). When the symmetric combination of a set of these orbitals is the only one occupied, then a three-centred 'face' bond is formed which contributes to the molecular stability. Such bonds are found in the $Ta_6Cl_{12}^{2+}$ cation. If more electrons are fed into each set of three orbitals, then a diamagnetic molecule will only result if four electrons are added. Further, occupation of these three orbitals by six electrons is essentially equivalent to having three non-bonding lone pairs of electrons. This is the situation which exists in Pt_6Cl_{12}, the thirty-two electrons added to the forty in the $Ta_6Cl_{12}^{2+}$ structure occupying anti-bonding orbitals to give a molecule held together by the chlorine bridges alone. This conclusion finds support in the Pt–Pt distance of about 3·4 Å (compare Ta–Ta in $Ta_6Cl_{12}^{2+}$ which is about 2·9 Å).

Clearly, there is little to choose between the two crystal forms of $(PtCl_2)_n$ from the point of view of energy. In both, each platinum is surrounded by four chlorine atoms at the corners of a square and is bonded to neighbouring platinum atoms by them, all other platinum electrons being effectively non-bonding.

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Tritium Enrichment by Gas Chromatography

GAS–SOLID chromatography has proved to be a promising method for enrichment of tritium in hydrogen gas samples[1]. Previous work has shown that tritium can be enriched with a concentration factor of about 20 in hydrogen samples of up to 300 ml. and with a tritium loss of up to 5 per cent in a relatively small system. Many problems involving low-level tritium determination, however, require much larger samples of hydrogen (or its equivalent as water) to obtain sufficient tritium for detection and it was, therefore, of interest to see how far the gas chromatographic system could be extended in this direction.

An important primary consideration is the size of the 'low background' counter ultimately intended for the determination of tritium. Two types have been considered here, one of low volume (approximately 40 ml.)[2], which implies the necessity of high concentration factors, and the other of relatively large volume (1·5–2·0 l.)[3] where a lower factor can be tolerated.

In the former case, an apparatus with a three-column system was used[1]. The first column was made of copper tubing with an inner diameter of 10 mm wound in a spiral and with an active length of 2 or 4 m. The second, also of copper, had an inner diameter of 5 mm and an active length of 160 cm, while the third or 'retention' column was a glass capillary of 1·2 mm inner diameter with an active length of 35 or 70 cm. In all cases, activated Linde molecular sieve 5Å was used with a grain size of 0·5–0·8 mm for the first column and 0·3–0·5 mm for the other two. As before[1], the elution-displacement technique was used with helium as the eluting gas and the columns cooled in liquid nitrogen. The results of experiments using various combinations of column lengths are shown in Table 1.

Table 1. TRITIUM RECOVERY FOR VARIOUS THREE-COLUMN SYSTEMS

Experiment	First column (m)	Second column (cm)	Third column (cm)	Sample volume (ml.)	Hydrogen fraction activity (per cent)	Tritium Volume (ml.)	fraction Activity (per cent)	Enrichment factor
1	2	160	35	1,290	1·2	12	98·8	106
2	2	160	70	1,530	0·3	25	99·7	61
				2 000	2 8		97·2	78
3	4	160	35	1,810	1·2	12	98·8	149
				2,120	3·7		96·3	170
4	4	160	70	3,060	3·8	25	96·2	118

It can be seen that 3 l. of hydrogen can be handled with an acceptable tritium loss. The final volume of tritium-hydrogen fraction is defined by the capacity of the third column and was 12 and 25 ml. for the 35 cm and 70 cm columns respectively. Some increase in the size of this third column would probably make it possible to deal with samples of more than 3 l. Any further increase in the size of the first column introduces the technical difficulty of satisfactorily winding copper spiral from large-diameter tubing.

Large volume counters (1·5–2·0 l. capacity) with an acceptable low background have been described and are produced commercially. In this case, a two-column system has proved to be satisfactory. The first column (of glass)

consists of three sections in line—the first two are 50 mm inner diameter and 28 cm long and the third 20 mm inner diameter and 30 cm long. These are connected by short lengths of tubing of 8 mm inner diameter and filled with molecular sieve material with a grain size of 0·5–0·8 mm. The second, or 'retention', column was made of a spiral of copper tubing (5 mm inner diameter) with an active length of 160 cm and filled with molecular sieve material of 0·3–0·5 mm grain size, corresponding to a hydrogen capacity of 900 ml. A specially long Dewar vessel was constructed for cooling the first column and this was lowered automatically over a period of about 100 min. Using a 10-l. sample of hydrogen (corresponding to 8 g of water) a final hydrogen sample of 900 ml. was obtained containing 98 per cent of the tritium. The whole process, including reduction of 8 g of water with magnesium at 600° C, took about 3 h.

By increasing the length of the retention column to 4 m it was possible to process 20 l. of hydrogen, which yielded a final fraction of 2 l. containing 96 per cent of the tritium. In all experiments, a concentration of approximately 0·015 μc. tritium/l. of hydrogen was used.

I wish to thank Dr. G. B. Cook, head of this Laboratory, for many helpful discussions and Dr. M. B. A. Crespi for his advice.

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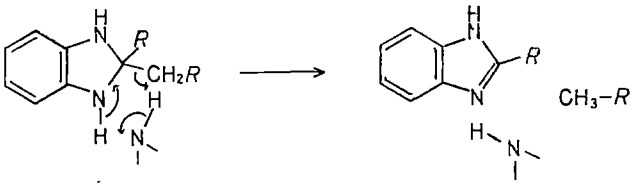
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Mechanism of the o-Phenylenediamine Cleavage of Ketones

The thermal reactions of *o*-phenylenediamine with carbonyl compounds have been investigated for a number of years[1–3]. Recently, Elderfield[4–6] has extensively investigated the cleavage of ketones with this reagent, and has found that the reaction affords 2-alkylbenzimidazoles and alkanes in high yields. Investigations of the scope of the degradation have indicated that the more substituted group is eliminated as the alkane[7–9]. The mechanism of this elimination is not, however, known at the present time.

Initial experiments in this laboratory have indicated that benzyl ketones are more readily cleaved than aliphatic or aromatic ketones. This fact has led us to suggest that the initial intermediate, 2-benzyl-2-alkylbenzimidazoline, either rearranges to 1-benzyl-2-alkylbenzimidazoline which then decomposes to the alkane and benzimidazole or degrades to the final products by means of a bimolecular elimination. In an investigation of the former hypothesis, the second intermediate, 1-benzyl-2-phenylbenzimidazoline, was prepared by reacting N-benzyl-*o*-phenylenediamine with benzaldehyde; this compound was then pyrolysed in the usual manner: the benzimidazoline (2·86 g, 0·01 M) was heated in a 'Pyrex' distilling tube. The temperature of the oil bath was raised slowly to 180° C and this temperature was maintained for 12 h. During this time, toluene did not distil from the reaction mixture. After the pyrolysate was allowed to cool, it was washed with methyl alcohol; this treatment gave 1-benzyl-2-phenylbenzimidazole (2·04 g, 72 per cent). It is evident, therefore, that 1-benzyl-2-phenylbenzimidazoline is not an intermediate in the *o*-phenyldiamine reaction.

The latter hypothesis, namely, the bimolecular elimination pathway, was also investigated. This mechanism was suggested because recent investigations[10,11] on the pyrolytic decompositions of esters have indicated a bimolecular pathway, and Elderfield *et al.* have been able to catalyse the cleavage reactions with both acids and bases[6,9]. The latter result has led us to investigate amines and benzimidazoles as possible catalysts in these reactions. These investigations have shown that the decomposition temperature (initial gas evolution) of 2-benzyl-2-phenyl benzimidazoline can be lowered from 235° C to 200° C by adding small amounts of diphenylamine, benzimidazole, 2-phenylbenzimidazole or 2-methylbenzimidazole to the pyrolysis mixture. A typical experiment is as follows: benzyl phenyl ketone (1·06 g, 0·01 M), and 2-phenylbenzimidazole (0·194 g, 0·001 M) were heated in a 'Pyrex' distilling tube. The temperature of the bath was slowly raised to 195–205° C; at this temperature gas evolution was evident. When the reaction was run in the absence of the benzimidazole, the decomposition temperature was found to be 230–240° C. Based on the catalytic activity of these compounds, the mechanism of this reaction may involve a bimolecular elimination that can be formulated in this manner:



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BIOCHEMISTRY

Operon Hypothesis: New Evidence from the 'Constant Proportion' Group of the Embden–Meyerhof Pathway

A number of examples have now been reported in which a group of enzymes is synthesized in such a manner that the relative activity of each of the enzymes within the group is constant. An important example of this is the 'constant-proportion' group in the Embden–Meyerhof pathway, which has been extensively investigated by Vogell *et al.*[1] and by Pette *et al.*[2]. These workers examined a wide range of materials, extending from baker's yeast to various mammalian tissues, and found that the activity of the five enzymes, triosephosphate isomerase to enolase, formed a pattern which is quite reproducible. They suggest that this indicates a "co-ordinate control of enzyme synthesis" by the cell at the molecular level, a subject which has been the focal point of much recent attention.

A most significant observation, however, which appears entirely to have escaped notice is that the actual pattern reported by Vogell *et al.* in fact represents equimolar amounts of these five enzymes. In Table 1, column 4 shows the calculated 'activity pattern' of equimolar ratios of the enzymes, and column 5 the findings of Pette *et al.* The figures in both columns are expressed as a ratio to the activity of glyceraldehyde phosphate dehydrogenase. Despite the large cumulative errors which may appear in calculations involving several independent measure-

Table 1. ACTIVITY PATTERN OF THE CONSTANT-PROPORTION GROUP IN THE EMBDEN–MEYERHOF PATHWAY: A COMPARISON OF THE CALCULATED VALUES WITH THE MEASUREMENTS OF PETTE *et al.*

Enzyme	Molecular weight	Specific activity (μmoles substrate/h/mg protein)	Calculated activity pattern	Measurements of Pette *et al* (ref. 2)
Triosephosphate isomerase	Approx. 5×10^{4}*	2.95×10^{5} (ref. 3)	Approx. 19	6–12
Glyceraldehyde phosphate dehydrogenase	1.4×10^{5} (refs. 4 and 5)	5.5×10^{3} (ref. 3)	1	1
Phosphoglycerate kinase	3.4×10^{4} (ref. 6)	1.85×10^{4} (ref. 3)	0·82	0·6–1·2
Phosphoglycerate mutase	5.7×10^{4} (ref. 7)	7.8×10^{3} (ref. 7)	0.58	0.6–1.2
Enolase	8.5×10^{4} (ref. 8)	2.72×10^{3} (ref. 3)	0·30	0.15–0·3

* This is an approximate value obtained in these laboratories by the method of Andrews (ref. 9) using gel-filtration on 'Sephadex *G* 200'.

ments (particularly by different workers), there is a remarkably good correlation between the calculated and the experimentally derived values.

We may conclude, therefore, that the cell necessarily produces molecules of each of these five enzymes in precisely equal numbers. This is of particular interest because the Embden–Meyerhof 'constant-proportion' group appears to be an example of a group of proteins the synthesis of which is controlled by a single 'operator' gene. Although a number of such groups have been investigated, particularly in bacteria[10,11], figures for the stoichiometric relationship of the rates of synthesis of the individual proteins within any particular group have not been published. It is therefore possible that this finding is valid for operon groups in general.

Should this be true, then a considerable simplification may be made to the present model of protein synthesis; namely, that one single molecule of 'messenger' RNA is synthesized from the DNA strand per operon rather than a separate molecule of 'messenger' RNA for each structural gene. This RNA molecule would obviously duplicate the 'commas' presumed to exist on the DNA molecule and would thus induce breaks in the polypeptide chain during the actual protein synthesis.

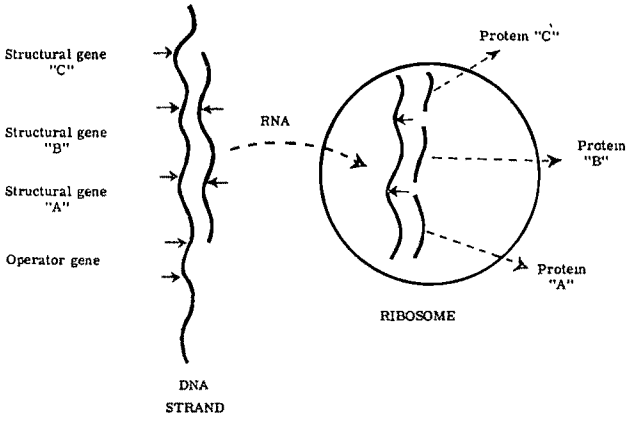


Fig. 1. A modified scheme for protein synthesis, necessitating equimolar production of each protein common to a single operon

A simple and unequivocal explanation thus becomes available for two aspects of the co-ordinate control of enzyme synthesis which are at present matters of conjecture. First, the remarkably constant ratio between the rates of synthesis of proteins within a group of this type becomes self-explanatory; secondly, the operator genes are no longer required to initiate RNA synthesis in structural genes which are geographically separated from them on the chromosome. With the techniques which are at present in use for the study of RNA synthesis, it should not be impossible to distinguish between the presently accepted model and that which is proposed in this communication.

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Colour, Magnetic Resonance and Symmetry of the Cupric Site in Blue Protein

THE blue copper-proteins are characterized by very intense optical absorption near 600 mμ and unusually low cupric hyperfine splitting ($A_{\parallel}$) as observed by electron paramagnetic resonance. The nature of the copper-protein binding and the relationship between the colour and the electron paramagnetic resonance spectrum, while the subject of much investigation and speculation, have remained obscure[1–4]. However, almost all investigations have centred on ceruloplasmin and laccase, which contain eight and four atoms of copper per protein molecule, respectively, with only about half this number in the cupric state. It seemed, therefore, that these two proteins are not best suited for further investigation, particularly since it has been shown that blue proteins which contain only one cupric ion per molecule also have intense optical absorption near 600 mμ and small $A_{\parallel}$ values[5,6]. It was with the foregoing in mind that an investigation of the blue protein from *Pseudomonas aeruginosa* was undertaken. This protein has a broad absorption with a maximum at 625 mμ (molar extinction coefficient of approximately 2,000) and an $A_{\parallel}$ value of 60×10^{-4} cm^{-1}. The protein was isolated according to the method of Horio[7], with some modifications, and investigated in solution at room temperature and in frozen solution at −160° C by optical, electron paramagnetic resonance and optical rotatory dispersion methods.

The electron paramagnetic resonance spectrum of the native protein, *p*H 6·8, in frozen solution is in agreement with the published spectrum[5]. In the *p*H range 3–8 there is no change in the electron paramagnetic resonance spectrum, and there are only small fluctuations in the optical density at 625 mμ. In the *p*H range 8–12, the blue colour decreases in intensity very slowly, the rate of change of optical density increasing as the *p*H is raised. This change is reversible, the optical density being restored on decreasing the *p*H to a neutral value. In proportion to the decrease in optical density, the intensity of the electron paramagnetic resonance absorption decreases without any other change in the features of the spectrum (Fig. 1). Since the line width and shape of the absorption do not change (for *p*H less than 12), the amplitude is a measure of the intensity. The effect of urea at *p*H 6·8 was determined at several concentrations, and the resulting microwave versus optical absorptions are included in Fig. 1. The three points in the case of 7·7 M urea represent the time course of the process.

The severe reduction of electron paramagnetic resonance signal in alkaline solution and high urea suggests that the copper is reduced from cupric to cuprous under these conditions. To test this hypothesis, one drop of 10^{-2} M potassium ferricyanide in buffer at *p*H 9·6 was added to

1 ml. of protein solution at *p*H 9·6 which had lost 63 per cent of its optical density at 625 mμ. The optical density and the electron paramagnetic resonance spectrum were restored immediately to their original values, thus supporting the hypothesis. Potassium ferricyanide restores neither the colour nor the electron paramagnetic resonance spectrum of the protein in 7·7 molar urea, a fact which points to the stabilization of the cuprous state in the denatured protein.

The reduction of copper to the cuprous state is most probably effected by —SH groups, there being no other known reducing agents present. This reaction occurs either because the oxidation of sulphydryl groups is favoured at a high *p*H, or because of an increase in the redox potential of the cupric–cuprous couple. A change in the latter redox potential does not seem likely in alkaline solution (*p*H less than 12) in which the configuration of the protein is apparently not changed; whereas it is very likely on urea denaturation which changes the secondary structure. These considerations are supported by measurements of optical rotatory dispersion.

At neutral *p*H, this blue protein (in contrast to ceruloplasmin[8]) shows a marked Cotton effect in the visible region (Fig. 2). Therefore, the cupric ion is in an asymmetric environment in agreement with the views of Brill *et al.*[4]. As the *p*H is increased, this Cotton effect becomes weaker. It is absent in the protein denatured by 7·7 M urea. Also, in the *p*H range 8–10, the change in the optical rotation in the visible region is not accompanied by any change in the ultra-violet (this is consistent with the idea that the secondary structure of the protein is maintained); however, at 7·7 M urea the optical rotation in the near ultra-violet changes to larger negative values (indicating less helical structure).

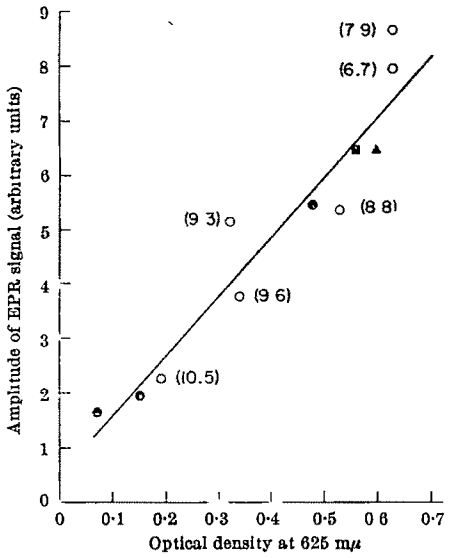


Fig. 1. Amplitude of electron paramagnetic resonance signal (arbitrary units) plotted against the optical density at 625 mμ. ○, No urea (the number next to each point is the *p*H of the solution); ■, 2 55 M urea; ▲, 5·1 M urea; ●, 7·7 M urea. All urea solutions were at *p*H 6·8 and the concentration of protein in all solutions was 3×10^{-4} M

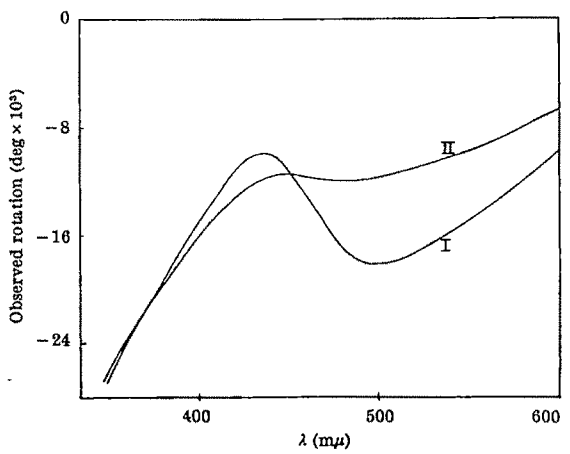


Fig. 2. Optical rotatory dispersion curves. (I) *p*H 7 9; (II) *p*H 9·6. The protein concentration was 3×10^{-4} M in both cases

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Intracellular Distribution of the Different Forms of Lactic Dehydrogenase

There is, at present, considerable interest in the investigation of the properties and metabolic roles of the five molecular forms of lactic dehydrogenase (LDH). A preponderance of forms I and II was found in tissues (for example, heart) which function under relatively aerobic conditions, whereas a preponderance of forms IV and V was observed in relatively anaerobic tissues (muscle, liver)[1,2]. In a conference on lactic acids recently held in New York, however, it was brought out in discussion that the intracellular distribution of the different forms of LDH had not then been investigated[3].

In this communication we report data on the electrophoretic fractionation, on 'Cellogel' strips, stained with MTT tetrazolium, of the five molecular forms of LDH present in the nuclear, mitochondrial, microsomal fractions and in the supernatant of rat liver. Preparations of subcellular structural components were obtained in isotonic sucrose solution, fractionated on an 'International' refrigerated centrifuge and on a 'Spinco' *L* ultracentrifuge. The protein content of the single fractions was adjusted to a similar level. In total liver homogenate the principal band is the one which migrates most negatively (LDH_5), while only a very small band is present in the adjacent cathodic zone (LDH_4). These electrophoretic patterns are also present in the case of the nuclear and microsomal fractions and in the supernatant of the rat liver. Mitochondrial preparations always show an anodic band (corresponding to LDH_1, present in other organs). This band I is clearly greater than band IV (Fig. 1). The presence of LDH_1 could not be ascribed to erythrocyte contamination because the liver was previously carefully washed and because the erythrocytes should have been precipitated in the process of separation of the nuclei.

The fact that in the mitochondria, that is, in subcellular structures with a high aerobic metabolism, the first type of molecular form of LDH is clearly evident supports the view that this form is a catalyst geared for activity in an aerobic environment[1,2].

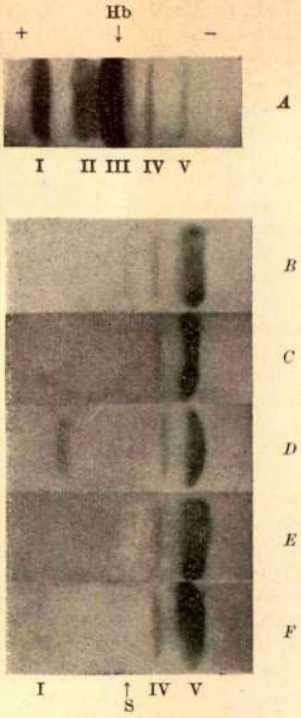


Fig. 1. Electrophoretic separation of molecular forms of LDH in rat liver. *B*, Total homogenate; *C*, nuclear fraction; *D*, mitochondrial fraction; *E*, microsomal fraction; *F*, supernatant. Cathodic bands predominate (LDH_4 and LDH_5), but in the case of the mitochondrial fraction an anodic band, corresponding to the LDH_1 of a haemolysate (*A*), is clearly evident

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Triglyceride Accumulation in Liver

A number of experimental conditions, including various types of stress[1] and several treatments including administration of adrenocorticotrophic hormone (ACTH)[2], ethanol[3], carbon tetrachloride[4] and ethionine[5] induce an increase of the level of liver triglycerides.

The synthesis of triglycerides in rat liver depends largely on the availability of plasma free fatty acids (FFA) derived from lipolysis of triglycerides in adipose tissue[6]. The recent findings that drugs such as nicotinic acid[7], salicylic acid[8,9] and 3,5-dimethylpyrazole[10] can block FFA mobilization from adipose tissue suggested a possible new method for preventing or controlling the development of liver triglyceride accumulation in the aforementioned experimental conditions.

In a first experiment 180-g male Sprague–Dawley rats, fed *ad libitum* up to the beginning of test, were injected intraperitoneally with nicotinic acid (200 mg/kg), salicylic acid (300 mg/kg) or 3,5-dimethylpyrazole (15 mg/kg) and at the same time subcutaneously with ACTH ('Acthormone', Richter) at a dose of 200 I.U./kg.

4 h later the animals were killed by decapitation. Plasma FFA were analysed according to Trout *et al.*[11] and liver triglycerides were determined according to Van Handel and Zilversmit[12] after extraction with chloroform–methanol (2 : 1). The results reported in Table 1 show that the tested drugs reduce plasma FFA and prevent liver triglyceride accumulation.

Table 1. Effect of Drugs lowering Lipid Mobilization on Triglyceride Levels in Liver

Drug	ACTH (200 I.U./kg subcutaneously)	Plasma FFA μequiv./l. ± *S.E.*	Liver triglycerides mg/100 g ± *S.E.*
—	−	189 ± 12	554 ± 79
—	+	805 ± 26	1,912 ± 90
Nicotinic acid	+	650 ± 53	876 ± 68
—	+	906 ± 14	1,798 ± 94
Salicylic acid	+	547 ± 28	810 ± 72
—	+	883 ± 25	2,408 ± 232
3,5-Dimethylpyrazole	+	650 ± 95	600 ± 10

Drugs and ACTH were injected at the same time.
Rats fasted during the treatment—ACTH was given 4 h before killing.

In a second series of experiments, 3,5-dimethylpyrazole was given together with other treatments able to increase liver triglycerides. As shown in Table 2, 3,5-dimethylpyrazole was an effective antagonist in all the experimental conditions selected. Similar results not reported here in detail were also obtained with salicylic and nicotinic acids.

Table 2. Effect of 3,5-Dimethylpyrazole on Various Types of Triglyceride Accumulation in Liver

Treatment	3,5-dimethylpyrazole (15 mg/kg intraperitoneally)*	Plasma FFA μequiv./l. ± *S.E.*	Liver triglycerides mg/100 g ± *S.E.*
Exposure to cold†	−	600 ± 10	830 ± 63
Exposure to cold	+	559 ± 33	662 ± 30
Ethanol 2·1 ml./100 g per os	−	483 ± 30	1,340 ± 197
Ethanol 2·1 ml./100 g per os‡	+	346 ± 15	562 ± 79
Ethionine 150 mg/kg i.p.	−	916 ± 115	1,420 ± 160
Ethionine 150 mg/kg i.p.‡	+	653 ± 160	604 ± 50
Carbon tetrachloride 2·5 ml./kg per os	−	676 ± 10	1,427 ± 40
Carbon tetrachloride 2·5 ml./kg per os‡	+	473 ± 25	970 ± 80

* 3,5-dimethylpyrazole was given together with the treatment causing triglyceride accumulation.
† Rats were exposed to cold for 5 h and fasted during the treatment.
‡ Rats fasted 18 h before the beginning of the treatment. Ethanol was given 8 h before killing. Ethionine and carbon tetrachloride were given 5 h before killing.

In a last experiment, 3,5-dimethylpyrazole was injected intraperitoneally when accumulation of liver triglycerides was already induced either by ACTH or carbon tetrachloride. Table 3 shows the results obtained.

Table 3. Effect of 3,5-Dimethylpyrazole on Already Induced Accumulation of Liver Triglycerides

Treatment†	3,5-dimethylpyrazole (15 mg/kg intraperitoneally)*	Plasma FFA μequiv./l. ± *S.E.*	Liver triglycerides mg/100 g ± *S.E.*
ACTH 200 I.U./kg s.c.	−	685 ± 51	1,444 ± 195
ACTH 200 I.U./kg s.c.	+	191 ± 9	702 ± 106
Carbon tetrachloride 2·5 ml./kg per os‡	−	1,056 ± 114	1,831 ± 70
Carbon tetrachloride 2·5 ml./kg per os‡	+	414 ± 43	1,899 ± 142

* 3,5-dimethylpyrazole was given 30 min before killing.
† ACTH and carbon tetrachloride were given 5 h before killing. Rats fasted during the treatment.
‡ Rats were fasted the night before the experiment.

It is evident that after 30 min from the administration of 3,5-dimethylpyrazole there is a considerable decrease of liver triglycerides in the case of accumulation induced by ACTH but not in the case of carbon tetrachloride. This difference could be explained on the basis of observation that carbon tetrachloride[13], as opposed to ACTH, blocks the transfer of triglycerides from liver to plasma. The block of liver triglyceride synthesis due to the decreased availability of plasma FFA therefore results in a rapid decrease of liver triglycerides only when, as after ACTH treatment, the transfer of triglycerides from liver to plasma is still occurring.

The data here reported show that drugs preventing release of FFA from adipose tissue are able to prevent and, in some cases, to lower liver triglyceride accumulation even if the cause of such an increase of liver triglycerides is not an increased lipid mobilization.

These results may have a more general implication and might explain by means of a common mechanism why several conditions such as spinal transection and adrenalectomy[14] and a number of drugs such as nucleotides[15] and adrenolytic agents[14] prevent accumulation of triglycerides.

It is suggested that drugs and procedure preventing fatty liver should be tested also for their capacity to lower plasma FFA.

We thank Dr. G. C. Gerritsen (Upjohn Co.) for a sample of 3,5-dimethylpyrazole and Miss A. M. Codegoni for technical help.

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Sex-associated Protein in Liver Tissue of the Rat

A protein component has been described[1-3] which appeared to be present in liver tissue of the male rat but absent from that of the female. The response of this substance to varied endocrine manipulation suggests that its presence or absence is dependent on the sex hormones. testosterone potentiating and oestradiol inhibiting the formation of the protein. Recently[4], this work has been confirmed and extended to show that progesterone also has an effect causing the appearance of the protein in the female and an increase in the amount found in the castrated male.

Investigations on this unusual protein have continued. The protein has been purified to the extent that a highly specific antiserum has been prepared. The reagent produces a single precipitin band when diffused in agar against the 105,000*g* supernatant[5] from liver homogenates. The sensitivity of the agar-diffusion techniques is such that it has become possible to detect the protein in livers of normal female rats. The concentration ratio is about thirty-two times less in the female than that in the male. Both single[6] and double[7] diffusion quantitative agar techniques give good agreement with this figure which, nevertheless, lacks precision because of its logarithmic nature. Stability problems associated with preparing the highly purified protein have prevented the accumulation of sufficient material to use more sophisticated quantitative methods and, thereby, to arrive at the absolute amounts present in tissue.

It would be of considerable interest to know if the sex-associated protein exists as a circulating entity in the blood. Thus far, it has not been detected there either chromatographically or by the much more sensitive immunochemical techniques.

It would seem that the adrenal glands are not directly concerned with this sex-associated phenomenon. The adrenalectomized female still responds to testosterone treatment, although rather poorly compared with the intact animal. Adrenalectomy seems to cause no significant change in the amount found in the male. The adrenalectomized male responds similarly to the intact male when treated with oestradiol.

Animals have been treated with oestradiol and testosterone simultaneously with the same dose schedule and proprietary preparations used previously[3]. Interestingly, the sex-associated component disappeared rapidly in the male similarly to that seen when oestradiol was given alone. At about 20 days after treatment, however, the longer lasting testosterone repository apparently caused a rapid reappearance of the component. A similar phenomenon occurred in the female, in which case the sex-associated protein began to be present in substantial amounts after about 20 days. These observations strengthen the argument for a direct-acting antagonism between the hormones and for a reciprocal dose relationship.

The physiological significance of the sex-associated protein is still unknown. Intuitively, it may be assumed to have considerable importance as judged by the comparatively large amounts found in adult male liver[3,4]. Experimentally, the protein may be of considerable value in investigating physiological processes in the liver. With the elimination of a necessary adrenal involvement, the evidence favours that of a direct response to sex hormones. In itself this provides a means for studying a highly specific response to hormone action in addition to studying the consequences of the action. Sensitive immunochemical techniques will be most valuable in further investigations on this sex-associated phenomenon.

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Carcinogenic Activity *in situ* of Further Steroid Compounds

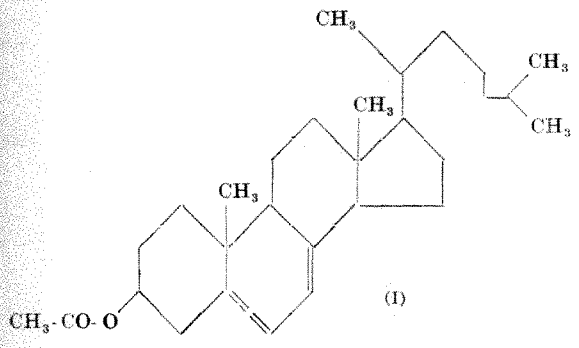
There is more and more evidence coming from several research groups that a number of steroid molecules with no hormonal competence nevertheless possess carcinogenic properties. Ghiron[1], and later Cook *et al.*[2], obtained sarcomas with deoxycholic acid by subcutaneous injection in mice; cholesterol itself has been shown by Hieger[3] to produce a 12 per cent incidence of sarcomas, as against 0·8 per cent in control mice injected with the solvent alone. More recently, we recorded the activity of apocholic acid, a dehydration product of cholic acid; it gave sarcomas in 4 out of 42 mice, as compared with 0 per cent in 350 mice injected with the solvent alone[4]. Tumours have also been recorded by Bryson and Bischoff[5] in Marsh mice given testicular injections of cholesterol α-oxide. We wish now to report on the definite sarcomagenic activity of two further steroid molecules, one with a biochemical function, 7-dehydrocholesterol [in the form of its acetate (I)], the precursor of vitamin D_3, and the other, 3β-acetoxy-bisnor-Δ^5-cholenic acid (II), a product of the artificial oxidative degradation of several sterols and bile acids[6].

Sixty 3- to 5-month-old mice (28♀ and 32♂) of strain XVII *nc/Z* (Radium Institute) received subcutaneously, in the right flank, ten injections (with a 10-day interval between each) of 0·5 mg of 7-dehydrocholesterol acetate dissolved in 0·2 c.c. sterile neutralized olive oil. The first tumour was detected at the site of injection, 210 days after the first injection, in one male mouse out of the 52 surviving animals. Two other tumours developed in 392 and 482 days, in a male and female mouse respectively. In all

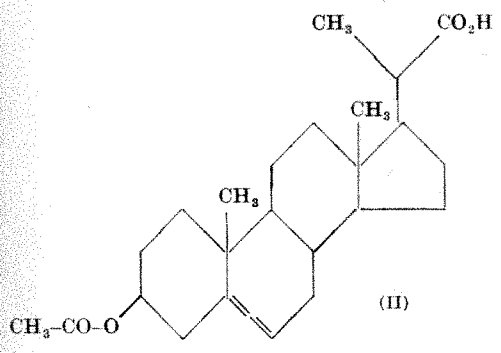
three cases the tumour was a typical fibrosarcoma. Forty-one animals survived for longer than 500 days.

A second group of 60 mice (28♀ and 32♂), of similar age and strain as for the first group, was given 10 injections of 0·5 mg of 3β-acetoxy-*bisnor*-Δ^5-cholenic acid, in the same experimental conditions. After 526 days an *in situ* sarcoma developed in a female mouse; by that time, 38 animals still survived. A second sarcoma was observed in a male mouse after 609 days.

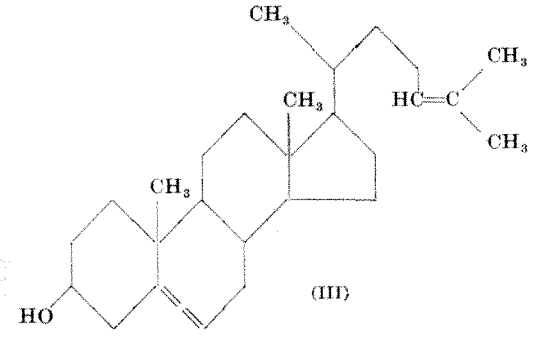
In a third group comprising 71 mice (23♀ and 48♂), treated in the same conditions with 24-dehydrocholesterol (desmosterol) (III) (0·5 mg; 10 injections), no tumour was obtained; 36 of the animals survived for over 650 days.



(I)



(II)



(III)

Although the yields in tumours (5 per cent for compound (I) and 3·3 per cent for compound (II)) were low, evidence that 7-dehydrocholesterol acetate and 3β-acetoxy-*bisnor*-Δ^5-cholenic acid act, in our strain of mice, as true carcinogens rests on the facts: (*a*) that spontaneous sarcomas of the soft parts have never occurred in that strain; (*b*) that in our laboratory, subcutaneous injections of the solvent alone (that is, neutralized olive oil) have always failed to produce any sarcomas *in situ*, as observed in more than 530 animals; and (*c*), that most of the steroid molecules tested by us previously, as well as 24-dehydrocholesterol reported here, are completely inactive in our experimental conditions.

Both 7-dehydrocholesterol and 24-dehydrocholesterol are minor constituents of the tissues of higher animals and man; the weak but definite sarcomagenic activity of 7-dehydrocholesterol is a further point in favour of the existence of endogenous carcinogens in normal tissues. As for 3β-acetoxy-*bisnor*-Δ^5-cholenic acid, its close chemical relation to the bile acids suggests that further compounds of this type should be tested for carcinogenic activity.

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An Investigation of Plant Seeds for Adenosine Deaminase

Adenosine deaminase (adenosine aminohydrolase, *EC*3.5.4.4) of plant origin was first reported in a commercial sample of malt diastase[1]. Later, its presence in extracts from barley rootlets was claimed[2]. Another study, however, failed to detect deaminase activity in any of a number of plants examined[3]. On the other hand, most animal cells[4] and many micro-organisms[5] are known to contain enzymes capable of deaminating adenosine.

In this investigation, the seeds of twenty plants were tested for deaminase activity, with particular attention being given to barley.

The seeds were ground in a sterilized coffee mill and 20 per cent aqueous extracts prepared from the powder. Enzyme activity was measured by determining ammonia release from an incubation mixture composed of 2 ml. 0·5 per cent adenosine in 0·1 M phosphate buffer *p*H 7·0 and 1 ml. of seed extract[6]. Substrate and seed-extract controls were always included. Activity was recorded in micro units (μu.).

Barley seeds, both fresh and germinated, had activity after 15 h incubation, but no activity could be detected with short incubation times of 1–3 h. Germination always resulted in increased activity but there were considerable fluctuations in the activity of different extracts of the same seed sample. Addition of 0·1 ml. saturated benzoic acid caused 60 per cent reduction, while 0·1 ml. saturated thymol completely suppressed activity. Filtration of the extract through a bacterial membrane filter of average pore size 200 mμ resulted in a 56 per cent reduction in activity, while a second filtration produced a further reduction to 29 per cent of the original.

A bacteriological examination kindly carried out by Prof. O'Mullane, Department of Dairy Bacteriology and Microbiology, University College, Cork, showed that the unfiltered seed extract was heavily contaminated with micro-organisms. Although there was diminution in their number after filtration, the second ultra-filtrate was still appreciably contaminated.

The deaminase in an extract of calf mucosa showed no reduction of activity with these treatments. It is evident, therefore, that plant and animal cell extracts differ in the nature of their deaminase activity.

From the foregoing findings it appeared that developing micro-organisms might be the cause of activity in barley seed extracts. To test this, the activity/time relationship in a plant and animal extract was compared. Activity in each case was measured at 3-h intervals during a 24-h incubation period at 37° C.

Fig. 1 shows that the mammalian extract gave a steady release of ammonia resulting in almost identical activity

values throughout the 24 h. The barley extract, in contrast, showed no activity at 3 h, only slight activity after 6 h, followed by a rapid increase, terminating in a plateau of almost constant activity after 15 h. The graph (Fig. 1) shows a striking similarity to a typical bacterial growth cycle with a lag phase, followed by a logarithmic growth phase and terminating in a stationary phase. It demonstrates that enzyme is being formed during the incubation. As cell-free extracts were used, enzyme formation by the plant was ruled out, so that the appearance of activity must be due to micro-organisms developing during the incubation. The fact that there was no ammonia release at 3 h means that deaminase activity, if present, in the barley is below 1 μU. or less than 1/10 of that found in blood plasma, the animal tissue with lowest activity.

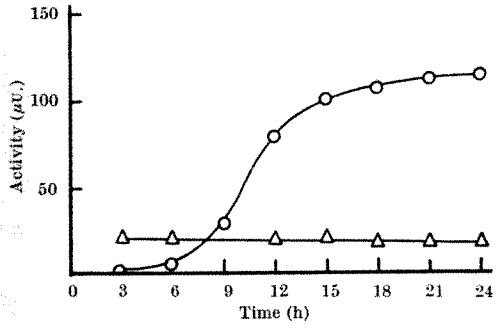


Fig. 1. Comparison of adenosine deaminase activity in extracts of barley seed and of calf mucosa. ○, Barley seed; Δ, calf mucosa. Assays were performed in 0·33 per cent adenosine in 0·1 M phosphate buffer at *p*H 7·0 at 37° C. Results are expressed as specific activity. Values for the calf mucosa are multiplied by 10^{-3}

Two samples of dried barley rootlets (combings) were assayed in the same way. One extract showed no activity throughout the 24-h incubation. The second extract had no activity at 6 h, after which low activity appeared up to 18 h, followed by a steep increase at 21 and 24 h.

Fresh barley roots were grown in water culture. An extract of the roots was prepared by homogenization. No activity was evident at 3 h, but after 9 h activity appeared and increased steeply until the termination of the experiment. These results, similar to those with seeds, provide no evidence for the presence of adenosine deaminase in either dried or fresh barley roots.

The following seeds were assayed for adenosine deaminase activity: Family (1) Gramineae: wheat, oats, barley; (2) Cypraceae: sedges; (3) Junaceae: rushes; (4) Liliceae: onions; (5) Leguminosae: peas and beans; (6) Compositae: lettuce; (7) Umbelliferae: celery; (8) Ranunculaceae: buttercups; (9) Cucurbitaceae: vegetable marrow; (10) Cruciferae: white turnips; (11) Papaveraceae: poppy; (12) Ericaceae: heather; (13) Gerianiaceae: geranium; (14) Malvaceae: mallow; (15) Chenopodiaceae: beet; (16) Solanaceae: tobacco; (17) Polygonaceae: buck wheat.

Extracts were prepared as previously described, but incubations were conducted at a temperature of 25° C.

A selection of the results is given in Fig. 2 which includes, for comparison, a similarly treated extract of calf mucosa. With only two exceptions, the seed extracts gave either no ammonia release or a very slight amount after 12 h.

In all cases, except those seeds giving negligible ammonia release, the graph of ammonia formation with time was curved in contrast to the animal extract, which gave a straight line. Mallow and celery gave some ammonia release at 3 h, but the course of the subsequent release indicates that here, too, micro-organisms were responsible for the deaminase activity.

These results call into question the claims that adenosine deaminase is present in barley. Its presence in malt diastase[1] cannot be taken as proof of its occurrence in barley seed, because malt diastase is prepared from

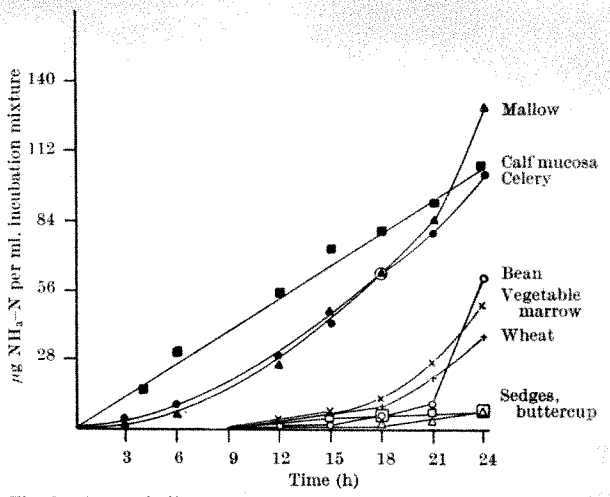


Fig. 2. Ammonia liberation from adenosine by seed extracts. Assay conditions were identical to those used in Fig. 1, except that the incubation temperature was reduced to 25° C. The calf mucosa was diluted 1 : 350,000

germinated barley and, therefore, likely to be extensively contaminated with micro-organisms. Doubt must also be expressed about its reported presence in barley rootlets in view of the long incubation times used and the semi-quantitative nature of the assay method used[2].

Unequivocal proof for the existence of adenosine deaminase in any type of higher plant is still lacking, and the conclusion that adenosine deaminases are of much less widespread occurrence in higher plants than in other forms of living matter seems inescapable.

We thank the Medical Research Council of Ireland; the European Research Office, U.S. Department of the Army, for financial support; and the Department of Education for a training grant to one of us (V. J. H.).

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Effect of Aeration on the Consumption of Ethanol by the Isolated Perfused Rat Liver

In a particular series of experiments designed to determine ethanol consumption by the isolated perfused rat liver it was observed that consumption patterns differed from that obtained in the intact animal. The following experiments were conducted to show how normal consumption patterns can be obtained by changing the aeration media of the blood from the usual 95 per cent oxygen, 5 per cent carbon dioxide, to a mixture containing 18 per cent oxygen, 5 per cent carbon dioxide, and 77 per cent nitrogen.

The liver perfusion apparatus and details of the operation have already been described[1,2]. Young male albino rats weighing between 200 and 300 g were used in all the experiments. The perfusate consisted of freshly drawn blood from rats, and to this was added 24 mg/100 ml. of heparin. The volume of the perfusate was maintained at 90–100 ml. throughout the 4-h experiment. The temperature of the system was maintained at 38° C and the aeration medium used was either 95 per cent oxygen, 5 per cent carbon dioxide or 18 per cent oxygen, 5 per cent carbon dioxide and 77 per cent nitrogen. The rate of loss

of ethanol by evaporation in the aerating 'lung' was determined experimentally.

To determine the ethanol concentration samples were removed from the perfusate at 10-min intervals during the first 90 min of the experiment, and thereafter at 15-min intervals, and immediately transferred to a chilled, air-tight vial. The ethanol vapour in the volume above this aliquot was allowed to equilibrate at 37° C and was afterwards analysed utilizing a Loenco gas chromatograph, model 70, equipped with a hydrogen flame ionization detector. Adequate precautions were taken to prevent any loss by condensation when transferring this measured gas sample to the system for analysis. The operating conditions were as follows: a 6 ft. × 0·125 in. stainless steel column packed with 20 per cent 'Carbowax 20 *M*', on HMSD 'Chromosorb *W*' 60/80 mesh, was maintained at 78° C, and utilized helium as the carrier gas, with a flow rate of 30 ml./min. The retention time of ethanol was 2·25 min. The ethanol concentrations were calculated by measuring peak area and comparing these areas to those of suitable ethanol standards treated in the same manner as the unknowns.

The data illustrated in Fig. 1 show the effect of different aerating media. Eight experiments were conducted using 95 per cent oxygen, 5 per cent carbon dioxide and an initial concentration of 240 mg of ethanol per 100 ml. of perfusate. Concentrations of ethanol in the perfusate are plotted against time. It is apparent that the concentrations vary exponentially with time for these particular experiments. This is shown more clearly in Fig. 2, in which the logarithm of the concentration is plotted against time. In this plot the data appear as two distinct straight-line segments of differing slopes with a distinct break at an ethanol concentration of approximately 40 mg/100 ml. of perfusate. Such a dependence indicates that the consumption of ethanol depends primarily on the concentration of ethanol in the blood and that a distinct change in the mechanism of consumption occurs at the break point. The time taken for the concentration to fall to one-half of its original value ($T\frac{1}{2}$) is a recognized measure of the rate of consumption for this type of curve. The $T\frac{1}{2}$ for the upper segment of the curve was 45 min, and for the lower segment was 20 min. In these calculations a small correction has been made for the loss of ethanol by evaporation during the aeration process.

Five experiments were conducted using 18 per cent oxygen, 5 per cent carbon dioxide, 77 per cent nitrogen gas mixture; quite different results are obtained, as indicated by the remaining data in Fig. 1. A straight line can be drawn through these points, indicating that ethanol is now disappearing at a constant rate which is

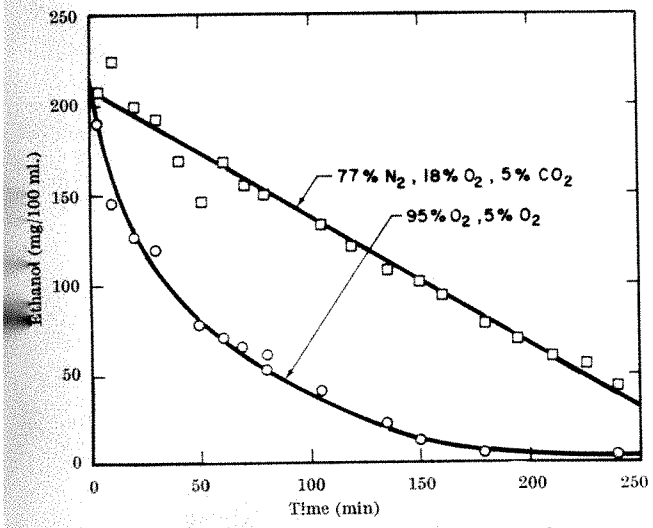


Fig. 1. A linear plot of ethanol concentration in the perfusate

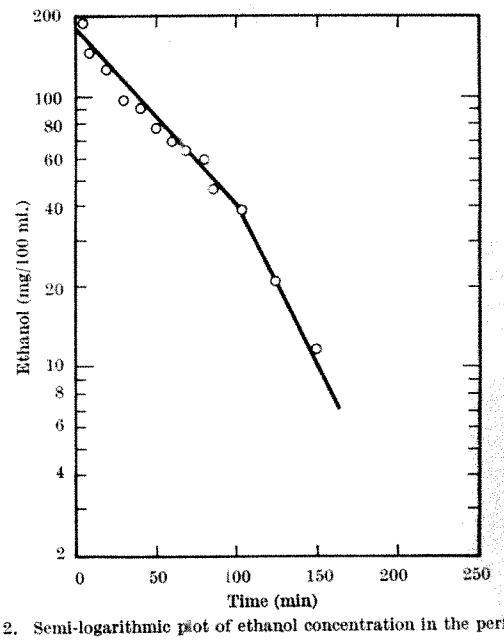


Fig. 2. Semi-logarithmic plot of ethanol concentration in the perfusate

independent of the ethanol concentration. When corrected for evaporation losses, the ethanol consumption indicated by these data is 36 mg/100 ml. per 10 g of liver per hour, and is within the range published for the intact rat[3–5]. In these experiments there is no doubt regarding the differences in magnitude and shape of the curves involved. The use of 95 per cent oxygen, 5 per cent carbon dioxide atmosphere results in a non-physiological mode of consumption.

At this stage of the investigation, no clear answer can be given as to the mechanism involved in the consumption of ethanol for these different aeration media. However, some inferences seem reasonable. Bauer *et al.*[6] have indicated that there is a fourfold increase in the consumption of oxygen by the isolated perfused rat liver when the oxygen in the aerating media is changed from 18 per cent to 95 per cent. It seems reasonable to postulate that the rate-limiting step in the metabolism of ethanol by the liver may be controlled by the availability of oxygen. Since Theorell[7] and his group have indicated that the rate of oxidation of reduced nicotinamide-adenine dinucleotide ($NADH_2$) formed when ethanol is oxidized to acetaldehyde is the rate-limiting step, this assumption seems reasonable. It is evident that further information on the levels of $NADH_2$ and NAD in these perfused livers would greatly aid in the understanding of the mechanisms involved. A pertinent feature of these experiments is the demonstration that an isolated organ in an artificial system can utilize ethanol at rates similar to that found in the intact animal.

In this investigation, these 'normal' rates were only observed if the oxygen partial pressure of aeration media approached that of air. These findings suggest that caution must be exercised in the interpretation of data arising from investigations utilizing media of abnormally high oxygen partial pressures.

I thank Mrs. B. Ahlstrom for her help in this work.

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PHYSIOLOGY

Sciatic Nerve Activity evoked by Sensory-motor Cortex Stimulation during Paradoxical Sleep

The cortico-spinal system undergoes consistent modifications during the desynchronized phase of sleep. The integrated pyramidal activity rises to a level even higher than that reached in wakefulness[1]; the pyramidal tract neurones change their temporal pattern of discharge[2]; the integrated pyramidal activity increases synchronously with rapid eye movements and myoclonic twitches[3]; and the sciatic nerve responses to liminar pyramidal stimulation are abolished[4].

The aim of the present investigation was to study the behaviour of sciatic nerve responses to sensory-motor cortex stimulation during sleep and wakefulness, in order to obtain further information on the function of the cortico-spinal system during these physiological phases.

The experiments were performed on unrestrained, unanaesthetized cats with chronic implanted electrodes. Bipolar stimulating electrodes were applied subdurally on the surface of the sensory-motor cortex of one side. Bipolar electrodes left in place around the sciatic nerve of the opposite side could record the spontaneous and evoked activity of a large population of lumbo-sacral motoneurones. The sensory-motor cortex was stimulated with bursts of from two to five stimuli at 300/sec, 0·05–0·5 msec, 1–3 V delivered every 1–2 sec. The spontaneous and evoked activity in the sciatic nerve was recorded on an 'Ampex' tape-recorder during wakefulness, synchronized sleep and desynchronized sleep, averaged on a *CAT* 400 'Mnemotron' and then photographed on an oscilloscope. The electroencephalogram (from frontal areas), the electromyogram (from nuchal muscles) and the oculogram indicated the stages of wakefulness and sleep which occurred during the experiments.

During wakefulness without apparent movements of the animal, a spontaneous activity was present in the sciatic nerve. A train of from two to five stimuli delivered to the sensory-motor cortex evoked a complex response on the neurogram with a latency of 12–18 msec from the first stimulus. Synchronous with the electrical potentials, which fluctuated in amplitude, a slight flexion of the hindleg of the same side was generally observed. Fig. 1*A* shows an average of fifty responses during wakefulness in which three main deflexions are seen. During synchronous sleep the evoked activity in the sciatic nerve was usually slightly reduced in amplitude, although did not change in its latency and pattern (Fig. 1*B*). It can be seen that the first and second deflexions are reduced in amplitude as compared with wakefulness. When the animal went into an episode of paradoxical sleep (with desynchronized EEG, flattening of nuchal muscles and rapid eye movements, REM) the evoked activity was usually tonically reduced in amplitude with phasic marked increase generally synchronous with the bursts of REM. The averaged values were often higher than those observed during wakefulness (Fig. 1*C*), the increase being proportional to the amount of REM present during the paradoxical sleep episode. It can be seen that the latency of the discharges does not change, while the increase in amplitude affects particularly the second and third deflexions of the neurogram. Both the second and the third deflexions also appear to be slightly shorter in duration than those seen in Fig. 1 *A* and *B*. The results indicate that the excitability of the corticospinal system is tonically depressed during synchronized and desynchronized sleep, whereas it increases during wakefulness and REM periods of desynchronized sleep. Since the sciatic nerve activity evoked by liminar pyramidal stimulation is abolished during paradoxical sleep[4] the phasic enhancement of activity evoked by sensory-motor cortex stimulation during this phase may be due to an increase of cortical excitability.

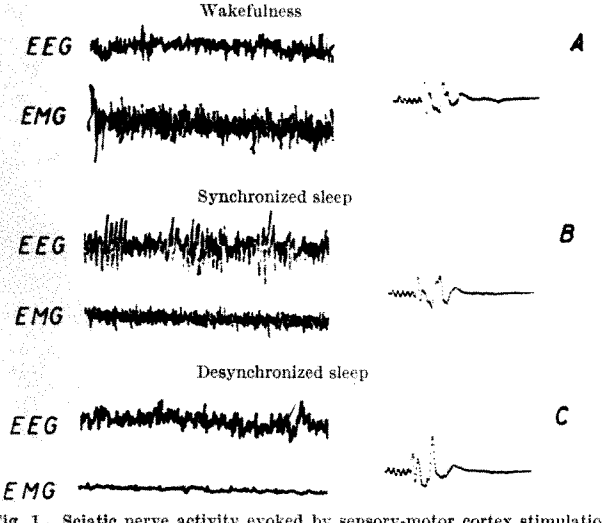


Fig. 1. Sciatic nerve activity evoked by sensory-motor cortex stimulation during wakefulness, synchronized and desynchronized sleep. Chronic unrestrained, unanaesthetized animal with frontal EEG and nuchal EMG. *A*, *B* and *C*: fifty averaged sciatic nerve responses evoked by stimulation of the contralateral sensory-motor cortex with bursts of 5 impulses at 300/sec, 0·07 msec, 3 V during wakefulness (*A*), synchronized sleep (*B*) and desynchronized sleep (*C*). Sweep duration 125 msec

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Electrocardiograms of Lambs in Selenium Deficiency

Studies have been made of lambs taken from their dams at 4 weeks of age and weaned, by stages, on to a selenium-deficient diet of the type used by Schwarz[1] for the development of dietary hepatic necrosis in rats. Lambs on this diet develop muscular dystrophy, the lesions appearing in both the cardiac and skeletal musculature; the livers do not become necrotic as they do in the rat. Control lambs fed the same diet, supplemented with an oral drench of 10 mg of sodium selenite per week, do not develop muscular dystrophy.

Electrocardiography on these lambs reveals the development of a characteristic abnormality. As the disease progresses the *T*-wave increases in amplitude until it finally dominates the record; at this stage the elevated *S–T* segment is typical of the pattern of myocardial infarction in man (Figs. 1 and 2). As the abnormal electrocardiograph develops progressively over a period of

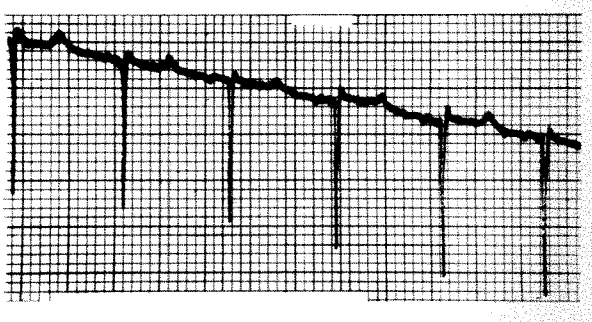


Fig. 1. Electrocardiogram of a lamb maintained for a few weeks on a selenium-deficient diet. At this stage no abnormality has developed in the electrocardiogram pattern

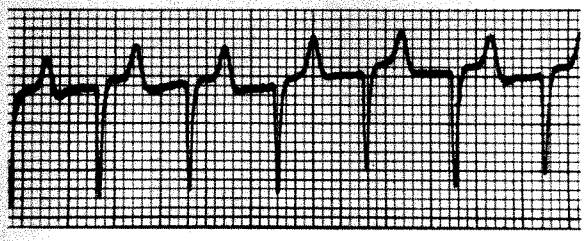


Fig. 2. Electrocardiogram of the same lamb as is shown in Fig. 1. However, the lamb is at this point clinically dystrophic and the electrocardiogram pattern shows the typical elevated *S–T* segment of myocardial infarction

2–3 weeks before the deficiency syndrome leads to death, it is possible to monitor the course of the deficiency symptoms.

It remains to be seen whether the same type of electrocardiogram change occurs in 'white muscle disease', a malady of sheep that are grazed in particular areas and which responds to selenium therapy[2].

I have already reported[3] that similar changes occur in selenium-deficient rats.

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Glucose Metabolism of Central Nervous Tissues in Rats with Experimental Allergic Encephalomyelitis

THE incorporation of carbon from glucose-^{14}C and acetate-1-^{14}C into lipids of the central nervous system has been shown to be greater than normal in rats with experimental allergic encephalomyelitis (EAE)[1,2]. In order to explore further the metabolism of tissues from animals with this demyelinative condition, glucose uptake, carbon dioxide production and lactate accumulation *in vitro* were measured in brain stem and spinal cord slices from rats in the acute paralytic stage of experimental allergic encephalomyelitis. As controls, similar slices were investigated from both normal animals and those injected only with Freund's adjuvant.

Fourteen Wistar rats, 6 weeks old, were injected in the hind foot pads with 0·5 ml. Freund's adjuvant (obtained from Difco) containing 85 mg homogenized guinea-pig spinal cord. The controls were litter-mates matched in regard to weight and sex, fourteen of which were injected with 0·5 ml. Freund's adjuvant; the remaining fourteen served as normal controls. When paralysis of the hind feet became apparent in the group receiving nervous tissue homogenate (12–15 days after injection), the animals were killed by cervical fracture, the brain stem and spinal cord removed, sliced and incubated in Warburg flasks containing 3 ml. Krebs–Ringer phosphate with 3 μc. uniformly labelled glucose-^{14}C in 3 mg glucose. Potassium hydroxide was present in the centre well for carbon dioxide absorption. The flasks were incubated under oxygen in a Dubnoff Metabolic Shaker at 37° for 1 h. At the end of this time 0·2 ml. perchloric acid was tipped in from the side arm and the flasks incubated 30 min longer. The potassium hydroxide was removed from the centre well and the absorbed carbonate precipitated by the addition of barium acetate. The barium carbonate was plated and counted in a gas-flow Geiger–Müller counter using an end window. The medium and slices were removed from the flask, centrifuged, and the medium decanted. Subsequent washings of the slices were added to the medium. Aliquots of the medium plus washings were used for lactic acid measurements according to Barker and Summerson[3] and for glucose determinations according to the carbohydrate method described by Svennerholm[4].

Table 1 shows glucose uptake in the EAE brain stem and spinal cord to be very close to the normal. A slightly higher uptake of glucose was shown in the Freund's adjuvant-injected controls, however, and this was significantly different from the normal in the spinal cord ($P < 0·01$), but not in the brain stem. The lactic acid level of the incubation medium was markedly higher than the normal in both the brain stem and spinal cord of the EAE animal ($P < 0·01$). These higher levels are significant also when compared with the Freund's adjuvant-injected control. No significant difference was seen between the Freund's adjuvant control and the normal control.

The level of specific activity in the carbon dioxide produced by the brain stem was not significantly lower than the normal, but was significantly different ($P < 0·01$) from the Freund's adjuvant controls. The spinal cord carbon dioxide specific activity was, however, significantly less ($P < 0·01$) than both groups of controls.

Two explanations for the lowered specific activity of the carbon dioxide are possible. A lowered production of carbon dioxide by the slices seems most plausible when the high lactic acid levels are taken into account. It is possible also that other non-labelled constituents are being oxidized in the EAE animal, thus resulting in a decreased specific activity of the carbon dioxide.

If the former explanation is considered, the present results would indicate that an abnormal carbohydrate metabolism is present in nervous tissue from the EAE animal. This abnormality is not seen in glucose uptake, but seems to exist somewhere between the lactic acid and carbon dioxide stages of carbohydrate metabolism or the tricarboxylic acid cycle. The enzymes required in this pathway are known to be located in the mitochondria. Other preliminary evidence[5] would indicate that the mitochondria are involved in the increased rate of lipid synthesis previously described in the EAE animal[1,2]. In electron microscope pictures Roizin has shown mitochondria of the central nervous system of the EAE guinea-pig which are abnormal in appearance[6].

At present it is not known whether these abnormal metabolic relationships may be related to the demyelinative condition. It is possible that the paralysed nerves may utilize glucose differently, or that paralysis may

Table 1. LEVELS OF GLUCOSE UPTAKE, LACTIC ACID PRODUCTION AND SPECIFIC ACTIVITY OF BARIUM CARBONATE IN EAE, FREUND'S ADJUVANT-INJECTED CONTROLS AND NORMAL CONTROLS
Each group represents fourteen animals

	Glucose uptake (mg/100 mg tissue ± *S.D.*)	Percentage of normal	Lactic acid production (mg/100 mg tissue ± *S.D.*)	Percentage of normal	$BaCO_3$ (c.p.m./mg/100 mg tissue ± *S.D.*)	Percentage of normal
EAE						
Brain	0·211 ± 0·058	100	0·189 ± 0·028*	122	4,028 ± 77††	89
Spinal cord	0·132 ± 0·032	97	0·187 ± 0·021*	150	2,138 ± 419*	87
Freund's adjuvant control						
Brain	0·249 ± 0·087	119	0·166 ± 0·041	110	4,853 ± 663	107
Spinal cord	0·158 ± 0·040*	116	0·123 ± 0·015	99	2,484 ± 464	101
Normal control						
Brain	0·210 ± 0·047		0·151 ± 0·020		4,525 ± 886	
Spinal cord	0·136 ± 0·035		0·125 ± 0·013		2,447 ± 350	

* $P < 0·01$. † $P > 0·1$.

result from deficiencies in the fundamental energy sources. A more detailed examination of the tricarboxylic acid cycle in the nerve tissue of the EAE animal is now in progress.

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PHARMACOLOGY

Possible Neurotoxicity of Diisopropyl Phosphorofluoridate in Guinea-pigs

Neurotoxicity (demyelination and axon degeneration) caused by various organophosphorus compounds is said to occur only in certain species[1,2]. According to Davies[1], guinea-pigs show no delayed paralytic effect after treatment with one of the neurotoxic compounds. We have found that guinea-pigs treated with diisopropyl phosphorofluoridate (DFP) show delayed paralytic effects. This report outlines the gross signs of neurological damage observed.

Ten male guinea-pigs each weighing about 800 g were treated subcutaneously with 1 mg/kg of DFP per day for 4 days. This amount of DFP was not sufficient to cause convulsions. However, the animals were highly excitable for the 4 days and subsequent few days.

The development of symptoms generally occurred as shown in Table 1. As might be expected, there are overlaps in the times at which the symptoms were first noticed.

The peculiar gait of the two guinea-pigs (walking with raised quarters) appeared to be due to spasticity of the hind limbs. Otherwise, the paralysis was flaccid. The walking on the hocks indicated that the lower muscles of the hind limb were initially weakened and that afterwards the muscles of the entire limb became paralysed.

Table 1. Development of Symptoms in DFP-treated Guinea-pigs

Days	Symptoms
1–4	Injections of 1 mg/kg daily; excitable
5–7	Excitable
10–14	Walked on hocks of hind legs. Sores on hocks. Pronounced thumping sound when walking, heavy-footed
15–20	Ataxic—hind quarters wobbled when walking
20–35	In two animals, ataxia became worse until hind limbs were completely paralysed. These animals scrambled with fore limbs when trying to move, but the hind quarters were completely paralysed. These two died, one at 28 days, the other at 35 days. Both showed large loss in body-weight. Two animals walked with hind quarters higher than the front quarters
35–50	Started to recover
75 days	All seven survivors appeared normal except one, which still had a weak right hind limb. One was killed for histological examination

This description is a composite picture and not all animals showed the severest signs. The least affected showed symptoms no worse than the sores on hocks and heavy-footedness (two animals).

We believe that the neurological signs shown by the guinea-pigs treated with DFP indicate that neurotoxicity is brought on by organophosphorus anticholinesterases in this species. It must be emphasized that the neurological lesions which one would have expected to accompany the symptoms described were not observed.

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HAEMATOLOGY

Synthesis of α_2-AP (Acute Phase) Globulin of Rat Serum by the Liver

The appearance of a new protein in rat serum in a wide variety of pathological and physiological states has been reported by several investigators in recent years[1,2]. The protein which we have designated α_2-AP (acute phase) globulin[1] has also been referred to as slow α_2-globulin (SA_2G) (ref. 1), abnormal serum component[1], reproduction-associated protein[1], and α_2-glycoprotein (α_2-GP) (ref. 2). The presence of the protein in serum has been detected by immunological procedures[1,2] and by filter paper-[1] and starch-gel-electrophoretic analyses[1]. The present communication reports the demonstration of the synthesis of α_2-AP globulin by the liver during the acute phase of an inflammatory response.

An anaesthetized, adult, male rat of Sprague–Dawley origin, weighing about 400 g, was injected with 1·0 ml. of a sterile solution of spirits of turpentine, N.F., in corn oil (v/v) as previously described[3]. Twenty-four hours later the rat was exsanguinated by cardiac puncture under ether anaesthesia and the liver removed aseptically. Tissue culture and radioimmunoelectrophoretic techniques were the same as those described in an earlier publication[3], with the exceptions that the film was exposed for 3 weeks, and an antiserum specific for α_2-AP globulin was used. Antiserum was prepared by immunizing rabbits with pooled sera from 'turpentine-stimulated' rats. The antiserum specific for α_2-AP globulin was obtained by absorbing the original with normal rat serum[1].

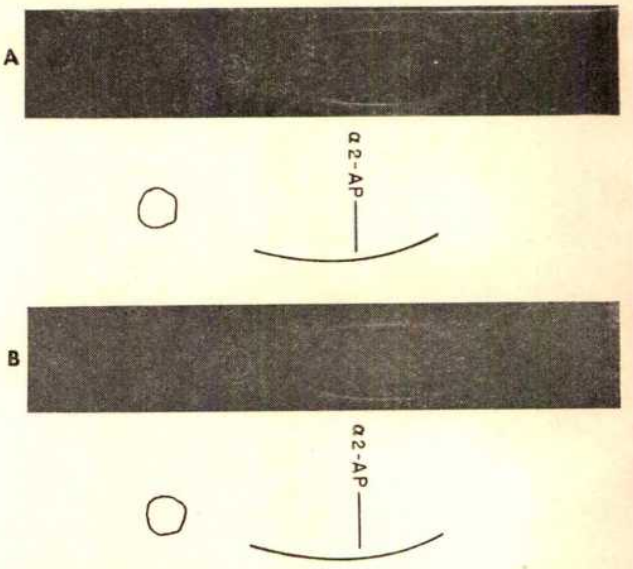


Fig. 1. *A*, Photograph and schematic representation of immunoelectrophoretic analysis of mixtures of tissue culture fluid and carrier serum proteins from donor rat using monospecific antiserum. *B*, Photograph and schematic depiction of autoradiograph of same slide

Fig. 1*A* shows a photograph of the immunoelectrophoretic analysis of the concentrated tissue culture fluid with carrier proteins using the monospecific antiserum. Fig. 1*B* is the autoradiograph of the same slide. Hepatic synthesis of α_2-AP globulin was unequivocally demonstrated by the radioactive labelling of the specific precipitin arc.

When the unabsorbed antiserum was used to develop the immunoelectrophoretic patterns, incorporation of the radioactive label into twelve other proteins was observed, as noted in a previous report[3].

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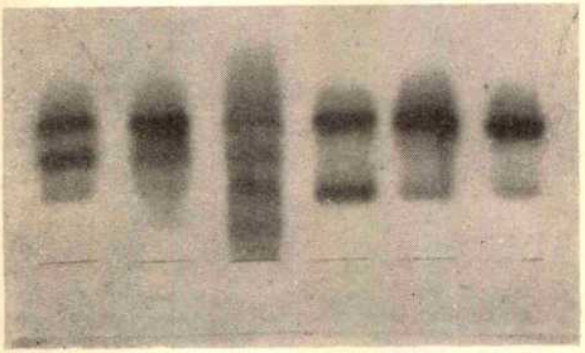
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New Variant of Haemoglobin G, Haemoglobin G_{Paris}

Since the first haemoglobin G was described in 1954 (ref. 1), several varieties have been observed. Structural analysis has shown that the electrophoretic mobility could be due to various amino-acid substitutions in the α- as well as in the β-chain. The only similarity between all these haemoglobins is that the net charge differs from that of normal haemoglobin and that they migrate close to haemoglobin S, but a little faster in alkaline buffer.

This communication describes a new variety of haemoglobin G, which we propose to call haemoglobin G_{Paris}. Different techniques were used: starch-gel electrophoresis, using the *tris*-borate-EDTA buffer, *p*H 8·4 (ref. 2), and phosphate buffer, *p*H 7·4 (ref. 3), starch-block electrophoresis with barbital buffer, *p*H 8·6 (ref. 4), hybridization both with abnormal haemoglobins and interspecific[5,6], and fingerprinting, according to Ingram as modified by Baglioni[7].

The propositus was a 5-year-old Negro child from West India. He was admitted early in 1964 to this hospital for a corticoid-resistant thrombocytopenic purpura. He was moderately anaemic, having 3,500,000 red blood cells/mm³, but there were no cellular abnormalities. Moreover, no abnormality was found in the blood of the mother. The father was not available for blood analysis.



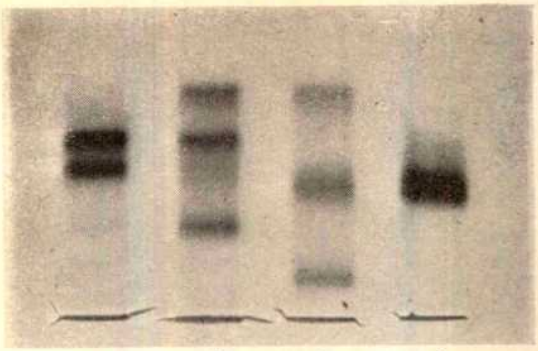
a *b* *c* *d* *e* *f*

Fig. 1. Starch-gel electrophoresis. Buffer *tris*-borate-EDTA, *p*H 8·4. *a*, A.S.; *b*, propositus; *c*, A.G.C.; *d*, AC; *e*, high A_2; *f*, normal A



a *b* *c*

Fig. 2. Hybridization experiment between S and (supposed) G. *a*, Normal A; *b*, mixture after hybridization; *c*, mixture without hybridization



a *b* *c* *d*

Fig. 3. Interspecific hybridization with canine haemoglobin. *a*, A + canine mixture; *b*, the same after hybridization; *c*, mixture between canine and (supposed) G after hybridization; *d*, same mixture without hybridization

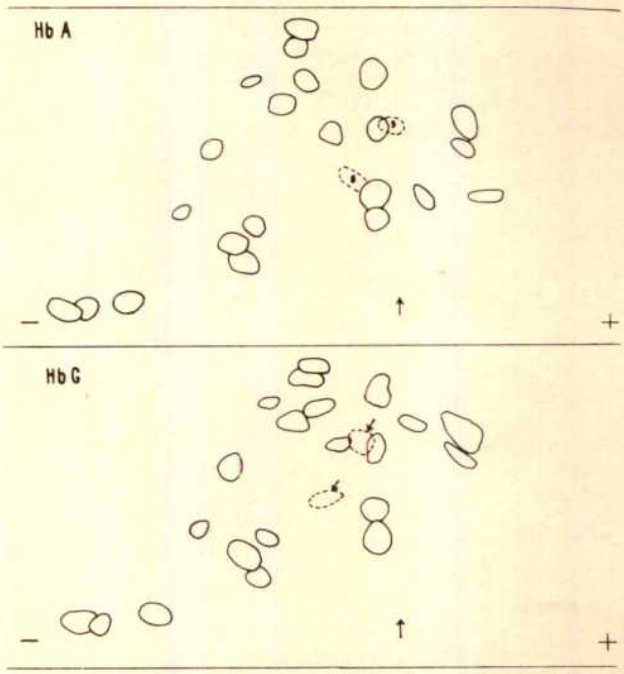


Fig. 4. Comparison of fingerprints of A and G haemoglobins. Peptides 3 and 9, which are displaced, were identified as methionine and histidine by specific stainings

We were able to observe by starch-gel electrophoresis two major components, one of them migrating more slowly than A, similar to haemoglobin G. Two minor components were also detected by *o*-dianisidine staining; one of them was A_2 while the other had a lower R_F. The result strongly suggested that the abnormality was located on the α-chain[8]. This was confirmed by hybridization experiments, and when haemoglobin G and haemoglobin S were dissociated together it was possible to obtain haemoglobin A and a slow abnormal hybrid.

$$\alpha_2^G\beta_2^A + \alpha_2^A\beta_2^S \quad \alpha_2^G\beta_2^A + \alpha_2^A\beta_2^S + \alpha_2^A\beta_2^A + \alpha_2^G\beta_2^S$$

Interspecific hybridization with canine haemoglobin have evidence for a fast hybrid $\alpha_2^{can}\beta_2^A$ with a mobility similar to that of the hybrid obtained with normal haemoglobin A. Conversely, the slow hybrid $\alpha_2^G\beta_2^{can}$ was slower than the hybrid of the normal haemoglobin, α_2^A.

Finally, fingerprinting showed displacement toward the cathode of two peptides, peptide 3 (α *T* IX) and peptide 9 (α *T* IX + free lysine or α *T* VIII). The latter peptide is similar to the former, but has only one supplementary

lysine 61 residue; tryptic digestion between the lysine and peptide α *T* IX was incomplete.

The α *T* IX peptide contains 29 residues (62–90). Only one haemoglobin G has so far been described (haemoglobin $G_{Philadelphia}$)[9] in which asparagine is replaced by lysine. But the pattern described is not similar to that which we found. The presence of one more lysine gives rise to three peptides instead of two, and the new peptides *G-3b*, *G-3b* ox., *G-9a*, show chromatographic as well as electrophoretic displacement.

In our patient, both peptides 3 and 9 were electrophoretically, but not chromatographically, displaced, and we only found two peptides. Our results are not compatible with the substitution of a neutrally charged amino-acid by one with a positive charge. Therefore, the most plausible mechanism would be the substitution of a negatively charged amino-acid by a neutral one; this substitution could well occur at the level of aspartic acid 64, 74 or 85.

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IMMUNOLOGY

Failure of the Antibody Method to demonstrate a Circulating Hormonal Factor after Partial Hepatectomy

Although the extremely rapid regeneration of the liver that occurs after partial hepatectomy has been known for many years[1], the mechanism remains obscure. Many authors have suggested that the cell proliferation is due to a hormonal factor which appears in the blood following this procedure.

The evidence for this is based on the following claims: (*a*) Injections of serum taken from partially hepatectomized rats stimulate mitoses in a normal rat, or accelerate regeneration in another partially hepatectomized rat[2–5]. These claims have, however, been denied[6–8]. (*b*) Partial hepatectomy in one parabiont causes signs of regeneration in the partner[9,10]. Several groups have failed to confirm these findings[11–14]. (*c*) An autograft of liver with a satisfactory new blood supply will show growth stimulation if a partial hepatectomy is performed on the remaining original liver mass[15,16].

It is clear that, although the evidence is conflicting, the presence of a circulating hormonal growth-stimulating factor after partial hepatectomy cannot be excluded. It was considered possible that such a factor might be a protein or polypeptide, in which case it should be practicable to neutralize this by the action of a specific antibody.

Adult male Wistar rats were subjected to a 65 per cent partial hepatectomy by the standard method[17]. 24 h later, when mitotic activity in the liver is just beginning[18], blood was obtained from either the aorta or the portal vein; the latter source was used in view of the possibility that the hypothetical factor might be produced in the viscera drained by the portal vein. Rabbits were immunized with these sera mixed with Freund's adjuvant, according to the schedule of Peetoom[18]. 10 days after the final injection the rabbits were bled. The antisera were tested on an Ouchterlony plate against normal rat serum and post-hepatectomy serum, and were then absorbed with normal rat sera to remove non-specific antibodies.

In order to test the biological activity of these absorbed sera, rats and mice were partially hepatectomized between 2.0 and 4.0 p.m. Serum was injected into the peritoneal cavity at the finish of the operation, then at 10.0 a.m. and 4.0 p.m. the following day, and again at 10.0 a.m. on the second post-operative day. The last injection was accompanied by the subcutaneous injection of colchicine (100 μg for mice, 300 μg for rats). At 4.0 p.m. (that is, 48 h after the partial hepatectomy) the animals were killed, and the liver remnants, after weighing, were fixed in Bouin's fluid. The regeneration on a wet-weight basis and the mitosis count were estimated according to methods described earlier[20].

After several months' immunization, pooled antisera from three rabbits produced marked precipitation reactions with normal rat serum when tested on the agar plate. Normal serum and post-hepatectomy sera gave the same precipitin patterns with the antiserum. This does not, of course, exclude the presence of a specific factor in the post-hepatectomy serum, as an antigen–antibody complex may be soluble, or if insoluble, it may be present in amounts too small to form a visible precipitate.

Table 1. Effect of Antisera on the Regenerating Liver of Mice and Rats 48 h after Partial Hepatectomy

Experimental groups	No. of animals	Percentage of regeneration (wet weight) ± *S.E.*	Percentage of mitosis count ± *S.E.*
Mice			
Control (saline)	36	63·3 ± 3·8	3·06 ± 0·37
Normal human serum	36	73·1 ± 4·1	2·09 ± 0·44
Combined aortic (4) and portal (13) antiserum	17	74·6 ± 6·5 (0·20 > *P* > 0·10*)	2·01 ± 0·56 (0·20 > *P* > 0·10)*
Rats			
Controls (no injections (6) and normal serum (6))	12	63·6 ± 4·7	4·75 ± 0·55
Portal antiserum	10	60·8 ± 9·8	6·92 ± 1·05 (0·10 > *P* > 0·05)

* Compared with saline control.

The biological effects of the antisera are summarized in Table 1. The mice were injected with a total of 2·0 ml. of absorbed antiserum and the rats with 4·0 ml. of serum. It will be seen that no significant effect was produced. Regeneration on a wet-weight basis was slightly elevated in the mice and slightly depressed in the rats; neither change is statistically significant. The mice showed a fall in the percentage of parenchymal cells in mitosis; this also is not significant, and in any event injection of normal serum produced a similar value. In the rats there was actually a very slight rise in the mitosis rate.

Many workers have shown that antibodies can be produced to a wide variety of hormones. It is true that frequently the antigen used has been a relatively concentrated hormone; however, in some instances hormone-rich serum has been used as the antigen; for example, for the production of antigonadotrophin by the injection of crude pregnant mares' serum[21]. Hence the failure to demonstrate biologically active antibodies in the present experiments must be considered as strong, but not conclusive, evidence against a circulating protein or polypeptide factor stimulating liver regeneration. The existence of a non-protein hormone obviously remains open, and other possibilities are that partial hepatectomy causes a lowering of concentration of an inhibitor normally present in serum, or that hormonal factors in the circulation do not play a part in the mechanism of liver regeneration.

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Complement Fixation and *p*H

IBE and Wardlaw have re-investigated the effect of *p*H on the activity of complement[1]. They confirmed previous reports[2] that the haemolytic activity of complement decreases with increasing *p*H and, in addition, presented evidence that complement fixation increases with increasing *p*H. They were able to increase the complement fixation titre of an antigen four-fold by raising the *p*H of the reaction mixture from 7·15 to 8·56. Stein and Van Ngu[3], however, showed that complement fixation titres can be increased by reducing the amount of complement used in the test. Thus, increased complement fixation sensitivity at relatively high *p*H may be due to loss of complement activity rather than to increased complement fixation. Experiments were devised to test this hypothesis.

Veronal buffer of *p*H 7·3 was prepared using the method of Kabat and Mayer[4]. Similar buffer was made to *p*H 8·3 by adding less hydrochloric acid. The conductivities of the two buffers differed by less than 2 per cent. Two chessboard (two-dimensional) complement fixation tests were performed with bovine serum albumin (BSA) and rabbit anti-BSA, one at *p*H 8·3 and one at *p*H 7·3. Both tests were performed with five 50 per cent units of complement. To supply this number of units in each test, more guinea-pig serum had to be used at *p*H 8·3 to compensate for the reduced activity of complement at the higher *p*H. Standard complement titrations were performed to determine the dilution of guinea-pig serum yielding five 50 per cent units of complement at each *p*H. Except for the differences in *p*H and absolute amount of guinea-pig serum used in each test, all other conditions were the same. Under these conditions the results of the two chessboard titrations were identical.

The next experiment was a direct test of the hypothesis that increased sensitivity of complement fixation tests at relatively high *p*H is due to reduction of complement activity. Four antibody titrations were performed with the optimal concentration of antigen found in the two-dimensional complement fixation tests. Parallel rather than serial dilutions were used and the extent of lysis in each reaction mixture was determined spectrophotometrically. Two of the antibody titrations were carried out at *p*H 7·3, one of them with five and the other with four 50 per cent units of complement. Similarly, two titrations were carried out at *p*H 8·3, one with five and the other with four 50 per cent units. The results in Table 1 show that the complement fixation antibody titres vary with the number of units of complement in the test. The apparent increase in complement fixation sensitivity at *p*H 8·3 was precisely duplicated at *p*H 7·3 by reducing the number of 50 per cent units of complement in the test from five to four. The choice of four 50 per cent units of complement was based on the observation that five 50 per cent units of complement at *p*H 7·3 are reduced to four at *p*H 8·3. Thus, the apparent increase in complement fixation sensitivity can be accounted for entirely by the reduced activity of complement at high *p*H. It must be concluded, therefore, that a change in *p*H in the range tested has no intrinsic effect on the efficiency of complement fixation. Sensitivity may be increased by alterations in procedure (for example, changes in *p*H or ionic strength), which inactivate complement, but the same result may be achieved under better control merely by using less complement.

Table 1

No.	Complement				Percentage of haemolysis			
					As 1/5,760–Ag 1/13,600		'1/5-control'	
	*p*H of complement fixation test	50 per cent complement units at *p*H 7·3	50 per cent complement units at *p*H 8·3	Guinea-pig serum dilution	Duplicates	Average	Duplicates	Average
1	7·3	5	4·0	1/109	56·6 49·1	52·8	56·6 56·6	56·6
2	8·3	5	4·0	1/109	39·0 35·6	37·3	33·8 26·9	30·3
3	7·3	4·0	—	1/137	31·6 33·8	32·7	25·4 25·4	25·4
4	8·3	—	5	1/86	52·5 45·0	48·7	55·0 55·0	55·0

No. of units of complement in each test is underlined.

A single lot of guinea-pig serum was used throughout. Complement titrations were performed in a reaction mixture of 7·5 ml. at *p*H 7·3 and at *p*H 8·3. The titres were 219 50 per cent complement units at *p*H 7·3 and 173 50 per cent complement units at *p*H 8·3, a ratio of 5·0 : 4·0.

Complement fixation tests shown in Table 1 were performed with 0·4 ml. BSA 1/13,600; 0·4 ml. anti-BSA 1/2,880, 1/5,760 or 1/11,520; and 0·5 ml. of the appropriate complement solution. The total volume of each reaction mixture, following the addition of 0·2 ml. optimally sensitized sheep erythrocytes, was 1·5 ml. (ref. 5). BSA 1/13,600 was the optimal antigen dilution found in the chessboard titrations which showed an antibody titre of 1/5,760. The reaction mixtures containing anti-BSA 1/2,880 showed no lysis, those containing anti-BSA 1/11,520 showed complete lysis, and those containing anti-BSA 1/5,760 are represented in Table 1. Anti-complementary control mixtures containing only antiserum or antigen, in the presence of three 50 per cent complement units, showed complete lysis. Stock BSA contained 2·5 mg protein/ml.

Each '1/5-control' contained 1/5 the amount of complement solution used in its corresponding test. These controls contained neither As nor Ag. '2/5 controls', containing 2/5 the amount of complement solution, were almost completely lysed. 3/5, 4/5, 5/5 controls were completely lysed.

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New Methods of identifying Serum Siderophilin using Radioactive Iron

In earlier investigations of the immunoelectrophoretic identification of the iron-binding protein siderophilin or transferrin, pure preparations of the protein have been used[1–3]. The preparation of siderophilin is, however, difficult in spite of the fact that methods have been perfected to do this; for example, precipitation with rivanol[2,4]. Wuhrmann and Jasinski[8] were able to show siderophilin in paper electrophoresis by labelling it with radioactive iron. Paper electrophoresis, however, only allows the classification of siderophilin into a specific group of serum proteins, that is, the β-globulins.

In the present experiments, radioactive iron was used for detecting siderophilin in immunoelectropherograms of sera. The method made possible not only the classification of siderophilin into a specific group of proteins, but also the exact identification of the siderophilin arch.

Precipitating sera were obtained from rabbits weighing about 3,500 g, which were immunized with fresh rat and horse sera. Bentonite was used as adjuvant[5]. Adjuvant was mixed with an equal volume of serum. Three days after the last (fifteenth) injection, the serum levels of precipitins were checked in the immunized animals, which were then bled. The sera (with merthiolate added in the proportion 1 : 10,000) were stored at −20° C. Equine precipitating serum No. 512 (from the Pasteur Institute, Paris) was used in the study of human sera.

The isotope iron-55 (half-life 2·94 years) as the chloride in weak solution of hydrochloric acid (Radiochemical Centre, Amersham) was used. To 1 ml. of serum, 0·2 μc. of iron-55 was added, and the mixture was incubated in the refrigerator for 1 h.

The method of Scheidegger[6], as modified by Hirschfeld[7] using agar gel, was adopted. Mixtures of sera with iron-55 were separated electrophoretically for 2·5 h. The precipitating sera described above were used to precipitate proteins. The preparations were washed and dried and, after making autoradiograms, were stained with amide black.

Foton–Roentgen 'Super Films' were used for autoradiography. The preparations obtained by immunoelectrophoresis were covered by roentgen films of dimensions equal to those of the preparation and were exposed for 4 weeks.

After saturating the serum with radioactive iron, immunoelectrophoretic preparations and their autoradiograms were obtained and compared with each other. In the autoradiograms, iron-binding protein gives bright arches.

In Fig. 1, the immunoelectrophoretic pattern of normal human serum (*b*) and the autoradiogram of the same preparation (*a*) are illustrated. The autoradiogram contains a single bright arch, which is an exact reflexion of the arch of iron-binding protein precipitated in the immunoelectropherogram. The arch lies in the β_1-globulin region and is situated near the ditch containing the precipitating serum. Its localization in our experiments is analogous to

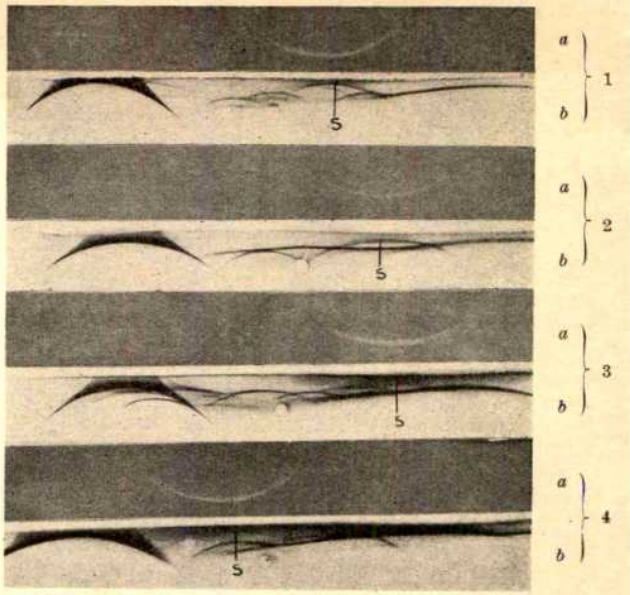


Fig. 1. *a*, Autoradiogram of siderophilin of human serum; *b*, immunoelectropherogram of normal human serum

Fig. 2. *a*, Autoradiogram of siderophilin of horse serum; *b*, immunoelectropherogram of normal horse serum

Fig. 3. *a*, Autoradiogram of siderophilin of rat serum; *b*, immunoelectropherogram of normal rat serum

Fig. 4. *a*, Autoradiogram of siderophilin of sheep serum; *b*, immunoelectropherogram of normal sheep serum

that obtained by Grabar and Burtin[1], Polonovski *et al.*[3], and Kistler *et al.*[2], who utilized pure preparations of siderophilin.

Siderophilin in the autoradiogram of horse serum is also situated in the β_1-globulin region (Fig. 2), and its arch is identical with the β_1-globulin arch in the immunoelectropherogram nearest the ditch containing the immune serum.

The siderophilin in rat serum shows different electrophoretic mobility, lying in the β_2-globulin region (Fig. 3). In the same way as in the preceding serum preparations, the arch of siderophilin of rat serum lies nearest the ditch containing precipitin.

Preliminary experiments showed that the electrophoretic mobility of sheep-serum siderophilin corresponds to that of the α_2-globulins (Fig. 4).

For technical reasons, the isotope iron-55 (half-life, 2·94 years), which has very weak radiant energy, was used in our experiments, which made necessary long exposure of the photographic films. The exposure time can be shortened to several days[8], using the isotope iron-59 (half-life, 47 days), which has much higher energy of β-radiation than iron-55.

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PATHOLOGY

Evidence for the Cell-free Transmission of chemically induced Mammary Carcinoma in the Rat

THE possibility that chemically induced cancer might be the result of the activation of a latent virus has been considered in the past. Some evidence has been reported[1] which indicates transmission of chemically induced tumours by subcellular materials prepared from the latter. Data implicating a virus aetiology of this class of tumours have been inconclusive[2].

In the investigations described here, we have attempted to produce mammary carcinomata in the rat with cell-free preparations derived from the same type of tumour induced in the rat by administration of 7,12-dimethylbenzanthracene (DMBA), as originally described by Huggins *et al.* and by Geyer *et al.*[3].

Female rats of the random-bred Sprague–Dawley strain, approximately 50 days old and weighing 130–150 g, were given a single oral dose of 20 mg DMBA. About 65 per cent developed single or multiple mammary carcinomata within a 6-month period. These tumours were used in the experiments described here.

Tumour tissue preparations from one rat were never pooled with those derived from another rat; however, in some instances, multiple tumours from the same rat were combined. Aseptic procedures were followed at all times. The tumour tissue was rinsed once in Hanks's balanced salt solution, minced with scissors, and the mince was divided into two portions. One portion was ground in a mortar with 'Alundum' in 0·15 M potassium citrate solution containing 1 mg per cent hyaluronidase. This extract was freed from tissue debris and cells by three cycles of centrifugation at 1,900*g*, 2,400*g*, and 12,000*g*, for 20, 20, and 10 min each, respectively. The supernatant from the third cycle of centrifugation was centrifuged at 73,000*g* for 1 h. The resulting pellet was suspended in 0·05 M potassium citrate to give a concentration of 0·5–1·0 g equivalents of the original tumour tissue per ml.

The second portion of the same tumour mince was rinsed twice with Hanks's solution and gently agitated as a suspension in a 0·25 per cent solution of trypsin for 1 h at 37° C. The trypsinized cells were washed twice in Hanks's solution and suspended in complete medium to a concentration of 5–10 × 10^5 cells per ml., and grown on glass in Brockway Flint prescription bottles at 37° C for 2–4 weeks. (The complete medium consisted of 55 per cent minimum essential medium, 30 per cent beef amniotic fluid, 15 per cent calf serum (heated at 56° C for 30 min), 100 U. penicillin per ml. and 100 μg streptomycin per ml. The *p*H was adjusted with a 7·5 per cent sodium bicarbonate solution. All ingredients were obtained from Microbiological Associates, Inc., Bethesda, Maryland.) When the cell sheets from a number of bottles became confluent, the cells and medium were collected and pooled. This suspension was frozen and thawed three times. The suspension thus obtained was centrifuged in the same manner as the first portion of the mince. The final pellet was resuspended in a volume of 0·05 M potassium citrate equivalent to 1/10–1/20 of the original volume of the culture fluid.

Inocula prepared from each tumour by both processes were injected into new-born female Sprague–Dawley rats. Groups of 10–15 new-born rats were each injected with 0·1 ml. of the inocula subcutaneously, intraperitoneally, or, in a few instances, with 0·025 ml. intracerebrally. The injected animals were weaned when they were 3 weeks old and palpated for the presence of tumours at bi-weekly intervals. New-born rats of the same sex and strain, serving as controls, were injected either with similarly concentrated extracts of liver–spleen–lung tissue from tumour-bearing rats, of rat embryo cell cultures, 0·05 M potassium citrate or with 1–10 × 10^5 intact trypsinized cells from DMBA-induced tumours. None of the 800 rats given the foregoing control materials developed mammary carcinomata during 9 months of observation. On the other hand, when inocula derived from 54 DMBA-induced tumours were injected into a total of 1,180 new-born rats, fourteen mammary carcinomata (identified by histological examination) developed from thirteen of the DMBA tumours within 9 months. The results of these experiments, presented in Table 1, show that: (*a*) the incidence of tumour response of the injected animals was very low even in the positive groups; (*b*) the average latent period was about 6 months; (*c*) tumour production was equally successful with tissue culture preparations and tumour tissue extracts; (*d*) tumours also resulted from the injection of extracts that were filtered.

Table 1. PRODUCTION OF MAMMARY CARCINOMATA BY INJECTING NEW-BORN RATS WITH PREPARATIONS DERIVED FROM CHEMICALLY (DMBA) INDUCED MAMMARY CARCINOMATA OF THE RAT

DMBA-induced tumours: Source of inoculum	DMBA-induced tumours: Route of injection	Tumour response: Latent period (days)	Tumour response: Tumour*/total
Cell culture	intraperitoneal; subcutaneous†	100; 224	2/9
Cell culture	intraperitoneal; intracerebrally†	187	1/6
Cell culture	intraperitoneal; intracerebrally†	205	1/12
Cell culture	intraperitoneal	250	1/3
Cell culture‡	intraperitoneal	264	1/10
Cell culture‡	intraperitoneal	283	1/13
Tumour tissue	intraperitoneal	95	1/13
Tumour tissue‡	intraperitoneal	141	1/12
Tumour tissue	intraperitoneal; subcutaneous†	171	1/12
Tumour tissue	intraperitoneal	178	1/11
Tumour tissue	intraperitoneal	193	1/12
Tumour tissue	intraperitoneal	201	1/15
Tumour tissue	intraperitoneal	210	1/22

* Histologically, all tumours were papillary carcinomata or adenocarcinomata.

† Same animal injected by two routes.

‡ Filtered through 'Millipore *HA*' filter impermeable to bacteria (0·45μ).

The small number of tumours obtained in the experimental groups would seem to be significant, however, since none developed in control animals during the same period of time. Further investigations are being undertaken in an attempt to increase the tumour incidence.

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Deoxyribonucleic Acid Synthesis of Hydatidiform Moles in Organ Culture: an Autoradiographic Investigation

MIDGLEY *et al.*[1], using tritiated thymidine, have demonstrated that placental syncytiotrophoblast in the monkey originates from cytotrophoblast. Electron microscopic and immunochemical investigations of benign and malignant trophoblast have supported this concept and have also shown that the differentiated syncytiotrophoblast produces chorionic gonadotrophin[2–4].

A hydatidiform mole is a chorionic growth which morphologically and clinically represents an immature and pathologic trophoblast[5,6]. In the investigation reported here, an attempt was made to label selectively and to follow the maturation pattern seen in trophoblast

from vesicles of hydatidiform moles incubated in organ culture. Tritiated thymidine was chosen as the label, as it is incorporated into newly synthesized DNA in the normal cytotrophoblast[1].

Twenty vesicles of two hydatidiform moles were obtained in the delivery room from women with histories of 13 and 15 weeks of molar pregnancy, respectively. The vesicles were incubated in tissue culture medium 199 with 1 μc. ^{3}H-thymidine per ml. of culture.

Vesicles were recovered from the culture at intervals of 3, 6, 12 and 24 h, fixed in Bouin solution, embedded in paraffin, sectioned at 6μ and coated with Kodak '*NTB* 3' emulsion. After sufficient exposure at 4° C in plastic boxes, the slides were developed and stained with haematoxylin and eosin.

Irregular distribution of silver grain deposits was observed in the cytotrophoblast of vesicles lined by two rows of trophoblast cells, but no label was present in syncytiotrophoblast nuclei. Areas with hyperplasia or anaplasia of the trophoblast showed irregular labelling of cytotrophoblast nuclei in different areas, but there was no evidence of labelling in the syncytiotrophoblast. Labelling of the trophoblast greatly increased in those vesicles that had been left for 24 h in organ culture. A large number of Hofbauer cells and other stromal cells were also labelled by radioactive thymidine (Fig. 1).

When the percentage distribution and variance of the marked nuclei were plotted against the incubation time of the vesicles, an increased proportion of the labelling was found. The same proportion of label was also observed in thin areas of trophoblast as in areas of hyperplasia (Table 1).

These findings suggest that the cytotrophoblast of hydatidiform moles, although it exists in an irregular form, contributes to the formation of syncytiotrophoblast, and that inactive-looking portions of trophoblast seem to be potential areas for developing hyperplasia, as they incorporate thymidine in the same proportion as does hyperplastic trophoblast. Although it might be expected to be active, the latter does not show any greater proportion of any label than do thin areas of trophoblast. It is possible that this finding indicates an alternating capacity for cellular reproduction in these tissues. The presence of labelled nuclei of stromal cells indicates an active incorporation of thymidine; the proportion found was not expected, as mitoses are infrequent in these cells.

Table 1. Percentage of Localization of Tritiated Thymidine in Nuclei of Hydatidiform Moles

Time (h)	'Normal'* Cyto-trophoblast	'Normal'* Syncytio-trophoblast	Hyperplastic Cyto-trophoblast	Hyperplastic Syncytio-trophoblast	Stroma cells
3	5–50	0	5–85	0	3–20
6	10–70	0	10–80	0	10–40
12	20–90	0	20–80	0	20–50
24	20–90	0	20–90	0	10–50

* 'Normal' cytotrophoblast is the inactive form in vesicles.

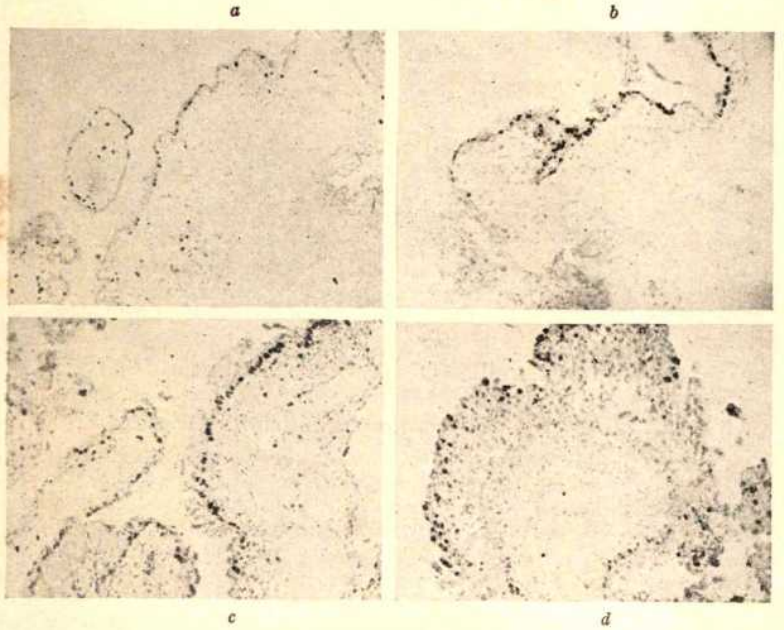


Fig. 1. *a*, Vesicle of hydatidiform mole with a thin layer of trophoblast showing label in cytotrophoblast and some stromal cells (3 h incubation). *b*, Heavy labelling in cytotrophoblast from a small area of hyperplasia and thin trophoblast; absence from small area of hyperplasia (6 h incubation). *c*, Labelling of cytotrophoblast and some stromal cells; syncytiotrophoblast remains unmarked (12 h). *d*, Vesicle with hyperplasia showing irregular label in cytotrophoblast (24 h)

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Nucleic Acids of Malignant and Benign Splenic Hyperplasias in Mice

Cerecedo *et al.*[1–3] and Hilf *et al.*[4], studying the effect of sarcoma 180, melanoma *S*91 and stressing agents on the nucleic acid content of different organs of the mouse, found that the RNA/DNA ratio usually dropped with the growth of the tumours.

In transplanted murine leukaemias the RNA and RNA : DNA ratios were increased in the spleen[5]. Moreover, when the nucleic acids of splenic cells of spontaneous and transplanted leukaemias were investigated, only the RNA was found to be increased in the former, while both DNA and RNA as well as the nitrogen content were augmented in the latter[6]. In induced leukaemias, Rich *et al.*[7] observed that splenic RNA was higher than in the controls.

The work reported in this communication was devised to investigate whether there is any significant quantitative difference in the nucleic acid content of malignant hyperplasias as compared with benign processes, and if so, how do these changes develop with time.

Groups of 5–10 male *BALB* mice about 3 months old were intraperitoneally injected with a cellular homogenate of either a leukaemia (*L*423) or a lymphosarcoma (*L*370). Animals were killed at 2 h, 1, 3, 5, 7 and 10 days after the injection.

Splenic nucleic acids were extracted according to the technique developed by Schneider[8]; RNA was determined by the orcinol reaction as modified by Ceriotti[9] and DNA by a colorimetric method based on the diphenylamine reaction described by Dische[10]. Each determination was repeated three times and compared with the corresponding standard solution; the results are expressed in mg per mg wet tissue. Three groups of controls were used: (1) spleens of normal mice injected intraperitoneally with normal *BALB* splenic cells; (2) spleens of mice injected intraperitoneally with 0·2 ml. of Freund's adjuvant; in this case specimens were obtained over a period of 90 days; (3) spleens of

mice injected subcutaneously with 0·2 ml. of a 1 per cent phenylhydrazine hydrochloride (PH). When the determination of nucleic acids in the spleens of those animals transplanted with *L*423 and *L*370 had been carried out and related to the evolution of the process, it was observed that while RNA disclosed a gradual increase, significant only in the case of *L*423, DNA had a tendency to decrease although the drop was not significant. These modifications in the nucleic acids were more clearly demonstrated by the RNA : DNA ratio, which rose with time in *L*423 (F, 21·3; $P < 0.01$) and *L*370 (F, 9·73; $P < 0.05$) (Fig. 1).

When the same determinations were carried out in those animals injected with normal splenic cells, no changes in the nucleic acids were detectable ($P > 0.4$; > 0.3).

When PH was administered, although DNA and RNA both showed a non-significant transitory increase, the RNA : DNA ratio disclosed a progressive and significant fall (F, 12·8; $P < 0.05$) (Fig. 1).

When Freund's adjuvant was injected, although the splenic RNA showed a slight tendency to increase while the DNA tended to decrease, neither of them varied significantly. However, the RNA : DNA ratio showed a transitory and significant increase in the first 10 days (F, 10·4; $P < 0.05$) (Fig. 1), after which it levelled off again. In Fig. 2 the various RNA : DNA ratios are plotted against the log spleen weight, and since the slopes of both benign hyperplasias are similar their values have been pooled. The remaining slopes are significantly different (F, 5·67; $P < 0.01$). When the values of RNA : DNA ratios are adjusted to a mean log spleen weight common to all groups, their difference is highly significant (F, 7·8 ; $P < 0.01$). This means that for the same log spleen weight the RNA : DNA ratio is greater in the two malignant groups.

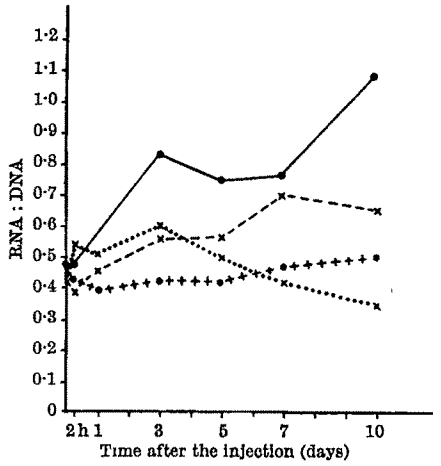


Fig. 1. RNA : DNA ratios in benign and malignant hyperplasias plotted against time Fitting the curves by lineal regressions the significance of the slopes are: Lymphosarcoma (*L*370), F, 9·73, $P < 0.05$ (●——●); leukaemia (*L*423). F, 21 3, $P < 0.01$ (× — ×); Freund's adjuvant (FA), F, 10·4, $P < 0.05$ (● + + + + ●); phenylhydrazine hydrochloride (PH), F, 12·8, $P < 0.05$ (× ···· ×). Note that phenylhydrazine hydrochloride has, in part, a negative slope

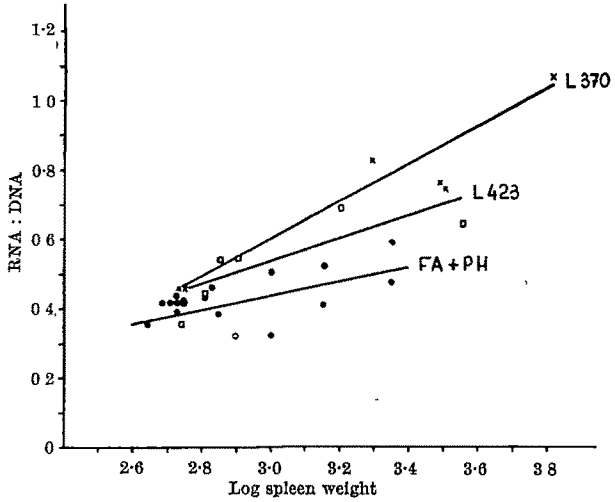


Fig. 2. RNA : DNA ratios in benign and malignant hyperplasias plotted against log spleen weight. These curves proved linear and their slopes are significantly different (F, 5·67, $P < 0.01$). The values of Freund's adjuvant and phenylhydrazine hydrochloride have been pooled because their slopes are not significantly different

It is possible to assume, according to these experiments, that the increases observed in animals injected with *L*423 and *L*370 are related to the malignant nature of the processes and not only due to splenic hyperplasia or rapid growth, as postulated by Peterman[6]. In fact, mice treated with PH showed an increase in spleen weight comparable with or even greater than that seen in the malignant groups. It is also of interest to note that with *L*423 splenic RNA showed a gradual and progressive increase; however, Peterman[5], working with a transplantable leukaemia, reported that RNA, although increased, did not change with the evolution of the disease. In conclusion, the malignant processes studied in these experiments show a definite modification in the RNA : DNA ratios which seems to be characteristic of malignancy since no similar degree of alteration was observed in the benign hyperplasias when related either to spleen weight or time of evolution.

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BIOLOGY

Normal Distribution of Cell Generation Rates

The distributions of generation time are markedly skewed, but the reciprocal of the generation time, termed 'generation rate', was reported to have a normal distribution for a bacterium, yeast, a strain of human tissue culture, and the protozoan *Tetrahymena geleii*; these results provided evidence for the generalized nature of this distribution in widely different kinds of cells in balanced growth[1]. Although other measurements had not always given a normal distribution of generation rates, this was to be expected since the sources of experimental bias were not then completely understood. There are several kinds of bias that must be avoided[2]. Furthermore, some experiments in the literature have given no clear test of the nature of the distribution, since data are insufficient, with rather fewer than 100 cell divisions measured. Nevertheless, it has been asserted that the skewed character of the generation-time distribution has decreased as experimental techniques have advanced[3], and this would require that the generation rates depart from a normal distribution. This communication presents further evidence for the validity of the normal distribution for generation rates.

In an extensive study of the individual generation times of another protozoan, *Euglena gracilis*, grown either in a salt medium (790 cells) or in a complex organic medium (548 cells), Cook and Cook observed skewed distributions of generation time[4]. Their data (kindly sent to me) provide excellent evidence for normal distributions of generation rate for *Euglena* in each of these media, and are replotted in Fig. 1. It may be seen from this that there is no significant departure from the normal distribution for generation rates as small as three standard deviations below the mean (in each case the lower three points are the sums of one, two, and two observations respectively) and for generation rates as large as two standard deviations above the mean. The remarkable agreement at rapid rates was unexpected, since it seemed obvious that some maximum rate must exist for cell reproduction. Clearly, these data strongly support the normal distribution of generation rates, modified only at large generation rates by the requirement of a minimal time for the completion of the cycle of cell division.

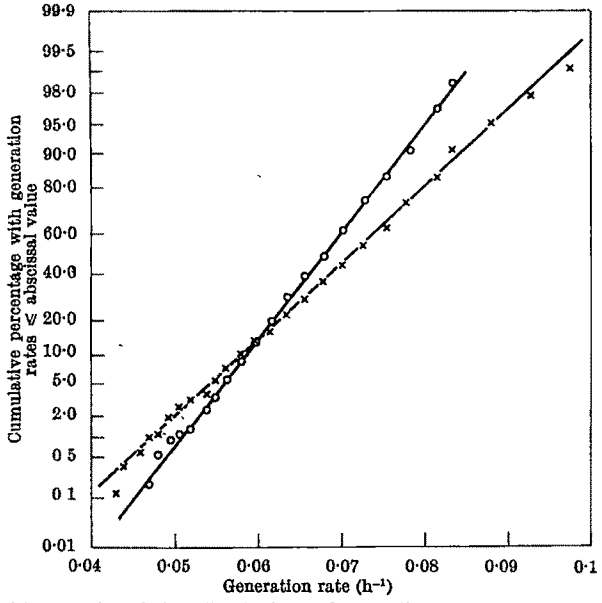


Fig. 1. Cumulative distributions of generation rates for *Euglena gracilis*. The data of Cook and Cook[4] are plotted as cumulative distributions of generation rates on arithmetic probability coordinates. ×, Cells growing in a salt medium; ○, cells growing in a complex organic medium

It should be pointed out, however, that the cell interdivision times are not independent from one generation to the next: the data for *Escherichia coli*[1] fail to show the required increase in standard deviation of the sums of the times of successive divisions[5]. A more complete analysis is in progress.

Finally, the results of Cook and Cook partially modify an earlier suggestion of mine that the control of cell division might tend to fall into either of two classes under conditions of balanced growth[2]. The average values of the coefficient of variation of generation rate ($C.V.$) that had been observed for the two classes (along with their standard errors) were 9·4 (±0·2) per cent and 18·8 (±0·6) per cent. For *Euglena* grown in organic medium the value of $C.V.$ is 10·6 (±0·3) per cent, which tends to fall into the first group above. However, the value with the salt medium is 16·0 (±0·4) per cent, which does not fall into either group. Thus, it is apparent that the variability in cell division can be modified by environmental conditions, even for cultures in balanced growth.

These results can readily be understood if the generation time depends on several or many reactions, any one of which might be transiently rate-limiting for cell replication. For cells growing in a complex organic medium the number of different kinds of reactions that the cell must carry out will be reduced by the exogenous supply of compounds that would otherwise necessarily be synthesized. Here, the ready availability of these compounds would reduce the time necessary for cellular replication, eliminating the contribution of these reactions to the over-all generation time and thereby decreasing the value for $C.V.$

The clustering of values of $C.V.$ which remains when cells are allowed to grow at nearly maximum rates suggests, further, that the residual variability in generation rates might arise essentially from the variability remaining in a single process; for example, the time needed to replicate DNA. The mechanism by which some cells achieve tight control of cell division (that is, $C.V. \approx 9{\cdot}5$ per cent) while others have their cell division only poorly controlled ($C.V. \approx 19$ per cent) is not known. However, further evidence that there are two discrete states of the control of cell division has been obtained for the yeast *Saccharomyces cerevisiae:* either of two values of $C.V.$ is obtained over a temperature range[6].

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Prolactin and Serum Osmolality of Hypophysectomized Killifish, *Fundulus heteroclitus*, in Fresh-water

The presence of the pituitary[1], or, more specifically, the presence of the erythrosinophilic cells of the proadenohypophysis[2], is necessary for fresh-water survival in the euryhaline cyprinodont, *Fundulus heteroclitus*. Failure, with symptoms of asthenia, is correlated with a fall in serum chloride. Hypophysectomized *F. heteroclitus* can be maintained in fresh-water by intraperitoneal injections of *Fundulus* pituitary brei. Crude pituitary preparations from other species may be partially effective (perch, rat). Replacement therapy with synthetic or partially purified hormones led to the discovery that mammalian adenohypophyseal preparations with prolactin activity can prevent failure[3,4]. Purified ovine prolactin was highly effective, and significant prolongation or maintenance of survival was observed with bovine prolactin and with primate growth hormone preparations containing prolactin activity. Ovine prolactin also prolongs fresh-water survival in hypophysectomized *Poecilia latipinna*[5], *Xiphophorus maculatus*[6], and *Tilapia mossambica*[7].

Preliminary data, reported in this communication, demonstrate that injections of ovine prolactin, at a suitable dose, enable hypophysectomized *F. heteroclitus* to maintain normal serum osmolality. Serum was taken from selected groups of fish the failure or survival of which in fresh-water has been the subject of a separate communication[4]. Osmolality was estimated on 6–15 μl. samples, from individual fish, by comparison with standard sodium chloride solutions, using a 'Mechrolab' vapour pressure osmometer equipped with Hamilton microsyringes. The results are expressed in terms of equivalent osmolality (Table 1).

Normal values for serum osmolality of hypophysectomized *F. heteroclitus*, maintained in salt-water, averaged 0·372. This is in the expected range for intact fish under similar conditions. Effective treatment with ovine prolactin (105 mU./g, alternate days) resulted in essentially similar values for the serum of hypophysectomized fish maintained for 28 days in fresh-water. In contrast, low levels were observed in the serum of fish taken at the onset of asthenia (tilting) in fresh water. This datum was

obtained from fish that had received ineffective replacement therapy: four fish had been treated with bovine growth hormone and had failed in 6–10 days; five fish treated with arginine vasotocin (0·0225 mU./g daily) had failed even more rapidly. Monkey growth hormone, containing prolactin activity equivalent to 40 mU./g on alternate days, prolonged survival but failure ensued in 18–22 days. Ovine prolactin administered at a high dose (350 mU./g, alternate days) was less successful than the lower dose reported here: one fish that failed after 17 days had a serum osmolality of 0·252; another that was still alive after 22 days in fresh-water had a serum osmolality of 0·289. This may be near the lower limit for survival, since one fish receiving arginine vasotocin failed with a serum osmolality of 0·265, the highest recorded at onset of asthenia.

These results may be compared with Burden's[1] data for serum chloride: hypophysectomized *F. heteroclitus* in salt-water averaged 152 mM Cl/l., hypophysectomized *F. heteroclitus* at onset of asthenia in fresh-water averaged 66 mM Cl/l. It is possible that Burden's fish were taken in a more advanced state of failure, as the serum chloride was 43 per cent of normal, whereas, in our experiments, the serum osmolality was 63 per cent of normal. Further clarification would require the simultaneous determination of all major solutes contributing to serum osmolality.

The failure of teleostean neurohypophyseal peptides to prolong fresh-water survival in hypophysectomized *F. heteroclitus*, documented in the article already cited[4], is associated with a concomitant inability to maintain serum osmolality, reported in the present investigation. In contrast, ovine prolactin, which was highly effective, contained little or no activity that would indicate the presence of contaminating traces of neurohypophyseal hormones.

The mode of action of prolactin requires further investigation. Restoration of impaired function of the mucus-secreting cells may be suspected. Removal of the mucous coating of the skin severely disturbs salt- and/or fresh-water tolerance in various teleostean species[6]. Burden reported atrophic changes in the mucous cells of the gills in hypophysectomized *F. heteroclitus*. On the other hand, prolactin stimulates the secretion of 'discus milk' in *Symphysodon discus*[9], and a single injection causes a transitory increase in the abundance of mucous cells in the skin of *S. aequifasciata axelrodi*[10]. Another possible contributory action has been reported: prolactin increases urine flow and decreases urinary sodium concentration in hypophysectomized *Fundulus kansae*, maintained in hard tap water[11]. The impaired kidney function of hypophysectomized fish is, in this respect, restored to normal. Furthermore, in agreement with our findings on *F. heteroclitus*, prolactin enables *Poecilia latipinna* to maintain serum sodium at near normal levels, 24 h after hypophysectomy in fresh-water[12]. Osmoregulatory processes are more primitive in myxinoids, and the failure of prolactin to increase the diminished serum sodium and potassium of intact hagfishes adapted to 60 per cent salt-water[13] may have no bearing on teleostean problems.

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Table 1. SERUM OSMOLALITY OF HYPOPHYSECTOMIZED MALE *Fundulus heteroclitus**

Group	Serum osmolality, mOsm./kg water Mean	s†
(1) Five fish maintained in salt-water‡	371	±6
(2) Thirteen fish at onset of failure in fresh-water (see text)	232	±19
(3) Three fish treated with ovine prolactin, in healthy condition after 28 days in fresh-water	375	±24

* All experiments at *c.* 15° C.
† Standard deviation for small numbers.
‡ Hypophysectomized 7 months previously, used in an experiment with fish pituitary fractions and allowed to recover for 3 months before sampling; salinity of water at time of autopsy 34 per cent.

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Uncertainty of Leadership-rank in Fallow Deer

SOCIAL animals may have group 'leaders' which respond to external stimuli more rapidly than do other individuals, and thereby influence the behaviour of the whole group. Allee *et al.*[1] described leaders in a flock of pekin ducks, but noted only the frequency with which specific individuals led the rest of the group. Apparently no one has investigated ordering among the 'followers', so that it is not known whether the rest of a group tends to follow the leader in a definable sequence.

As part of a behavioural study[2] of the fallow deer (*Dama dama*), six pregnant does were penned and marked for individual identification at the Duke University Field Station for Animal Behavior Studies. When frightened, as by approaching persons, the does ran from one part of the enclosure to another along a length of fence. The sequence of individuals became stable shortly after their start and was noted at the midpoint of their travel by one of us (B. K. G.) on 101 occasions. Does were thus scored as rank one (first in line) to rank six on each occasion.

If each rank assignment is an x and there are n assignments the mean rank $(\bar{x})$ of each individual is:

$$\bar{x} = \frac{\sum_{n=1}^{101} x}{n} \qquad (1)$$

The uncertainty in rank of each individual (U_x) is computed from Shannon's[3] information equation in which the probability (p_i) of occurrence of rank i is estimated by the ratio of x's in i to the total number of x's (n), and the solution is in bits of uncertainty:

$$U_x = \sum_{i=1}^{6} p_i \, (-\log_2 p_i) \qquad (2)$$

Fig. 1 shows that stability in rank is very highly correlated with mean rank. Under the conditions of the experiment one can refer to a single group leader. However, other does do not merely comprise a group of followers; they tend to follow the leader in a linear sequence which becomes more variable as leadership-rank decreases. We cannot say from the present observations whether leadership-rank is related to dominance-rank in these individuals. Nor can we say whether the positive correlation of leadership-rank and rank-certainty is maintained in differently sized groups.

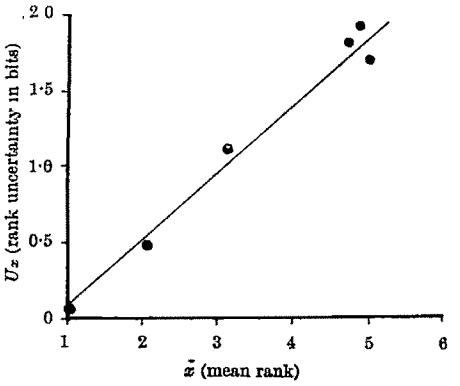


Fig. 1. The linear correlation between decreasing leadership rank ($\bar{x}$) and increasing uncertainty of rank (U_x). Each of the six points represents a particular doe of the herd. The line is fitted by eye

It is possible that differences in responsiveness—dependent primarily on individual physiological factors (for example, sensory capacities and response latencies) and psychological factors (for example, nervousness)—produce the observed sequences. Alternatively, the data may illustrate a parameter of social organization heretofore not predicted by behavioural theory.

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Metabolism of *Spirogyra* during the Conjugation Process

Metabolic changes corresponding with morphological change during the conjugation process of *Spirogyra* sp. were investigated.

The activity of endogenous respiration in intact cells was found to change during this process as seen in Fig. 1. The activities of NADH oxidase, NADH- and succinate-cytochrome *c* reductases, and cytochrome *c* oxidase were found to vary during the conjugation process in parallel

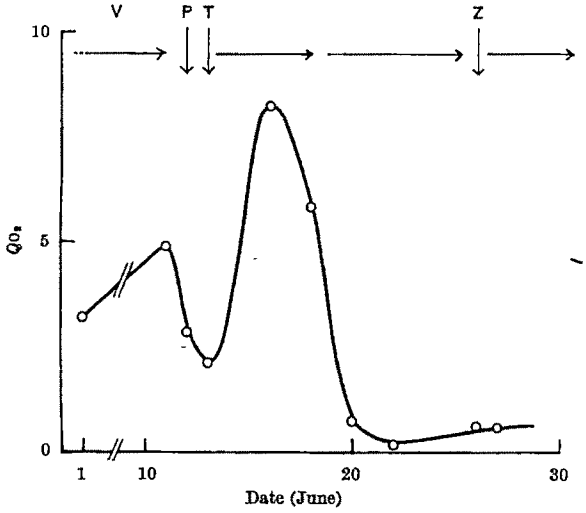


Fig. 1. Activity of endogenous respiration of *Spirogyra* sp. at different stages of the conjugation process. Conditions: intact cells, 200 mg wet weight; phosphate buffer, 0·02 M, *p*H 7·2; total volume, 2·0 ml; at 30° C

Table 1. Amounts of RNA and DNA in *Spirogyra* at Different Stages of Conjugation Process

Cells	Modified Ogur–Rosen method (refs. 1, 2)					Schmidt–Thanhauser method (ref. 3)				
	μg/mg dry wt.		μμg/cell		RNA/	μg/mg dry wt.		μμg/cell		RNA/
	RNA	DNA	RNA	DNA	DNA	RNA	DNA	RNA	DNA	DNA
*V**	6·17	1·37	840	187	4·5	6·20	1·13	845	154	5·5
*T**	5·86	1·61	1,167	321	3·6	6·69	1·73	1,132	344	3·8
*Z**	9·33	2·61	2,303	644	3·6	7·28	2·15	1,797	531	3·4

* *V*, Vegetative cells; *T*, conjugation-tube formed cells; *Z*, zygote.

with the change of respiratory activity. The amounts of oxygen evolved, the oxidative activity of reduced indigo carmine, and the activities of oxidation and reduction of cytochrome *c* in light, declined at the late conjugation stage both in intact and cell-free states.

The content of nucleic acids was also found to vary during the process (Table 1). The amount of DNA per mg dry weight of cells was increased by about 20 per cent in conjugation-tube formed cells (*T*-cells) and by about 200 per cent in zygote (*Z*-cells) compared with that of vegetative cells (*V*-cells), but there was not such a percentage increase of RNA. Calculation of the DNA content per cell showed that this was increased about 1·7-fold in *T*-cells and 3·4-fold in *Z*-cells compared with *V*-cells, while RNA was increased in *T*-cells and in *Z*-cells by about 1·4-fold and 2·7-fold, respectively. From these results one can see that the DNA and RNA per cell of zygote is approximately twice that of a *T*-cell. As shown in Table 1, the ratio of RNA to DNA in *T*-cells was the same as in *Z*-cells and less than in *V*-cells.

When the cells were growing and dividing normally, most of the cells observed by day were short in length (172–174μ) and elongated cells (342–344μ) were rarely observed. The width of both cells was about 115μ. The former were designated as 'short' cells and the latter as 'long' cells. It is of interest that *T*-cells were originated only from 'short' cells and not from 'long' cells, and DNA content of each 'long' cell was equivalent to that of a *T*-cell and about twice that of a 'short' cell. DNA in *T*-cells, half of which might be synthesized at the earlier conjugation stage, seems to be closely connected with conjugation-tube formation, because *T*-cells are formed without cell division and differ in length from 'long' cells.

Estimation of basic proteins in the larger particulate fractions (nuclear and chloroplast) showed a gradual increase during the conjugation process. From this fact, it may be assumed that the decline of enzyme activities, at the later stages of this process, is due to the action of some basic proteins depressing the formation of messenger-like RNA and of enzymes. Investigations on the properties of the basic proteins are in progress, and the details of this study will be published elsewhere.

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Variable Photosynthetic Responses of *Pinus resinosa* Seedlings to Herbicides

Herbicides have shown variable toxicity to young gymnosperms; the susceptibility of these plants greatly varies according to the chemicals and dosage used, the species, and the age of the plants[1–4]. Among the more obvious deleterious responses of young *Pinus* plants to certain herbicides are twisting and curling of cotyledons, distortion of old needles, chlorosis, and subsequent plant mortality. The incorporation of herbicides by the soil usually causes greater injury to plants than does the application of them to the soil surface[3,4]. Comparisons

of cross-sections of needles from young control *Pinus resinosa* and herbicide-treated plants showed marked effects on needle anatomy as a result of treatment[5]. Needles from control plants were approximately semicircular in outline. Their mesophyll cells were closely packed and infolded, and they contained large chloroplasts. In contrast, needles of plants treated with atrazine, simazine or prometryne were distorted and chlorotic. Their mesophyll cells were often rounded and the infolding characteristic of normal mesophyll tissue no longer occurred. Frequently the mesophyll cells became vacuolated or nearly empty and sometimes large gaps were present among them[5]. These experiments have now been extended in order to evaluate the effects of five structurally different herbicides on photosynthesis of 3–0 *Pinus resinosa* Ait. seedlings.

Experiments were performed in north-central Wisconsin, beginning in early June 1964, and continuing for up to 60 days. The length of time that a particular herbicide was tested depended on the plant response. If photosynthesis reached a negligible value after treatment measurements were then discontinued.

Herbicides tested included DCPA, EPTC, atrazine, 2,4-D, and monuron. Test plants, 15–20 cm in height, were grown out of doors in plastic pots containing sandy loam soil. Each treatment consisted of five replicates (plants) and five non-treated seedlings were used as controls. Each herbicide was applied at 20 lb./acre of active ingredient suspended in 50 ml. of water. This was carefully mixed into the soil of the potted seedlings. Plants were watered regularly. After treatment, each test plant was brought into the laboratory, at approximately weekly intervals, in order to measure the photosynthesis of the entire shoot; the seedling was then returned out of doors. Apparent photosynthesis was measured, for 10 min in a closed system, as the uptake of carbon dioxide, and for this purpose a Beckman 15*A* infra-red gas analyser was used. The seedling shoot was enclosed in a sealed metal photosynthesis chamber which had a glass window at the top. Light was provided by a single high-intensity lamp, and was 10,000 ft.-candles at the top of the chamber and 6,000 ft.-candles at the bottom. Temperature in the shoot chamber was maintained at 29°–30° C by using two layers of water filters.

The various herbicides showed marked differences in their effects on photosynthesis. In control plants the rate of photosynthesis increased gradually with each weekly measurement and by 5 weeks the uptake of carbon dioxide had increased by about half. This was apparently caused mainly by increased leaf area following bud opening and expansion of the new shoots. It is probable that the increase also reflected the increasing efficiency of chlorophyll and the improved conditions for photosynthesis—especially the increasing temperatures and the somewhat higher light intensities out of doors. Plants treated with DCPA showed very similar responses to control plants, and no significant differences were found between the two groups. Plants treated with DCPA showed a healthy green colour throughout the experiment.

In contrast to the treatment with DCPA, EPTC, atrazine, 2,4-D and monuron depressed photosynthesis to varying degrees. The photosynthetic rates of plants treated with EPTC increased very slightly for at least 2 weeks after the herbicide had been applied. However, as from the third week the photosynthesis decreased gradually until at 45 days the rate was only about a third of that shown by the control plants. The appearance of EPTC-treated plants was markedly different from that of plants treated with other herbicides. Thirty days after treatment with EPTC the new foliage showed slight chlorosis, while the old needles retained a healthy green colour. At 40 days, necrosis of new needles was observed—this being characterized by a bright, reddish-brown colour. Unlike the effects produced by treatment with other herbicides, this response progressed downward in the plant. Uptake of carbon dioxide was still detectable 57 days after treatment, by which time almost all the needles had turned brown and wilted.

Atrazine rapidly depressed photosynthesis, and no uptake of carbon dioxide was detectable after 4 weeks. Colour changes were already discernible 3 weeks after treatment and the 2-year-old needles appeared flaccid and yellow-green. At 4 weeks all needles were flaccid and yellow, and mortality soon occurred rapidly. Two of the plants were dead within 4 weeks and all five were dead within 5 weeks.

2,4-D also rapidly depressed photosynthesis, and the pattern of decline in photosynthesis was similar to that following treatment with atrazine. However, the colour changes were different, and plants treated with 2,4-D showed a whitish, bleached appearance in contrast to the yellow-green colour of those treated with atrazine. Photosynthesis was negligible 5 weeks after 2,4-D treatment. Some chlorophyll degradation was evident 2–3 weeks after treatment. At 4 weeks colour changes were conspicuous in the new needles and some abscission of leaves had occurred by that time. Leaf abscission did not ensue from treatment with any of the other herbicides.

Of all the herbicides tested, monuron depressed photosynthesis most rapidly. However, visible symptoms of damage to leaves following treatment with monuron were practically unnoticeable. Absorption of carbon dioxide could not be detected 11 days after treatment. At that time no change in leaf colour or condition was apparent. The needles were green even after 30 days, but after 40 days they were a weak green colour similar to those of the atrazine-treated plants.

These experiments once again emphasize the extreme variability of physiological responses of plants to different herbicides. Furthermore, they emphasize that the mechanism inhibiting photosynthesis varies from herbicide to herbicide, and that the depression of photosynthesis occurs with or without breakdown of chlorophyll.

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Role of the Leaf in Flowering of Late Pea Varieties

Late (*Sn Sn*) pea varieties respond to long days and vernalization by flowering at a lower node[1]. Paton[2] suggested that the product of leaf area and photoperiod governs the first node at which flowering occurs. If this is so, defoliation treatments should have an effect on the node of first flower (*F*). I have conducted several experiments to test this hypothesis. In general, the only conclusion to be drawn from the results (those for a typical experiment are shown in Table 1) is that leaves apparently have little effect on flowering.

Recently, Cathey and Borthwick[3] have shown that the ratio of red to far-red light reaching the cells may be the controlling factor in flowering, by governing the concentration of physiologically active phytochrome. This ratio would not be affected by defoliation treatments and it thus appears likely that *F* is a function of photoperiod only, not of leaf area as well, provided that a minimum area is present for photoreception. The stipules of the pea plant, which were left intact during the present experiments, may well be adequate for this purpose since they contribute up to half the photosynthetic area.

Table 1. Effect of Defoliation at Nodes indicated (Cotyledonary Node taken as 0) on Node of First Flower (*F*) and Total Shoot Height of the Late Pea Variety, Greenfeast*

Nodes defoliated		*F* (node No.)	Height (cm)
None—control		16·27	31·0
3,4	*E*	16·15	27·8
3,4	*L*	15·76	26·4†
3,4,5,6	*E*	15·60†	25 0‡
3,4,5,6	*L*	15·60†	22·8‡
3,4,5,6,7,8	*E*	15·97	23·0‡
3,4,5,6,7,8	*L*	16·02	22 2‡
3,4,5,6,7,8,9,10	*E*	16·12	23 2‡
3,4,5,6,7,8,9,10	*L*	16·03	21·0‡

* Plants grown in 16 h photoperiod. *E* and *L* denote whether leaves were removed when just unfolding (*E*) or when expanded (*L*). Nodes 1 and 2 bear only scale leaves.
† Significantly different from control at 5 per cent level.
‡ Significantly different from control at 1 per cent level.

Table 2. Effect of Cotyledon Excision at Different Times after Germination (Day 0) on Node of First Flower (*F*) and Total Shoot Height of the Late Pea Variety, Greenfeast

Plants grown under natural long days (about 14 h) Age when cotyledons excised (days)	*F* (node No.)	Height (cm)
4	17·13 ± 0·20	26·38 ± 2·81
6	18·16 ± 0·19	33·32 ± 1·88
8	18 63 ± 0·21	49·54 ± 1·87
10	18·96 ± 0·20	52·00 ± 1·18
12	19 28 ± 0·26	51·68 ± 1·41
Control, cotyledons intact	19·71 ± 0·26	53·67 ± 1·12

Observed correlations between (leaf area × photoperiod) and *F* in intact plants may be due to vegetative growth processes and flowering being similarly affected by environment.

Severe defoliation treatments reduce vegetative growth. Similar results may be obtained by removing cotyledons during the early stages of growth. However, unlike the removal of foliage leaves, removal of cotyledons has a marked effect on flowering, reducing *F* (Table 2 and ref. 4). Comparison of Tables 1 and 2 suggests two points. First, that the observed differences in *F* cannot be explained by alterations in the rate of node formation, since vegetative growth is affected similarly, but flowering differently, by leaf and cotyledon removal. Secondly, that foliage leaves and cotyledons have distinct roles in the flowering process. Cotyledons, as first proposed by Barber and Paton[5], contain a flower inhibitor. This is quantitatively transported to the apex during the first 2 weeks of growth, as shown in Table 2. Foliage leaves are obviously involved in the photoperiodic response, but in a qualitative rather than a quantitative fashion, possibly by supplying both phytochrome and a substrate for its action[6]. The relationship between the effects of foliage leaves and cotyledons is not yet clear.

These experiments were carried out while I was in receipt of the Imperial Chemical Industries of Australia and New Zealand research fellowship of the University of Tasmania.

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Chlorophyll Content of *Deschampsia flexuosa* Seedlings grown on a Calcareous and a Non-calcareous Soil

It is well known that many plants characteristic of acid soils become chlorotic when grown on calcareous soils. This phenomenon is usually referred to as lime-induced chlorosis and has been associated with impeded iron metabolism of the plants grown on calcareous soils. This communication describes an unusual alteration in the ratio of chlorophyll *a* to chlorophyll *b* in plants showing chlorosis. Seedlings of *Deschampsia flexuosa* were grown on a mor soil collected from the surface, 5 cm beneath a *Deschampsia flexuosa* litter layer, and on a protorendzina soil collected from carboniferous limestone ledges where the soil depth seldom exceeded 5 cm. Both soils were sieved and placed in plastic pots. Seeds were germinated on moistened filter paper and seedlings planted one per pot. The plants were grown in a glass-house with additional illumination from Phillips 'Daylight' fluorescent tubes. The soils were moistened daily with glass-distilled water. Plants were harvested at intervals over a period of 6 weeks. Chlorophyll was estimated in an 80 per cent acetone extract of the fresh leaves from the optical densities at wave-lengths 645 and 663 mμ (ref. 1).

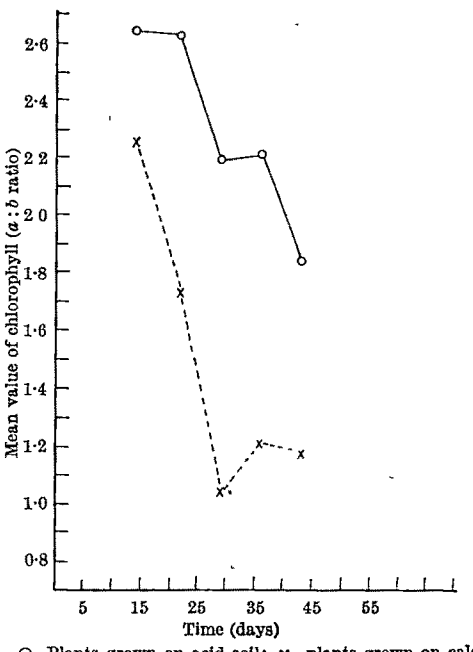


Fig. 1. O, Plants grown on acid soil; ×, plants grown on calcareous soil

In the first fortnight of growth there was a suggestion of a lower chlorophyll *a* : *b* ratio in the plants on the calcareous soil (Fig. 1). This became statistically significant after three weeks, due mainly to a lower chlorophyll *a* content (Fig. 2). Plants on both soils showed a fall in the chlorophyll *a* : *b* ratio with time but it is thought likely that the fall in plants growing on the acid soil represents an ontogenetic drift. By the end of the experiment there was a marked discrepancy in the growth of the plants in the two treatments. Plants grown on the acid soil had tillered several times while those grown on the calcareous soil had not. Chlorophyll *b* contents were similar in both treatments and showed no appreciable fall with time.

Some authors have implicated the bicarbonate ion as a causal agent in lime-induced chlorosis[2–4]. It was, therefore, decided to see if the bicarbonate ion had a similar effect on the chlorophyll content as did the calcareous soil. Seedlings were grown in water culture using the nutrient solution of Arnon and Hoagland[5] at one-fifth strength. Twenty m.equiv./l. sodium bicarbonate was added to one series of nutrient solutions and a similar concentration of sodium sulphate to the other. The *p*H's of the nutrient solutions were adjusted to *p*H 7·5. After 14 days, during which time the solutions were changed every 3 days, the plants were harvested and the chlorophyll contents determined. Chlorophyll *a* : *b* ratios were significantly lower in the bicarbonate treatment due mainly to a lower chlorophyll *a* content; for 18 deg. of freedom $t = 3{\cdot}83097$, $P = < 0{\cdot}01 > 0{\cdot}001$ (Table 1).

Table 1

	Chlorophyll *a**	Chlorophyll *b**	Chlorophyll *a* : *b*
Arnon/5 + 20 m.equiv./l. Na_2SO_4	1·04	0 53	1·97
Arnon/5 + 20 m.equiv./l. $NaHCO_3$	0·82	0·50	1 64

* Mean values in mg/g fresh weight.

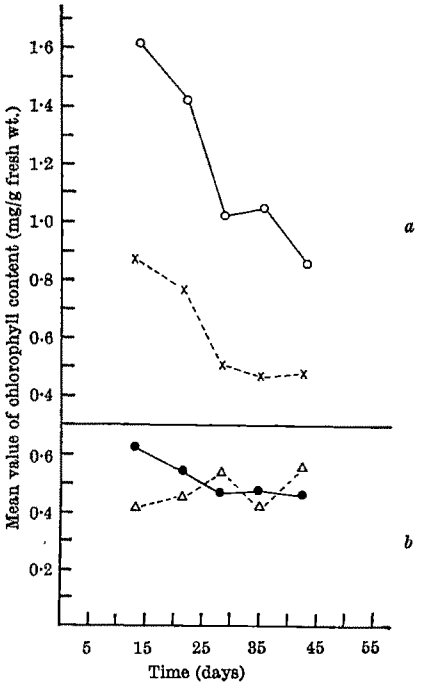


Fig. 2. *a*, Chlorophyll *a*. ○, Acid soil; ×, calcareous soil. *b*, Chlorophyll *b*. ●, Acid soil; △, calcareous soil

Thus the addition of bicarbonate ions to the nutrient solution caused a change in the chlorophyll *a* : *b* ratios in the same direction and in a similar manner to growth on a calcareous soil. Whether the bicarbonate ion is a direct cause of this change or whether it affects the availability and absorption of other ions from the nutrient solution was not determined. Plants grown on the calcareous soil developed a temporary purpling of the leaves symptomatic of phosphorus deficiency. No such purpling was recorded in growth on water culture. The work of Wallihan[6], showing that bicarbonate ions inhibit the uptake of ^{59}Fe by citrus roots, suggests that the present effects could be the consequence of a similar process. It is not possible to say whether the observed chlorophyll irregularities were produced by similar processes in the soil and in the water culture.

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ENTOMOLOGY

Effect of Zinc Ions on Mitosis in Wing Anlagen of *Ephestia kühniella* Z. *in vitro*

Some heavy metal ions, particularly zinc, have been shown to imitate the cytological effects of the insect molting hormone, 'ecdyson', in explanted dipteran salivary glands[1]. The use of such substances as hormone imitators would greatly facilitate research by providing readily available substitutes for the actual hormone. It is therefore worth while investigating this possibility further. This report describes the action of zinc on mitosis in cells of wing anlagen of meal moth larvae, *Ephestia kühniella* Z.

Experimental animals were mass-reared at 25° C until the seventh day of the last larval instar according to previously described methods[2,3]. The larvae were then anaesthetized with ether, immersed in 0·9 per cent sodium chloride, and the hind wing buds removed from the metathorax through dorsal and lateral incisions in the body wall.

Fixation and dyeing were achieved simultaneously by immersing the anlagen for 2 h in a solution of four parts aqueous aceto-carmine, three parts 94 per cent ethyl alcohol, and one part glacial acetic acid. The aceto-carmine was prepared by adding 1 g carmine to 100 ml. distilled water and 100 ml. glacial acetic acid, boiling the solution for 3 h and then filtering. The anlagen were mounted in a mixture of 1 : 1 'Celodal' (Merck A.G., Darmstadt) and glacial acetic acid. Mitoses were counted under a magnification of 500 times.

Test solutions were made by dissolving Merck analytical grade zinc chloride containing 1·2 per cent zinc oxide in distilled water and filtering before use. All solutions were brought to approximately the same molarity (0·15 M) by adding appropriate amounts of sodium chloride. Experiments were conducted either at room temperature (19°–22° C) or at a constant temperature of 25° C. Duplicate experiments under both conditions yielded nearly identical results.

The numbers of pro-, meta-, and ana-phases counted on the upper sides of right imaginal disks after immersion for various times in solutions of sodium chloride and zinc chloride are shown in Fig. 1. The left anlagen were fixed immediately after dissection and thus served as paired controls for the right disks. Preliminary tests showed that statistically equal numbers of mitoses normally occurred in left and right anlagen. Each point on the graphs in Fig. 1 represents the mean of five to twelve anlagen.

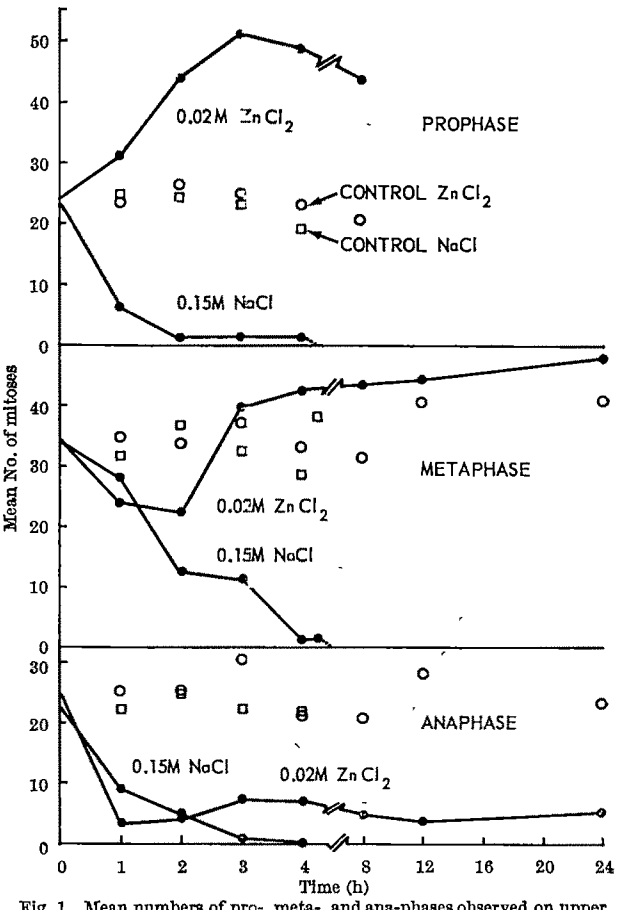


Fig. 1. Mean numbers of pro-, meta-, and ana-phases observed on upper sides of anlagen submerged for various times in 0·15 M sodium chloride and 0·02 M zinc chloride solutions. Control anlagen for experiments with sodium chloride (□) and zinc chloride (○) fixed immediately after dissection

Prophase nuclei disappeared rapidly in 0·15 M sodium chloride and were rare after 2 h. Conversely, the number of prophases in anlagen in 0·02 M zinc chloride rose steeply to a peak after 3 h when twice as many were present as in the controls. The numbers of metaphases in anlagen in both 0·15 M sodium chloride and 0·02 M zinc chloride decreased similarly during the first hour. In sodium chloride this decline continued until nearly all metaphases had disappeared after 4–5 h. However, in 0·02 M zinc chloride the reduction in number of metaphases ceased after 1–2 h and afterwards rose sharply to a level slightly above that of the controls. A *t*-test for difference between paired samples showed that the numbers of metaphases in anlagen after 1 and 2 h in 0·02 M zinc chloride were significantly smaller than in the controls and significantly greater from 3 to 24 h. Anaphases decreased quickly at first in 0·02 M zinc chloride and then remained at a relatively constant low level. In 0·15 M sodium chloride no anaphases occurred at all after 4 h. Mitosis may have been stimulated by the Zn^{++} at a concentration of 0·02 M zinc chloride and continued to be initiated for 3 h. A stimulation is not definitely proved, however. It may be merely that Zn^{++} initially supports continuing mitosis, somehow temporarily improving the poor sodium chloride medium, although other work[4] tends to indicate that a stimulation may be involved. Whatever the exact nature of the effect, it is accompanied by a blockage of mitosis in the metaphase due to disruptions of the mitotic figure by the Zn^{++}. Characteristic signs of zinc poisoning, such as shortening, clumping, and distortion of the mitotic figure, became increasingly obvious in anlagen after extended immersion in 0·02 M zinc chloride.

The increase in mitosis did not occur in anlagen in either 0·01 M or 0·1 M zinc chloride. At the higher concentration both metaphases and anaphases were blocked and their numbers remained the same as in the control anlagen. At lower concentrations only the metaphases were blocked.

Mitosis was favoured by Zn^{++} only when anlagen were exposed to the solution almost immediately after dissection. One hour later the cells had become incompetent to react to the Zn^{++}. This condition may be analogous to the irreversible 'rejuvenation' referred to by Kroeger[1], an unspecific response of the cell to mechanical or chemical wounding causing it to revert to a more juvenile stage of development.

How zinc may promote mitosis is not exactly known. It has been shown to stimulate RNA and protein synthesis in *Rhizopus nigricans*[4]. Accumulation of zinc on the chromosomes in prophase and its subsequent release in anaphase has also been recorded[5] and may be associated with the acquisition by chromosomes of ribonucleoprotein at prophase and its ensuing release at anaphase[6]. A structural function of zinc is suggested similar to the role of magnesium and other divalent metals in preserving the structural integrity of ribonucleoprotein particles and thus influencing the rate of protein synthesis[7].

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MICROBIOLOGY

Effect of Antibiotics on Some Rumen and Intestinal Bacteria

Antibiotics have been for some time added to ruminant feeds to increase the weight gains of the animals and they have also been advocated as remedies for bloat[1]. Some workers have also used antibiotics as additions to *in vitro* experiments on the basis that this would suppress the activities of the bacteria in mixed rumen contents and that residual enzyme activity would be caused by protozoa. (See, for example, ref. 2.) Antibiotics have also been used regularly to suppress bacterial growths in the *in vitro* culture of protozoa[3]. Little has, however, been done to test the susceptibility to antibiotics of defined strains of rumen bacteria, especially the strictly anaerobic types. Such anaerobic types have also been found in the intestines of ruminants and non-ruminants.

In the course of work on the effects of antibiotics on rumen function, we have screened a number of antibiotics for their action against some representative rumen and intestinal bacteria. The results are presented here. The strains of *Selenomonas ruminantium*, *Lactobacillus bifidus*, *Butyribibrio* sp. *Bacteroides amylophilus*, and an anaerobic lipolytic bacterium 5*S*[4], were isolated from the sheep rumen. The strain of *Ruminococcus albus* came from an air sample from a cowshed. *Veillonella gazogenes* was from the bovine rumen, while *Lactobacillus fermenti* and *Peptostreptococcus elsdenii* were taken from the rumens of young calves and *Streptococcus bovis* from the rumen of a young calf which had had aureomycin included in its feed. The strain of *Escherichia coli* was isolated from the intestinal tract of a four-day old guinea-pig. With the exception of the lipolytic bacterium, the organisms were grown in a medium the constituents of which were: mineral solutions *a* and *b*[4], 15 ml. each; centrifuged sheep rumen fluid, 30 ml.; Bacto yeast extract, 0·2 g; resazurin, 0·1 ml. of 0·1 per cent solution; sodium bicarbonate, 0·6 g; cysteine hydrochloride, 0·05 g; glucose, cellobiose and maltose, 0·1 g each; 70 per cent sodium lactate solution, 0·125 ml.; water to 100 ml. The medium for the lipolytic bacterium had 0·1 ml. glycerol instead of the sugars and lactate.

The media were prepared, dispensed and incubated under oxygen-free carbon dioxide in a manner similar to that previously described for such media[4]. The addition of the various sugars to the basal medium, and the presence of rumen fluid, were necessary to ensure that all the organisms would grow and that they would then be tested under similar conditions. The various organisms were kept on slopes of the foregoing media solidified by the addition of agar (2 per cent w/v), transferred from the stock slopes to tubes of liquid media, as above, and incubated for 24 h at 38° C. Slopes of the media of maximum surface area were prepared in 6 in. × 0·5 in. test-tubes under carbon dioxide by setting 4 ml. of medium in the tube at a small angle to the horizontal, and these slopes were inoculated at the bottom of the slope with two drops (0·05 ml.) of the 24 h cultures. The inoculum was spread over the surface of the slope with a wire spreader in a standard manner, all the manipulations being done under a stream of carbon dioxide. The slopes were then stored at room temperature for 15 min and then a sterile disk of filter paper containing antibiotic was placed in the centre of the slope, again under carbon dioxide, and the tube stoppered. The slopes were incubated upright at 38° C for 3 days and the growth noted on each day. Control slopes without antibiotic showed a uniform growth after 24 h. Antibiotic inhibition showed as the familiar circle of clear medium around the disk. The 'Tylosin' was tested in the form of factory-prepared disks containing 30 μg 'Tylosin' phosphate ('Tylan'); the other antibiotics were prepared as filter-sterilized solutions of concentration 1 mg/ml. and the disks used were of

Table 1. THE SUSCEPTIBILITY OF SOME RUMEN AND INTESTINAL BACTERIA TO ANTIBIOTICS AS DETERMINED BY INHIBITION OF GROWTH ON AGAR SLOPES

Antibiotic	Organism										
	S. bovis	*L. fermenti*	*P. elsdenii*	*V. gazogenes*	*E. coli*	*Bact. amylophilus*	*R. albus*	*Butyrivibrio* sp.	*L. bifidus*	*5S*	*Sel. ruminantium*
Penicillin *G*	+*	+−	+*	+	−	+*	+*	+*	+	+*	+*
Penicillin *V*	+	−	−	−	−	−	+	−	+*	+−	−
'Aureomycin'	+	+	−	+*	+*	+*	+	+	−	−	+
'Achromycin'	+	+	−	+	−	+*	+	+	−	+	+
'Ledermycin'	+	+*	−	+	+	+	+	+	−	+	+
'Erythromycin'	+	+	+	−	−	+	+	+	+	+*	−
Streptomycin (sulphate)	−	−	−	−	−	−	−	−	−	−	−
Dihydrostreptomycin	−	+−	−	−	−	−	−	−	−	+	+−
'Bacitracin'	−	+	−	−	−	−	+	+	+	−	−
'Novobiocin'	+*	+	−	−	−	−	−	+	+	+	+
Chloramphenicol	+	+	+	+	−	+	+	+	+	+	+
'Tylosin'	+	+	−	−	−	−	+	+	+	+	−

The antibiotic was added to a paper disk on the slope. *5S* indicates a strain of unclassified anaerobic lipolytic bacterium; +, inhibition of growth around disk; −, no inhibition. +−, small zone of lighter growth than rest of slope, no complete inhibition; * indicates that 'resistant' colonies grew in this inhibition zone after 1 or 2 days.

Whatman No. 1 filter paper, 6·5 mm in diameter and containing approximately 0·01 ml. of solution.

A positive result is shown in Table 1 whenever a clear zone was found, but the zones of inhibition varied in size with different antibiotics and organisms. The results support the use of chloramphenicol as an agent for suppressing the growth of bacteria in cultures of rumen protozoa and give a guide to the effects of other antibiotics on rumen function. The action of penicillin on strain *5S* could account for the marked reduction in lipase activity noted by Wright[2] in the rumen of a cow to which penicillin had been given orally. Wright also noted that streptomycin had no effect on lipase activity. In a number of cases, one or more colonies of the test organism appeared in the inhibition zone after more than one day of incubation, and this was especially noticeable in the case of penicillin *G*. Further work with liquid cultures showed that penicillin *G*, in concentrations up to 5 μg/ml., was completely destroyed in this medium after incubation for two days, and that higher concentrations lost a proportion of their activity. Growth of bacteria could appear at random in tubes of the liquid penicillin containing medium two days after inoculation. However, when sub-cultures were made from these tubes, or from apparently resistant colonies on agar slopes, into media containing penicillin, it was apparent that the organisms were not truly resistant to the antibiotic. The initial growth must have been due to chance survival of some of the inoculum until the penicillin concentration had been reduced below the inhibitory level.

We are grateful to T. H. Blackburn and S. O. Mann for some of the organisms used in this survey. We would also like to thank the Wellcome Foundation for a maintenance grant to one of us (I. E. A.), and the companies which provided the antibiotic samples.

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Adaptation of Bacteria from One Type of Hydrocarbon to Another

THE ease with which various bacteria assimilate hydrocarbons is known to vary with the structure of the hydrocarbon[1]. As part of an investigation of the fermentation of hydrocarbon material by bacteria we have investigated the relative rates of bacterial growth on liquid hydrocarbons of different structures. About thirty different kinds of bacteria, including *Pseudomonas fluorescens* and several species of *Corynebacterium*, have been isolated from soil samples taken from industrial oil and chemical spillage areas and from garden soils. The most rapid growth of fresh isolates from soil samples occurred with the normal paraffins $C_8 - C_{18}$, but growth on normal $C_5 - C_8$ hydrocarbons was much less rapid. This may be explained by the known toxicity of the lower alkanes to bacteria because of their lipid solvent properties. The hydrocarbons listed as follows are classified in decreasing order of ability to support bacterial growth:

Class 1. *n*-Paraffins (C_8–C_{18}).

Class 2. Branched chain alkanes (3-methylheptane; 2,2,4-trimethylpentane; pentamethylheptane; 2,2,4,4,6,-8,8,-heptamethylnonane; propylene tetramer).

Class 3. *n*-Paraffins (C_5–C_8).

Class 4. Cyclic hydrocarbons (*cyclo*hexane, methyl-*cyclo*hexane, decalin, benzene, *m*-xylene).

Bacteria isolated with the aforementioned hydrocarbons as sole carbon source were able to adapt from one hydrocarbon to another. However, there was a marked variation in the ease with which the different hydrocarbons were utilized by the same organism. Micro-organisms isolated on hydrocarbons low in the foregoing classification rapidly adapt and then grow well on a hydrocarbon higher in the list, but adaptation in the reverse sense did not apply. A typical set of experimental results is given in Table 1.

Table 1. BACTERIAL GROWTH AFTER ADAPTATION TO NEW CARBON SOURCES

Bacterial strain	Hydrocarbon used for isolation: Class	Substance	Hydrocarbon used to test adaptability: Class 1 Decane	Class 2 2,2,4-Trimethylpentane	Class 3 Pentane	Class 4 *cyclo*hexane
HD	1	Dodecane	+	+	−	−
CD	1	Dodecane	++	+	−	−
Q i/o	2	2,2,4-Trimethylpentane	++	+++	−	−
M1 i/o	2	2,2,4-Trimethylpentane	+++	++	−	−
SP	3	Pentane	+++	++	++	+
LP	3	Pentane	+++	+	+	−
Q c/h	4	*Cyclo*hexane	+++	+	+	++
T c/h	4	*Cyclo*hexane	++	+	+	++

+++, Between 5×10^8 and 5×10^9 cells/ml.
++, Between 5×10^7 and 5×10^8 cells/ml.
+, Between 5×10^6 and 5×10^7 cells/ml.
−, Less than 5×10^6 cells/ml.

All bacteria in Table 1 except *HD* are species of *Corynebacterium; HD* is a variety of *Pseudomonas fluorescens*. The cultures were incubated in static conical flasks containing 100 ml. of mineral salt solution (1·6 g KH_2PO_4; 0·8 g Na_2HPO_4; 0·5 g $MgSO_4.7H_2O$; 0·5 g NaCl; 5 g NH_4Cl in 1 l. of distilled water) and 2 ml. hydrocarbon at

30° C for 1 week. The growth was estimated as total cell count using a Thoma chamber.

I am grateful to Dr. D. S. Davies for his interest.

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VIROLOGY

Small-plaque and Large-plaque Variants of Papova Virus SV_{40}

Plaque size constitutes an important genetic characteristic of many viruses, including polyoma virus[1–3]. We have shown the existence of large-plaque (*LP*) and small-plaque (*SP*) variants for another Papova virus—SV_{40}.

The plaque-forming ability of nine strains of SV_{40} was investigated: *A*-426 (ref. 4), 128, 129, 130, 131 (all were isolated from *M. rhesus* kidney tissue cultures and kindly supplied by Dr. Deichman), 261 (isolated by Dr. Deichman from a hamster tumour induced by SV_{40}), *MR*-1, *MR*-2 (isolated in this laboratory from *M. rhesus* kidney tissue culture) and *I*-1 (isolated from hamster tumour formed by SV_{40}). All viruses were propagated in green monkey kidney tissue cultures. Plaques formed by these different virus strains were compared by inoculating green monkey kidney cell culture in 50-ml. bottles with about 50 p.f.u. in 0·1–0·2 ml. After 2 h adsorption at room temperature, the cell monolayer was overlaid with nutrient agar using a modification of the technique of Stinebaugh and Melnick[5] ('Difco' agar, 1·5 per cent; ten-fold Earle's solution, 10·0 per cent; skimmed milk, 10·0 per cent; calf serum, 1·0 per cent; sodium bicarbonate, 0·4 per cent; neutral red, 0·0017 per cent; in de-ionized water). The infected tissue cultures were incubated at 37° C. The timing of plaque appearance was followed and their size measured on a given day of incubation.

The diameters of the plaques formed by two virus strains on the fourteenth day after inoculation are shown in Table 1 (all plaques formed in the bottles were measured). Though great variability of plaque size is characteristic of SV_{40}, strain *A*-426 predominatingly formed larger plaques, while strain 128 formed smaller ones. The difference could easily be seen without special measurement. Moreover, the plaques of strain *A*-426 appeared 2–7 days earlier than did those of strain 128 (the exact interval varied for different batches of tissue culture cells).

Table 1. Comparison of the Size of Plaques formed by Two SV_{40} Strains on the Fourteenth Day after Tissue Culture Inoculation

Strain	No. of plaques measured	Percentage of plaques at the given diameters (mm)						Mean diameter (mm)
		< 0·5	0·5–0·7	0·8–1·0	1·1–1·5	1·6–2·0	> 2·0	
A–426	143	0·7	24·4	28·0	21·7	20·3	4·9	1·1
128	164	28·6	58·0	10·4	2·4	0·6	0·0	0·5

Investigation of the other seven strains of SV_{40} showed that strain 130 was identical with strain 128. This was so with respect both to the rapidity of plaque formation and the size of plaques formed (these strains were therefore termed *SP* strains); the remaining six strains were identical with strain *A*-426 (*LP* strains). Differences between *SP* and *LP* strains were constantly found in repeated assays, though they varied in degree.

It is possible that these differences between the two types of strains may be associated with the contamination of *SP* strains by a high concentration of a latent interfering virus. Simultaneous inoculation of tissue culture with equal doses of strains *A*-426 and 128 had, however, no effect on the time of appearance or on the size of plaques induced by the former (*LP*) strain. Differences in plaque formation cannot, therefore, be accounted for by contamination by an interfering agent.

All the strains examined were cloned, and the cloned lines were passed four times in green monkey kidney cell culture. All cloned lines were identical in plaque-forming capacity with their parent strains.

To establish that plaque size was a genetic characteristic of virus particles, we investigated clones obtained from small and large plaques of *SP* and *LP* strains (Table 2). Whatever the size of the plaque used for cloning, the resulting clones did not differ from their parent strains. Plaque size was variable within each strain, but could not be accounted for by the viral genome.

Table 2. Characteristics of Plaque Formation by Cloned Lines obtained from Large and Small Plaques of Large-plaque (*LP*) and Small-plaque (*SP*) Strains of SV_{40}

Strain	Original characteristic	Diameter of the plaque cloned (mm)	Number of clones	Clone characteristics*
A–426	*LP*	0·3–0·5	3	*LP*
		2·0–3·0	3	*LP*
129	*LP*	0·3–0·5	3	*LP*
		2·0–3·0	2	*LP*
128	*SP*	0·3–0·5	3	*SP*
		1·0–1·5	2	*SP*
130	*SP*	0·3–1·5	7	*SP*

* Rate of appearance and the size of plaques were determined by comparison with those of *A*-426 strain.

According to preliminary data, *SP* and *LP* strains do not differ in their sensitivity to agar polysaccharide and do not adsorb on to cells at different rates—as has been shown for polyoma virus variants[6]. Both *SP* and *LP* strains of SV_{40} produced transformation in cultures of human and rat embryo fibroblasts[7].

Further investigations should show whether it is possible to isolate *SP* variants from *LP* strain population and *LP* variants from *SP* strain population.

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CYTOLOGY

Microtubules in the Cytoplasm of Mammalian Platelets

Of late, and with increasing frequency, microtubules have been recognized as a cytoplasmic component in cells examined in thin section by electron microscopy (see review by Slautterback[1]). Their presence in the cytoplasm of human, guinea-pig and mouse platelets is recorded in this communication.

Using siliconized hypodermic needles and glassware and 3·8 per cent trisodium citrate (*p*H 7·4) as an anticoagulant, blood was collected from animals of both sexes. It was taken from the antecubital vein of eleven humans and, after an ether anaesthetic, by cardiac puncture from six guinea-pigs and from the retro-orbital sinus of eighty-four mice. That from six or twelve mice was pooled for subsequent processing. Aliquots of platelet-rich plasma, which had been separated from the blood by centrifugation, were fixed 2 h in 5 per cent glutaraldehyde[2] made up in Sörensen's phosphate buffer (*p*H 7·4), and were then subjected to further centrifugation. Platelet pellets so obtained were washed in the phosphate buffer, post-fixed 2 h in osmium tetroxide containing sucrose[3] (*p*H 7·4), then washed in water, dehydrated in ethanol, and embedded in 'Araldite'. Grey to pale gold thin sections

were cut with a glass knife. They were stained, first in a saturated solution of uranyl acetate in 70 per cent alcohol and then either with lead citrate[4] or with lead hydroxide[5] and examined in a Siemens 'Elmiskop 1' electron microscope.

Microtubules were observed approximately 500 Å deep to the plasma membrane in platelets from the three species. They were defined by an electron-dense wall some 70 Å thick that enclosed a less dense core and had an overall diameter of about 250 Å. The wall had smooth inner and outer surfaces and did not have the features of a unit membrane[6].

Their position in the cytoplasm was related to the shape of the sectioned platelet. Thus, in oval or elliptical sections, microtubules were found usually at opposite ends of the platelet profile. They were present either as groups cut in cross-section, or as five or six roughly parallel microtubules cut in longitudinal sections of varying length and, in either instance, were placed about 100 Å apart (Fig. 1). Some were surrounded by a zone of ground substance that was less electron dense than elsewhere, perhaps indicating a particular composition.

The number of microtubules in groups cut in cross-section varied from one end of an individual platelet to the other and also from platelet to platelet. On average, thirteen were present in groups in human and mouse platelets and ten in guinea-pig platelets. However, for reasons discussed by Fawcett and Witebsky[7] in relation to microtubule enumeration in nucleated erythrocytes, the accuracy of the counts is probably low. Groups of microtubules cut in cross-section were seen occasionally at sites other than the poles.

Guinea-pig platelets were often remarkably elongate. In these, several parallel microtubules cut in longitudinal section, or discontinuous fragments of single tubules passed from one pole to the other. Short segments of single tubules were also seen in the central zone of platelets that had transected tubules at either end.

Several parallel tubules, running deep to the plasma membrane and describing arcs of varying length, were present in round sections of platelets from all animals. Single tubules, which passed either towards or from those in the peripheral position, either followed a convoluted course in the central area of the section or crossed the diameter (Fig. 2).

Uncommonly, sectioned platelets had a squarish outline. In these, microtubules cut either in cross-section or longitudinal section could be seen in any corner.

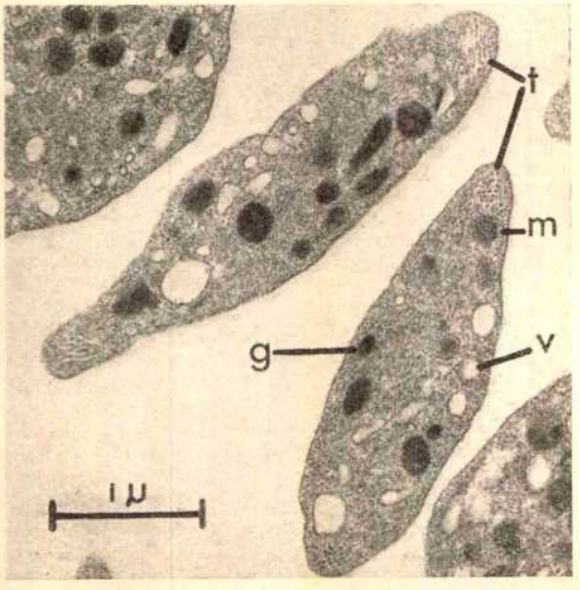


Fig. 1. Microtubules (*t*) present in cross- or longitudinal section at either pole of human platelets. Dense granules (*g*), vesicles (*v*), and mitochondria (*m*), also present in cytoplasm. (× 20,000)

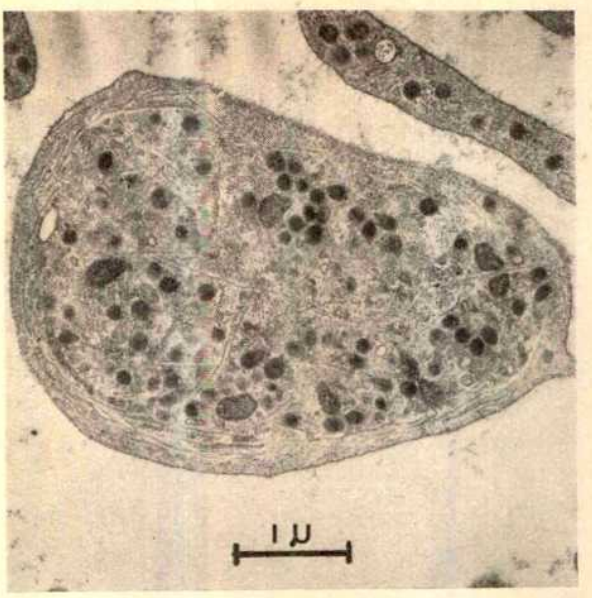


Fig. 2. Several roughly parallel microtubules describe a circumferential course approximately 500 Å deep to the plasma membrane of a guinea-pig platelet. Other single tubules have a radial arrangement. (× 15,000)

Human and mouse platelets showed occasional spikes or pseudopod-like structures projecting from their otherwise smoothly contoured outline. In some instances, several parallel microtubules cut in longitudinal section passed from either side of the platelet into the base of the spike, intermingled and continued towards its tip. It is not certain whether the two groups of tubules overlapped or fused. Furthermore, since it was usual for the tip of the spike to be out of the plane of section, it was not clear whether the two groups formed a loop at the apex, or came to a point like the walls of a tepee, or came into contact with the plasma membrane. Contact between microtubules and plasma membrane was not apparent elsewhere.

These observations, coupled with those made on rabbit platelets[8], suggest that microtubules may be present in the cytoplasm of all mammalian platelets. It is of interest that similar structures have been described forming an annular, paranuclear band in thrombocytes of the toadfish *Opsanus tau*[7].

Furthermore, the findings suggest that the anatomical arrangement of microtubules in mammalian platelets is in the form of a marginal band containing several tubules, with other single tubules running a radial course. Support for this conclusion is gained by the presence of circumferential and radial fibres in the hyaloplasm of platelets spread on films and examined with an electron microscope[9,10]; the difference in the recorded dimensions of fibres and microtubules being explained by the different methods of preparation used[11]. Added support is gained by comparison with analogous observations made on marginal bands found in nucleated erythrocytes from non-mammalian vertebrates[7,11].

The function of microtubules has still to be determined[1,7]. Perhaps their action on the plasma membrane causes spike formation. Alternatively, their extension into these cytoplasmic projections may be a secondary phenomenon. Investigation of microtubule morphology during the action of agents known to cause platelet aggregation[12] may give insight into their function and establish their role in thrombus formation.

I am indebted to Dr. E. DeSalis, Commonwealth Health Laboratory, Canberra, A.C.T., for human blood samples, and to Miss N. Carroll for technical assistance.

Note added in proof. Since submitting this communication, G. B. Haydon and D. A. Taylor (*J. Cell. Biol.*, 26, 673; 1965) have reported finding microtubules in hamster platelets, and O. Behnke (*J. Ultrastruct. Res.*, 13, 469;

1965) has described microtubules forming a marginal bundle in human and rat thrombocytes.

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AGRICULTURE

Field Responses of Cereals to Molybdenum

Reports of field occurrences of molybdenum deficiency in small-grained cereals are rare. Fricke[1] in Tasmania showed that the 'blue chaff' disease of oats could be cured by applying molybdenum. Lobb[2] and Fitzgerald[3] in New Zealand, and Mulder[4] in The Netherlands, have reported responses with field grown oats to molybdenum. Lobb[2] and Mulder[4] have also obtained field responses with wheat to molybdenum. It has been found that large areas of Australian soils are deficient in molybdenum that can be used for nitrogen fixation by Leguminosae, and for growth by Cruciferae, Compositae, Cucurbitaceae, Solanaceae and Linaceae[5]. The only field responses in the Gramineae in Australia are those reported by Fricke[1] with oats, and Noonan[6] with maize. It has frequently been demonstrated, for example by Johnson *et al.*[7], that members of Gramineae are relatively tolerant of low supplies of molybdenum and that molybdenum deficiency in cereals only occurs in extreme conditions of molybdenum deficiency.

This communication reports field responses to molybdenum by wheat and by oats in mainland Australia. The responses occurred in a region of the West Australian wheatbelt which receives 12–13 in. of rainfall annually. The soils concerned are sandy and lateritic podsols which are extremely deficient in native phosphate, deficient in nitrogen and often deficient in copper and/or zinc. Plant responses to molybdenum in this region have not previously been reported.

Ballidu oats were planted on June 2, 1962, east of Gutha. Observed in early August, the plants on the plots which were not treated with molybdenum appeared a slightly paler green than those on the plots treated with molybdenum, but no other differences were noticed. The results for grain yield are shown in Table 1 (Exp. 1). Highly significant increases in yields resulted from the application of molybdenum, and these increases were magnified by the application of 1 cwt. of ammonium sulphate/acre.

The wheat used in Exp. 2 was sown in 1960, east of Gutha. Molybdenum treatments caused no visible differences: grain yields are shown in Table 1. Application of molybdenum resulted in markedly increased yields only with ammonium sulphate (1 cwt./acre).

A further experiment with Gabo wheat planted on June 2, 1962, immediately adjacent to the oat plots, produced grain yields as shown in Table 1 (Exp. 3). Molybdenum markedly increased the yields, and the effect was again enhanced by treatment with 1 cwt. ammonium sulphate/acre. As with the oats, the only visible difference was a slight chlorosis of those wheat plants on plots which were not treated with molybdenum when compared with plants on plots which received molybdenum.

Table 1. Effect of Molybdenum on Oat and Wheat Grain Yields* at Gutha

	Exp. 1, Ballidu oats, Gutha, 1962	Exp. 2, Gabo wheat, Gutha, 1960	Exp. 3, Gabo wheat, Gutha, 1962
Superphosphate (150 lb./acre)	15·2	18·0	9·4
Superphosphate (150 lb./acre) + molybdenum trioxide (2 oz./acre)	17·2	17·8	11·5
Superphosphate (150 lb./acre) + ammonium sulphate (112 lb./acre)	12·2	18·8	11·9
Superphosphate (150 lb./acre) + ammonium sulphate (112 lb./acre) + molybdenum trioxide (2 oz./acre)	17·8	22·4	15·3
	Mo signif. at $P < 0·001$. S/A × Mo significant at $P < 0·05$. S/A effect N.S.	Mo signif. at $P < 0·05$. S/A significant at $P < 0·001$. S/A × Mo significant at $P < 0·001$	Mo signif. at $P < 0·001$. S/A × Mo interaction N.S.

* Yields in bushels/acre: 40 lb. to the bushel for oats; 60 lb. to the bushel for wheat.

Table 2. Effect of Molybdenum on Vegetative and Grain Yield of Wheat at Bodallin

	Exp. 4, Bencubbin wheat, Bodallin, 1964	
	Vegetative yield at 11 weeks. Dry matter in lb./acre	Grain yield (bushels/acre)
Superphosphate (180 lb./acre)	91·4	7·2
Superphosphate (180 lb./acre) + urea (24 lb./acre)	122·1	9·1
Superphosphate (180 lb./acre) + urea (50 lb./acre)	137·8	7·3
Superphosphate (180 lb./acre) + urea (76 lb./acre)	152·0	6·7
Superphosphate (180 lb./acre) + molybdenum trioxide (2 oz./acre)	119·5	9·6
Superphosphate (180 lb./acre) + urea (24 lb./acre) + molybdenum trioxide (2 oz./acre)	169·9	10·3
Superphosphate (180 lb./acre) + urea (50 lb./acre) + molybdenum trioxide (2 oz./acre)	176·3	9·7
Superphosphate (180 lb./acre) + urea (76 lb./acre) + molybdenum trioxide (2 oz./acre)	244·0	10·9
	Mo significant at $P < 0·001$; urea linear significant at $P < 0·001$; Mo urea linear significant at $P < 0·05$	Mo significant at $P < 0·001$

In 1964, at Bodallin, 180 miles south-east of the Gutha sites, striking responses in vegetative growth and grain yield of Bencubbin wheat resulted from the application of molybdenum. Within 5 weeks of seeding, differences in growth and colour due to molybdenum treatments were noticeable. Vegetative yields at 11 weeks after seeding are shown in Table 2.

Molybdenum application advanced maturity by approximately 10 days. Whiteheads barren of grain were abundant and scattered throughout those plots which were not treated with molybdenum. Grain yields are shown in Table 2.

That cereals respond so markedly to the application of molybdenum indicates that these soils contain little molybdenum that can be made available to plants. Numerous and widespread responses with pasture legumes in experiments carried out in 1964 have shown that extensive areas of molybdenum deficiency exist in the sheep and wheat growing area of South Western Australia.

This work was carried out with the co-operation of R. J. Doyle, R. J. Parkin, and J. A. C. Smith, advisers to the Wheat and Sheep Division, West Australian Department of Agriculture.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 7

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 5.15 p.m.—Prof. Theodor Bücher (University of Munich): "Dynamics of Liver Metabolism" (further lecture on March 9).*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. P. J. N. Norris: "An Introduction to Silicon Controlled Rectifiers".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. M. Finniston: "Experimenting in Experiments".

Tuesday, March 8

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 2.30 p.m.—Symposium on "Mathematics in Economics". Speakers: Prof. J. R. N. Stone, C.B.E., Dr. F. H. Hahn and Prof. J. D. Sargan.

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. R. E. Rowe and Mr. G. D. Base: "Model Analysis and Testing as a Design Tool".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (joint meeting with the Society of Instrument Technology, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. I. R. Young, Mr. A. T. Keefe and Mr. G. Moss: "A New Approach to Measurement and Data Transmission for Process Control Systems".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. W. S. Campbell: "Applications of Latex in Industry". 7 p.m.—Panel Discussion on "Natural and Synthetic Latices v. Polyurethane in Foam Applications".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. Blaauw (University of Groningen): "Distribution and State of Motion of the Interstellar Gas in the Solar Neighbourhood".*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Prof. Holger Arbman: "Early German Animal Style in the 5th–7th Centuries A.D.—III. The Late Vendel Styles in Scandinavia and Its Relationships with the Continent and the British Isles".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. R. J. Allwood and Mr. C. C. Norville: "The Analysis by Computer of a Motor Car Underbody Structure".

Wednesday, March 9

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 4 p.m.—Annual General Meeting, followed by Dr. G. H. Byford: "The Measurement of Human Reactions to Environmental Changes".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. W. Bright and Dr. P. L. Secker: "Applications of Electrostatics".

SOCIETY OF INSTRUMENT TECHNOLOGY (in conjunction with the British Computer Society, at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. R. G. Blake: "Use of a Digital Analogue Simulator"; Dr. L. J. Postle: "Analogue Computers and Chemical Plant Control System Design in Practice"; Dr. G. W. H. White: "The Calculation of Pole Zero Patterns for Process Control".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. Blaauw (University of Groningen): "Properties of the Youngest Stellar Population in the Solar Neighbourhood with special reference to the Problem of Star Formation".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. M. Court Brown: "Essays in Human Cytogenetics: I. Autosomal Structural Heterozygosity".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Prof. C. W. Oatley, O.B.E.: "The Scanning Electron Microscope and other Electron Probe Instruments".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Annual General Meeting followed by Dr. J. T. Martin: "Chemical Crop Protection in Britain".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the College of Technology, Roe Green, Hatfield), at 7.30 p.m.—Dr. G. A. Williams: "Some Aspects of the Chemistry of Free Radicals".

Thursday, March 10

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. S. V. Perry: "Biochemistry of Muscle".*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. B. C. Browne: "Gravity Surveying at Sea".

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Traffic Survey for Smaller Towns" introduced by Mr. A. E. Fieldhouse.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The Multi-Carrier Performance of Communication Satellite Repeaters", opened by Mr. W. L. Wright, Mr. K. U. Bolwell and Mr. P. N. Sargeaunt.

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The Application of Static Reactive Power Devices to Transmission and Distribution Networks", opened by Dr. E. Friedlander and Mr. L. A. Harris.

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Prof. R. W. van Bemmelen (University of Utrecht): "A New Model for the Earth's Geological Evolution".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. M. Court Brown: "Essays in Human Cytogenetics: II. The *y* Chromosome".*

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Implications of Metric Conversion".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6 p.m.—Dr. P. Kresse: "Inorganic Pigments in Building".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the Food Group, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. F. J. Ley: "The Use of Ionizing Radiations in Food Preservation".

Thursday, March 10—Friday, March 11

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m. daily—Discussion Meeting on "Circular Dichroism—Electronic and Structural Principles", organized by Prof. R. S. Nyholm, F.R.S.

Friday, March 11

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Prof. P. B. Beeson: "Bacterial Endocarditis".*

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 5 p.m.—Dr. K. L. Blaxter: "Some Effects of the Climatic Environment of Farm Animals".*

UNIVERSITY OF LONDON (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. M. Court Brown: "Essays in Human Cytogenetics: III. Population Studies: The Part and the Whole".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Sir Lawrence Bragg, O.B.E., M.C., F.R.S.: "Reminiscences of Fifty Years Research".

Saturday, March 12

NUTRITION SOCIETY (at the Medical School, Guy's Hospital, St. Thomas's Street, London, S.E.1), at 10.15 a.m.—Symposium on "Nutrition and Physical Performance".

Monday, March 14

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. A. Waldron: "The Explanation of Some Fundamental Phenomena of Modern Physics using a Ballistic Theory of Light".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. S. C. Brown: "The Ten-Element Band-Pass Filter Section and Its Applications".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Sky Above, the Mud Below" (colour film).

Monday, March 14—Friday, March 18

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Great Hall of Alexandra Palace, London, N.22)—Eighteenth Technical Exhibition.*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

UNIVERSITY DEMONSTRATOR (with a good honours degree or the equivalent in an appropriate subject and preferably some research and/or teaching experience) IN PLANT PATHOLOGY—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (March 9).

SCIENTIFIC OFFICER (with a good honours degree in zoology or applied entomology, and preferably some postgraduate experience) to carry out laboratory and field studies on the behaviour and performance of insecticides in soil to improve efficiency—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (March 11).

SENIOR LECTURER/LECTURER (preferably with previous computer programming experience, either academic or industrial) IN THE COMPUTER UNIT, to be responsible for the operational side of the University's computing service to consist of an Elliott 4130 system—The Registrar, University of Warwick, Coventry, Warwickshire (March 11).

ASSISTANT LECTURER OR LECTURER IN ZOOLOGY—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (March 14).

READER OR SENIOR LECTURER IN SOLID STATE ELECTRONICS; and LECTURERS (2) (one in the field of control systems) IN ELECTRONICS—The Registrar, The University, Canterbury, Kent (March 14).

LECTURER OR ASSISTANT LECTURER (with a special interest in either plant physiology or genetics) IN THE DEPARTMENT OF BOTANY—The Deputy Secretary, The University, Southampton (March 18).

SECOND CHAIR IN PHYSICS (applicants should have specialized in either experimental metal physics or in microwave physics)—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (March 18).

CHAIR OF PHYSICAL OCEANOGRAPHY—The Deputy Secretary, The University, Southampton (March 25).

ASSISTANT (honours graduate) IN THE DEPARTMENT OF BOTANY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (March 31).

CHAIR OF METALLURGY—The Registrar, The University, Sheffield (March 31).

CHAIR OF PHYSICS—The Registrar, The University, Sheffield (March 31).

LECTURER/ASSISTANT LECTURER (with a degree in medicine or science with suitable teaching and research experience, and preferably with a special interest in enzymology or biophysical chemistry) IN BIOCHEMISTRY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, March 31).

LECTURER/SENIOR LECTURER IN BIOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

RESEARCH ASSISTANT (graduate, man or woman, with a special interest in plant physiology or plant biochemistry) IN BOTANY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (March 31).

SENIOR LECTURER or LECTURER IN PHARMACEUTICS; a SENIOR LECTURER or LECTURER IN PHARMACOGNOSY; a SENIOR LECTURER or LECTURER IN PHARMACEUTICAL CHEMISTRY; and SENIOR LECTURERS or LECTURERS (2) in PHARMACOLOGY at the University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 31).

THIRD CHAIR OF PHYSICS—The Registrar, The University, Sheffield (March 31).

UNIVERSITY ASSISTANT LECTURER IN PURE MATHEMATICS—The Secretary to the Appointments Committee of the Faculty of Mathematics, The University, Silver Street, Cambridge (March 31).

LECTURERS or ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham, 15, quoting Ref. CE (April 1).

LECTURER IN BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, April 13).

LECTURER (with a good honours degree in electrical engineering, and industrial and research experience in the field of telecommunications and electronics) IN ELECTRICAL ENGINEERING at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, April 15).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (April 18).

CHAIR OF GEOGRAPHY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Kuala Lumpur, April 30).

LECTURER or ASSISTANT LECTURER (with a good honours degree, experience in modern semiconductor circuit design, and preferably an interest in communications engineering) IN ELECTRICAL ENGINEERING—The Secretary, The University, Edinburgh (April 30).

PROFESSOR OF GEOLOGY—The Registrar, University Registry, Oxford (April 30).

SECOND CHAIR OF ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (April 30).

ASSISTANT PROFESSOR IN ANATOMY—The Chairman of the Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

CHAIR OF OPHTHALMIC OPTICS AND HEAD OF THE DEPARTMENT—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. OOD.

DEVELOPMENT ENGINEER IN THE GEOPHYSICS DEPARTMENT, to accept responsibility for the design, construction and development of instruments involving both electronic and mechanical components, required in the Department's research programme—Prof. J. M. Bruckshaw, Geophysics Department, Imperial College of Science and Technology, London, S.W.7.

EXPERIMENTAL OFFICER or ASSISTANT EXPERIMENTAL OFFICER (plant physiologist or biochemist) to work on the nutrition of marine plankton—The Director, Scottish Marine Biological Association, Marine Station, Millport, Isle of Cumbrae, Scotland.

LECTURERS or ASSISTANT LECTURERS IN SOCIOLOGY—The Registrar, Bradford Institute of Technology, Bradford, 7.

POSTDOCTORAL FELLOW (physical chemist) IN THE DEPARTMENT OF CHEMISTRY, for work involving the measurement of the heats of chemical reactions taking place at elevated temperatures (300–1,000° C)—Dr. H. A. Skinner, Department of Chemistry, The University, Manchester, 13.

RESEARCH ASSISTANT (with an honours degree or equivalent) IN ANALYTICAL CHEMISTRY to work in conjunction with Dr. C. F. Bell, on the synthesis of co-ordination compounds of transition metals and the evaluation of their analytical uses—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

RESEARCH ASSISTANT (with a chemical or biochemical background) AT THE RESEARCH DEPARTMENT, Caterham, to work on aspects of tumour growth with special reference to hormone-dependent tumours—The Secretary, The Marie-Curie Memorial Foundation, 124 Sloane Street, London, S.W.1.

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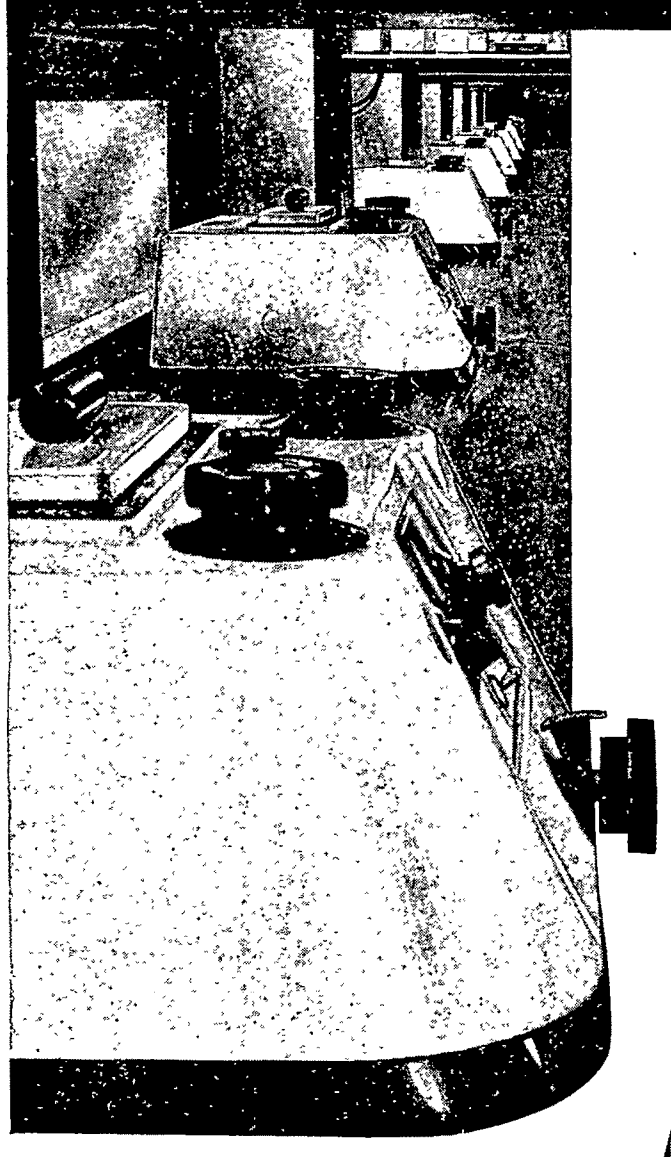
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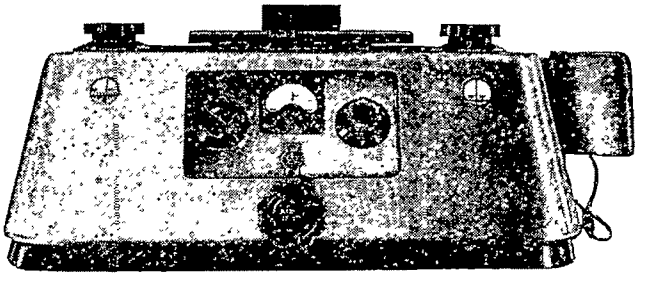




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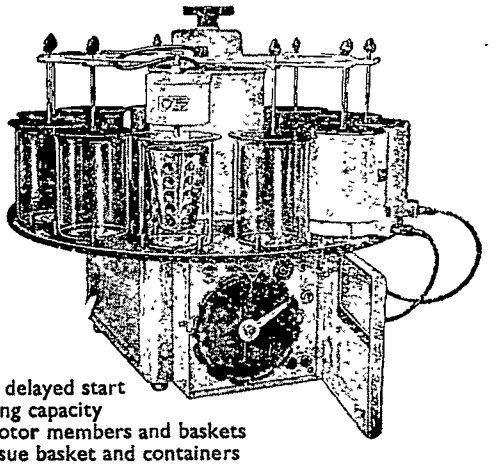
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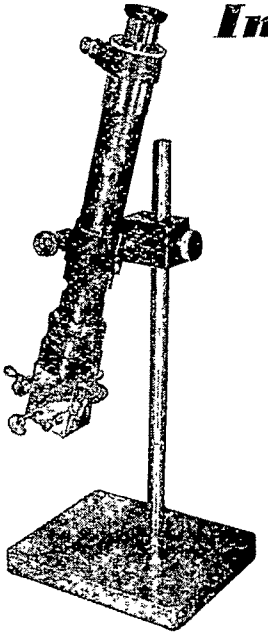
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THE FUTURE OF THE BRITISH AIRCRAFT INDUSTRY

THE great difficulties which face Defence Departments in attempting to assess the future needs of the Services for all forms of equipment were fully recognized by the sub-committee of the Estimates Committee which considered electrical and electronic equipment. Although the complexity of the techniques involved and their comparative novelty present special difficulties with electronic equipment, perhaps especially in the speed of advance, the Estimates Committee did not suggest that the position here is unique. It did suggest, however, that the present departmental arrangements contributed to the difficulties and, although the Committee did not venture into this aspect in detail, the seventh report of the Estimates Committee for the Session 1964–65 (see *Nature*, 209, 645; 1966) did not conceal its misgivings on this score, especially as regards the Ministry of Technology. It was not satisfied with the functioning in this field of either the Ministry of Aviation or the Ministry of Defence.

The key problem is the same in its essentials as that which confronted the Committee of Inquiry into the Aircraft Industry, appointed by the Ministry of Aviation, under the chairmanship of Lord Plowden, in December 1964, whose report has since been published*. That Committee was to consider the future place and organization of the aircraft industry in relation to the general economy of Britain—taking into account the demands of national defence, export prospects, the comparable industries of other countries and the relation of the industry with Government activities in the field of aviation. It was to make recommendations on any necessary steps and measures. The electronics industry was outside its terms of reference, although the interest of electronics firms in the fate of the aircraft industry is acknowledged in the Report and evidence was taken from them. Nevertheless, the terms of reference were widely drawn and the Committee recommended that some detailed aspects needed further investigation.

On the other hand, while on appointment the Committee was told that early decisions would be made on certain projects in the military aircraft programme, and the cancellation of the *P*1154 and of the *HS*681 was announced in January 1965, the fate of the *TSR*2 was not decided until April 1965. For some months, in consequence, witnesses in industry and in Government departments found it difficult to state their plans. Doubts about the military programme persisted even after April and remained unresolved until the results of the present defence review were known. In addition, several Ministerial statements about collaboration with other countries on future aircraft developments, such as the two Anglo-French projects announced in April 1965, and the aero-engine project recently negotiated with the United States, have accentuated the difficulties of the Committee.

The Report emphasizes at the outset the distinguished record of the British aircraft industry both in peace and war. For more than half a century it has made a major contribution to the defence, the economy and the technology of Britain. In recent years, setbacks and frustrations, including the cancellation of the major projects already mentioned, have diminished its prestige. In addition, British civil advanced jet aircraft have failed in general, despite their technical qualities, to realize the commercial expectations with which they were launched. Over the past 5 years, the main projects in the civil programme have together absorbed substantial private capital, as well as an increasing contribution from Government funds, and they do not, as a whole, promise a return commensurate with the investment.

The Plowden Committee was concerned with the consequent doubts about the future of the industry and its value to the community. Apart from the obscurity of the present outlook in the field of defence, it is difficult to predict with confidence the pattern of developments in civil aviation in the years ahead. The environment in which the industry operates is constantly changing and, accordingly, the Committee did not attempt to make recommendations on individual projects or on the detailed organization of the industry. It makes recommendations, however, regarding the relations between Government and the industry; but in spite of some discussion of the future of the Ministry of Aviation, it does not examine in any detail the relations between that Ministry and the Ministries of Defence or of Technology. The Committee does recommend an early review of this subject by the Government, since the efficiency of the aircraft industry is closely bound up with better arrangements for conducting aircraft business within Government departments. The Committee sought to define the basic principles which the Government and the industry should bear in mind when planning the future of the industry and when making decisions on individual projects in the coming years.

Looking first at the changing technology of the industry during the past 20 years, the Committee notes the increased performance of aircraft resulting from the gas turbine engine, the development of new materials to overcome the problems of supersonic flight, as well as the devising of new shapes with novel dispositions of wings, tail and engines. Striking advances have been made in aeronautical equipment: developments in electronics, radar and computers have revolutionized navigation and aircraft control systems, and there have also been advances in mechanical equipment and hydraulics.

All this has markedly increased the complexity of aircraft, which are now an intricate mass of electrical, electronic, hydraulic and mechanical equipment. As a result, a modern aircraft takes longer to develop and the costs of development and production are both much higher. Whereas the production cost of a *Spitfire* was about £10,000, a *Lightning* Mark I cost more than £500,000 and the *TSR*2 would have cost nearly £300 million to develop and nearly £3 million for each production aircraft. This is about twenty times more for development and ten times more for production than the *Canberra*, which the *TSR*2 was to replace. A modern sub-sonic civil air liner, including a new engine, could cost from £50 million to develop and anything from £1·5–£3 million for each

* Report of the Committee of Inquiry into the Aircraft Industry appointed by the Minister of Aviation under the Chairmanship of Lord Plowden, 1964–5. Pp. iv+139. (Cmnd. 2853.) (London: H.M.S.O., 1965.) 10*s*. net.

aeroplane, or two or three times as much as the airliner of a decade ago.

The problems of selection for customers and suppliers alike which these changes raise are rather in the speed with which technological changes succeed each other than in the character of the changes themselves. The penalties for faulty choices are heavy and, in general, fewer aircraft of each type are required than previously. The supplier of aircraft finds his resources tied up in fewer and increasingly expensive types; he is highly vulnerable if a project is suddenly cancelled. On civil projects the manufacturer himself normally finds at least half the costs of development, and if sales do not mature because some unexpected technical advance gives an advantage to a competitor, he may bear heavy losses. Timing and speed are thus increasingly important and there is a constant search for a new technological breakthrough to produce the next major advance.

This question of technology is considered more fully in a later section of the Report, in which the case for an aircraft industry is discussed. The activities of the British aircraft industry occupy some 8,600 qualified scientists, of whom 7,000 are employed in the industry itself, or 27 for 1,000 workers; this compares well with the average of 12 per 1,000 in British industry as a whole. The remaining 1,600 are engaged in aeronautical research and development or their administration in the Ministry of Aviation and its research establishments. The Plowden Committee was convinced that the new products and techniques developed by the industry found uses outside the industry and stimulated technological innovation generally. As examples of such indirect benefits or technological 'fall-out' it instanced the development of new materials, with high strength/weight properties, often coupled with resistance to high temperature, the development of digital computers, gas turbines, and high-pressure, light-weight hydraulic systems, as well as important advances in electronics, radar and radio.

While the importance of these indirect benefits was not to be doubted, it was more difficult to judge whether larger or even comparable benefits could be achieved by deploying these 8,600 scientists and technologists in other ways. For various reasons the Plowden Committee thought it improbable that any other single industry would have so pervasive an effect on the technological progress of the nation. Moreover, even if part of that effort could fruitfully be redistributed, it would not be easy to determine the objectives with assurance. Wholesale transfer of skilled research workers could not be achieved in the short or even medium term without serious loss, and the Committee had reservations as to how quickly and effectively the rest of British industry could absorb and use scientists and technicians from the aircraft industry. The caution of the Plowden Committee on this point is fully supported by the experience of the industrial research associations; it is their belief that the wider spread of technological progress in industry is limited not so much by the volume or nature of the new discoveries or advances being made by Britain's scientists and engineers, or even the areas in which they are being made, as by industry being unaware of them and unwilling to apply them.

While, therefore, the Plowden Committee concludes that disappearance of the aircraft industry, or its large and speedy contraction, would involve in the short term a significant loss to Britain's technology, it does not suggest that, in the long term, the technological 'fall-out' from the industry is a major justification for support to ensure its survival or maintenance at a particular level. The strength of this argument, moreover, depends on the general Government policy for technology; the greater the effort the Government is prepared to put into systematically encouraging the use of advanced technology throughout British industry, the less it need rely on the stimulus of the aircraft industry. Conversely, the nation cannot afford, in either the short term or long term, to tolerate weakness, whether in organization or in people, at the Ministry of Technology or at whatever Ministry may be charged with such responsibilities.

In this respect the Plowden Committee makes one constructive suggestion. It believes that arrangements to ensure that potentially valuable research reaches those British firms which could utilize it would increase the benefits from fall-out. It suggests, therefore, that the establishment of some kind of scientific clearing house merits consideration; however, it admits that the most spectacular transfers of technology will continue to result from one man's enthusiasm for a new discovery and from the determination and vision with which he applies it in apparently unrelated fields. These views, moreover, are substantially confirmed by the conclusions of a study by A. D. Little, Ltd., commissioned by the Committee, into the transfer of aerospace technology in the United States.

Some of these conclusions are worth noting. The experience in the United States provides valid examples of major transfers of technology from aerospace programmes to civilian sectors of the economy, and the existence and rapid growth of a large body of advanced technology in the aerospace sector have stimulated other sectors of the economy to consider the utility of technology in commercial life. Significant transfer of technology, however, has occurred only where a clearly recognizable correspondence was first demonstrated between technical opportunity, on one hand, and an economic or social need which could be satisfied with profit to industry, on the other.

The review also points out that a more formal and apparently comprehensive system of written communication is coming into existence in the United States to describe the developments in aerospace research, and this system is disseminated to industry at large. Much, however, remains to be done to develop the environment which can increase the efficiency of this process, and the review suggests that incentives for innovation within industry require re-examination. The transfer of technology will increase when the environment of the receiving side in industry and commerce is as open to innovation as the generating side. This is not so at present and the existing environment tends to discourage the movement of scientists and engineers with advanced knowledge of aerospace technology into other less venturesome sectors of the economy.

While such mobility is the most important factor in the transfer of technology, the A. D. Little review does not advocate the support of aerospace programmes on the ground of such stimulus to technology generally. Such stimulation is achieved more effectively by other forms of supporting civilian research and industrial innovation. The transfer of technology is no valid reason for maintaining an aircraft industry or a burdensome programme of space research. Little attention was paid to this aspect when the Report was discussed in the House of Commons on February 1. There was no suggestion that such technological transfer was a valid reason for maintaining an aircraft industry of a particular size but on the other hand, it was disappointing to find so little

attention being paid to the question of research or even to the structure of Government in so far as it affects the efficiency of the industry.

It was left for Mr. Keith Stainton to note the Committee's reference to the future of the Ministry of Aviation, but he merely pointed out that this had not been considered at all deeply by the Committee. Mr. Stainton also emphasized the importance of supporting the search for knowledge on which technical progress depends. The vital question was how this was done and he called for more analysis than was given in the Report of the areas of past research activity and general adequacy and competence to form a clear view of the future level of requirements. Even on present finance, research and development should be re-aligned but not curtailed. He urged that European co-operation should be developed on a thorough-going basis.

Mr. J. Stonehouse, Parliamentary Secretary to the Ministry of Aviation, replying on the debate, clearly recognized the importance of research. This was one reason why it was essential to widen the market for British aircraft, for example, by collaborative projects with our allies. At present the industry could not sustain its research and development costs and, as the Plowden Committee pointed out, was looking to the Government for a larger contribution than in the past towards the launching costs of any new project. Over the years between 1960 and 1963, research and development expenditure by the Government in the British aircraft industry on aircraft, excluding guided weapons, averaged about £100 million and total aircraft sales about £320 million. In the United States over the same period, expenditure on research and development of military aircraft averaged 635 million dollars and on procurement 5,500 million dollars a year. The Committee's rough calculations suggest that in recent years the degree of Government support given to the aircraft industry has been substantially higher than that given to British industry in general. Moreover, the higher the cost of research and development for an aircraft type, the more the price of the British version is likely to exceed that of the American.

The Plowden Committee's conclusion that the effort to establish a self-supporting British civil aircraft industry had not yet succeeded was not challenged in the debate in the House of Commons. The debate was in fact more especially concerned with the presentation in Section IV of the Report of the case for an aircraft industry. As already noted, the Plowden Committee did not base this on the transfer of technology, and while it thought that the defence benefit offered by the industry in affording an extra measure of national independence was of value at the present time, it also thought that the evidence suggested that the value of the benefit was likely to decrease and its cost increase. Nor did it consider that a case could be made out for the industry in terms of employment. It believes that new jobs would fairly quickly be found for workers from the aircraft industry if released, and, while a successful industry can make a genuine contribution to the nation's welfare, it does not regard this argument as validating the case for any particular level of support unless the very survival of the industry were in question.

From the point of view of stability the Plowden Committee concedes that there might be a case for some support to maintain the aircraft industry at a particular, lower level so that past failures would not debar it from achieving future success. Basic to this case, however, is a realistic prospect in the long run of successes outweighing failures so that such support could then be withdrawn. The Committee does not believe that in the long run the country would suffer from a major contraction in the industry, provided it was accompanied by adequate Government policies for technology. Nevertheless, Britain would suffer a serious loss if policies were followed which resulted in the speedy withering away of the industry, and the Committee does not believe that, in the long term, the benefits conferred by the aircraft industry will be so large or unique as to make it worth maintaining in existence regardless of cost; still less doing so at its present or at any particular level. Accordingly a coherent set of policies is required which offers the prospect of reducing support, the basic problem being to improve the relation between sales of the industry's products, their development and their initial production costs.

Four main policies are regarded as contributing to this. First, Britain should collaborate on a comprehensive range of military and civil projects with overseas partners. Secondly, these projects should be those for which development costs are reasonable in relation to the expected market. Thirdly, where development costs are disproportionate, as with the largest and most complex military aircraft and guided weapons, essential needs should be met by purchases from the United States. Fourthly, the industry's exports must be increased. In the view of the Committee a shift from total dependence on home production for Britain's defence needs is indispensable to these policies, and it appears that the Ministry of Defence is now ready to make such a shift.

Discussing next the possibilities of widening the market, the Committee observes that, while air traffic has grown year by year, the annual rate of growth has been declining since the Second World War and in the past few years has fallen to 12 per cent. Available evidence points to some further slow decline in the future. The increasing world demand offers little prospect for substantially increased sales for the British industry on its own, and the home military demand is unlikely to increase and may well fall. On collaboration with the United States the report emphasizes the free and intensive exchange of military scientific information encouraged by the close alliance between the two countries. Although on civil aircraft the exchanges are more restricted in view of the keen commercial competition, yet the British aircraft industry would be the poorer if the general flow of information were impeded.

Britain has also benefited from aviation research and development which successive American Governments have encouraged among their allies in Western Europe since the Second World War, often with generous financial assistance. The United States has shown a high regard for the inventiveness of Britain's scientists and several joint research and development projects have been launched between the two countries. There is little doubt that in those areas where Britain has a lead, or the promise of a lead, over the rest of the world, the United States Government will continue to encourage industry to co-operate with us in research and development.

While some, though not many, further joint projects may be expected, the United States has no over-riding need for a partnership in aircraft development and production. The technical resources of the American industry can meet all that country's main needs, and the domestic demand alone is normally sufficient to make production economic. Accordingly, the Plowden Committee believes that there is no likelihood of any agreement being reached on a comprehensive joint aircraft research and develop-

ment programme between British and American manufacturers. However, it considers that there should be scope for occasional worthwhile schemes for joint research and development as well as for the wider use of British engines and equipment in United States aircraft.

Association with Europe is another matter, and partnership between Britain and France offers many attractions. While the British and French industries are the largest in the free world, with the exception of the United States, and are reasonably complementary in capacity and evenly matched in competence, yet like all the national aircraft industries in Europe their home market is too small. None is strong enough alone to secure a reasonable share of world markets in the face of competition from the United States; even the combined market which Britain and France command might be insufficient to make a solely Anglo-French project economic. The Plowden Committee suggests that the best solution will be found in an association between the British and other European industries jointly to develop and produce as wide a range as possible of aircraft to meet the needs of Europe and sell competitively elsewhere in the world. It recommends that Britain should take the initiative in arranging a conference of European ministers of aviation with the view of formulating a common long-term policy for the manufacture and procurement of aircraft in Europe.

Beyond this the Committee considers that, if Britain is to retain a significant role in the manufacture of civil aircraft, the Government must take a larger share than in the past in financing new projects. Although the British industry cannot expect to win readily from the expanding world market a share large enough to make a major contribution to its own prosperity, it would be a mistake for British manufacturers to opt out of this field of activity. A more stable military programme than in the past will, however, be essential as the basis for the industry's contribution to a future civil aircraft programme. The Committee suggests that the Government should take a greater share of the responsibility for market studies and for formulating new requirements. While it advocates stringent tests before any project is undertaken, it does emphasize the importance of prompt decisions when the facts are known. It also comments that bolder decisions by the Government and manufacturers in the past might have paid dividends for the country. It concludes, however, that it would be undesirable for the Government to instruct the nationalized airlines to buy British or European aircraft exclusively.

The Committee did not examine the efficiency of the aircraft industry in any detail and its chapter on this subject merely suggests some aspects, such as management, which require closer examination. It formed a clear impression that productivity in Britain was not so good as it could be, or as it needs to be if the industry is to be successful. It therefore recommends that the Government should arrange with the industry for a joint full inquiry into its efficiency to determine the weaknesses and to recommend improvements. On the organization of the industry, the Committee was not convinced that existing competition between the new air-frame groups and between the main air-engine groups in the industry was either particularly real or particularly useful. It thought that the future of the defence programme and of international collaboration was at present too uncertain for it to make a firm recommendation on this fundamental point of organization, although all the evidence points to a reduction in the present size of the industry. It considered also that existing relations between Government and the industry had been a great source of frustration in the industry and had hampered the making of decisions and the efficiency of management.

The Committee suggests that what is required in this controversial field is a form of organization which will free the companies from the detailed control, both technical and financial, now exercised by the Ministry of Aviation and which it believes prevents efficient and dynamic management. The new organization would still allow the Government to contribute in varying proportions to civil aircraft projects but would leave the Boards of the air-frame groups to execute these projects without day-to-day control. It should also retain the greatest flexibility possible while the future pattern of the industry is evolving; retaining, for example, the advantage of private enterprise so far as possible, while still recognizing the dominating position of the Government both as provider of finance and as customer. The Government would thus be engaged in the working of the groups and be made directly aware of the consequences to the industry of any Governmental policies on research and development. While it sees no case for public ownership of the aero-engine and equipment companies, it thought that some degree of public ownership of the two main air-frame groups was essential to achieve a framework within which confidence in the future of the industry could be restored.

The Committee considered for this purpose both full ownership, such as nationalization, and a public share-holding. It did not, however, commit itself on this political issue, although it did draw attention to the possibilities of a combination of public and private enterprise. Equally, it did not express any view on the future of the Ministry of Aviation beyond recommending an early survey of the question by the Government. It regarded the question as outside its terms of reference; yet implied criticism of the existing arrangements is easily discernible, and had it been satisfied with the structure or competence of the Ministry of Technology it is difficult to believe that it would not have recommended transfer to that Ministry of whatever functions of the Ministry of Aviation could not widely and appropriately be transferred to the Ministry of Defence. This is fully in line with the comments of the Estimates Committee in its seventh report for the session 1964–65, dealing with electrical and electronic equipment.

The Plowden Committee does, however, add in conclusion a number of comments on the role of Government research establishments which for many years have played an important part in supporting the aircraft industry. These establishments are already engaging in work other than aeronautical, and since the three military projects were cancelled, a limit has been imposed on recruitment with the view of keeping employment at the present level. The Committee does not consider that the basic responsibility of the establishments for research and experimental work in support of the industry needs changing but recommends a shift of emphasis in the kinds of research in their programmes. In future, more emphasis should be placed on research that contributes to the more modest aircraft (especially civil) and weapons which the industry is likely to embark on. It instances the improvement of the aerodynamics of sub-sonic aircraft; the reduction of structural weight by new techniques of construction and new materials; the saving of weight on equipment and systems; improvements in reliability; and the development of more economical methods of construction.

The Committee urges that the specialized knowledge and expertise which establishments derive from their research work should be available to support the industry

in civil projects as needed, but that the establishments should not encroach on or duplicate the project work for which the industry is properly responsible. It also believes it is important that programmes should be kept consistent with the future role of the aircraft industry and recommends strengthening the industrial membership on the Aeronautical Research Council, and overhauling the Council's terms of reference and structure so as to secure a joint Government, industrial and scientific body capable of a more authoritative contribution to formulating the programme of the establishments. It suggests that a reduction over 5 years of 20 per cent in the present annual budget for intramural aerospace research and development would be realistic without detriment to the value and balance of the intramural programme.

In opening the debate on February 1 the Minister of Aviation, Mr. F. Mulley, while welcoming the Report, was distinctly non-committal on controversial issues and merely indicated the Government's views in general terms. When the Government took office the aircraft industry was absorbing a disproportionate share of Britain's resources; the Government accepted the Plowden Committee's general analysis of the situation and intended to help the industry by creating an environment in which the industry could save itself by its own resources. Priority would continue to be given to the policy of collaboration with Europe, but he accepted with reservation the recommendation that for the largest and costliest projects Britain should of necessity buy American. On civil aircraft he agreed that the general financial policy should be one of a 50 : 50 contribution from the Government, and on the size of the industry Mr. Mulley envisaged a specific contraction from the present 250,000 employees to about 200,000 in 1970, largely in the airframe side of the industry.

Mr. Robert Carr questioned the wisdom of being too specific about the reduction but, with few exceptions, criticism of the report and of the Minister's statement was balanced and constructive. Mr. Carr also emphasized the importance of greater continuity of control at every stage of a project, deprecating frequent changes of personnel. He also urged that the Ministries concerned should incorporate into the administrative hierarchy more staff with industrial and technological experience, and closer and more effective association of technical and financial assessments of a project from its conception. Closer formal association of the industry with the Government departments was essential in order to achieve cost effectiveness and there should be greater direct responsibility for execution of a project. More effective incentive contracts must be devised, and, together with some relaxation of security, Mr. Carr suggested stronger Parliamentary control by development of the Select Committee procedure.

Mr. Carr, whose suggestions were welcomed by Mr. Mulley, also strongly supported Anglo-European cooperation, as did later speakers in the debate. Some were critical of the Plowden Committee for its failure to examine more fully questions of efficiency and management, while recognizing the limitations which time placed on the enquiry. Mr. J. Cronin and Mr. N. Atkinson in particular pressed the issue of public ownership, but otherwise the debate was comparatively non-controversial. A welcome feature was the general recognition of the importance of effective association of technical experts in the control of the industry. Sir Ian Orr-Ewing put this particularly well in relation to the Ministry of Aviation, which he thought did not employ on a highly technical job in a scientific sphere enough people of a sufficiently high calibre.

Nevertheless, neither the Plowden Report nor the debate itself throws much fresh light on one of the most critical problems of the aircraft industry and of some other industries—that of accurate estimation of production costs in the face of rapid technological innovation. This is predominantly a problem of management, to which a higher standard of management may in due course be able to contribute to a decisive degree. It is also partly a problem of organization in which the structure of Government is an important factor, and this is one reason for undertaking a new and urgent examination of the place and function, not merely of the Ministry of Aviation, but of other departments concerned with civil research and development. It is also a matter of the effective use of the scientific and technical expert, and of the mechanics of scientific advice, and the Plowden report, like that of the Estimates Committee, could well stimulate the critical and constructive thought on those questions that is now so imperative.

PATENTS AND THEIR PROCEDURE

Patent Protection

The Inventor and his Patent. By Clifford Lees. Pp. x + 334 + 3 plates. (London: Business Publications, Ltd., 1965.) 50*s*. net.

PATENT Protection has been written by a patent agent who has been in practice for some years, with the object of explaining the patent system and its operation to business executives, free-lance inventors and others. With this object in view, the book has been well prepared. It is lucidly written and, indeed, in relation to its rather abstruse subject-matter it is most entertainingly presented, though it is sometimes difficult to be certain what precise audience or reader the author has in mind for some of the chapters.

The extent to which scientists and engineers find themselves involved in patent matters to-day is growing apace. They generally know little about the subject and are content not to know until the necessity arises. They then want to be told what is the right thing to do and to do it.

This book can accordingly be recommended to anyone who finds himself involved in, or concerned with, the subject of patents and wishes to acquire fairly rapidly the requisite background, though in some chapters, for example "Infringement. Opposition and Abuse of Monopoly", the detail is too great for the relatively casual enquirer, and those more knowledgeable would normally consult a more standard reference work.

In order to make the subject-matter more interesting to read and easier to absorb, much of *Patent Protection* has been contrived around a notional private inventor of a new design of potato peeler. In consequence, the practical implication in relation to the applied areas of chemistry and physics, not to mention electronics or electrical engineering, which are most likely to be of concern to the research scientist or engineer to-day, must to some extent be deduced by analogy.

Nevertheless, within the book's compass will be found both the theory and general practice of patent handling with adequate details of the system and procedure in Britain, as well as appropriate references to other such important patent countries as the United States, Germany and France.

The practical side of inventions is not neglected. There are some excellent chapters on practical development, selling and patent licensing. As the author rightly says, "success in this field is relative". It may vary from a modest income for the patentee to the establishment of a

new and flourishing business. It is debatable, however, whether there can be 'success' such as the completion of a prototype which does a job, or the solution of a technical problem, without subsequent manufacture and sale.

There are a few minor comments one might make on the accuracy of some details; for example, the author is inclined to underestimate the total cost of obtaining a United States or overseas patent, but perhaps these are not important in relation to the nature and purpose of this book, which, as the author emphasizes, can in no way stand as a substitute for the advice of the expert.

The book has a good index and some useful appendixes containing lists of libraries where patent specifications are available and of some research organizations who might be able to help or advise inventors.

B. J. A. Bard

A STUDENT'S APPROACH TO METAL JOINING

The Metallurgy of Welding, Brazing and Soldering
By J. F. Lancaster. (Institution of Metallurgists: Modern Metallurgical Texts, No. 3.) Pp. 291. (London: George Allen and Unwin, Ltd., 1965.) 36*s*. net.

WELDING, brazing and soldering are of fundamental importance in modern industry. Not only are they indispensable as constructional techniques, but also the mechanical behaviour of components ranging in scale from transistors to vast engineering structures often depend critically on the quality and design of the joints incorporated in them.

In spite of this, the study of welding metallurgy forms a disproportionately small part of many metallurgy and engineering curricula and there are scarcely any text-books written primarily for metallurgical and engineering students. In the light of this situation, *The Metallurgy of Welding, Brazing and Soldering* fulfils a very real need and Mr. Lancaster has proved a singularly fortunate choice as author. He not only has a broad grasp of the fundamentals of the processes themselves and the welding metallurgy of the different materials he considers, but he also has considerable experience of the different practical problems which may arise. He has made full use of this background to show, wherever possible, the relationship of basic principles and practical implications in a manner which might be copied to advantage by many other authors of engineering and metallurgical texts.

The book starts with a brief introduction to illustrate the historical development of the theory and practice of metal joining techniques. This is followed by a chapter outlining the general characteristics of fusion welding techniques in which the author considers the welding arc, process characteristics, drop detachment, heat flow, reactions in the weld metal, weld pool solidification and the development of residual stress. In the three following chapters fusion welding processes, solid phase welding and brazing, soldering and adhesive joining are considered.

Following chapters deal with the welding of carbon steel, ferritic alloy steels, austenitic and low-alloy steels, and non-ferrous materials, respectively. The treatment is necessarily brief in the space available and the author concentrates on defining the potential metallurgical problems which may arise during welding and showing how they may be avoided through a knowledge of the welding metallurgy.

The two final chapters are concerned with the basic principles involved in welding castings, clad steel and joining dissimilar metals and with a review of the means of controlling quality in welding respectively.

The book is very readable and plentifully illustrated by clear drawings, graphs and diagrams. The quality of the photographs and micrographs, however, leaves something to be desired. No doubt the choice of reproduction process depended largely on the need to keep the price as low as possible but in my opinion a higher quality would have been preferable even at the expense of an extra shilling or two.

Very few of the points made in the book will be seriously disputed but there is one which warrants some comment. Having started Chapter 6 by a refreshingly realistic definition of mild steel (up to 0·3 per cent carbon) the author goes on to say that this ". . . may normally be welded without any preheat or post welding heat treatment". This statement is certainly true for thin plates and sections but is not generally true of thicker plates, especially where the manganese content can approach or exceed 1 per cent.

In conclusion, this book can be thoroughly recommended for use by students embarking on the study of joining processes. However, its usefulness is by no means confined to technical colleges and universities. Practising engineers and metallurgists, even when directly concerned with welding, will find it valuable to have on their bookshelves, as I certainly shall myself.

R. G. Baker

DISLOCATIONS

Dislocations
By Prof. J. Friedel. (International Series of Monographs on Solid State Physics, Vol. 3.) Pp. xxi+491. (London and New York: Pergamon Press, Ltd., 1964.) 120*s*. net.

FRIEDEL has produced an outstanding treatise on *Dislocations* aimed at the research student in physics, in metallurgy and in engineering. That both theory and experiment on dislocations now dominate the study of the physical and mechanical properties of crystals is evident, as this very scholarly treatise ends with a bibliography of twenty-seven close-packed pages, more than a thousand references, most of which have appeared within the past twenty years. There is so much in this book that it is hard to summarize, the only deceptive feature being the relatively slight index. The line diagrams and the photographic illustrations are superb and will be the envy of every text-book writer. The clarity and lucidity of exposition owe much to these.

Elastic theory of dislocations, motions of dislocations, glide, vacancies, interstitials and dislocation climb are all treated in an adequate way, with a minimum of mathematics, but with authoritative thoroughness. The difficult field of imperfect dislocations is clarified by outstandingly lucid line illustrations, beautifully executed and notably clear. In this section twinning and epitaxy are neatly fitted in. The now well-known spiral growth theory, to which Frank has contributed so much, is then briefly summarized, and this completes Part 1.

Part 2, devoted to dislocation networks, deals sympathetically with a formidable subject. In a section on the Frank network, the intriguing subject of crystal whiskers is briefly surveyed, then comes the discussion the metallurgists will have been waiting for, namely, cold working, the relation between dislocations and hardening, annealing, recrystallization, grain boundaries and creep. The scholarship exhibited in these sections arouses admiration. Cleavage and crack propagation are next briefly discussed and here is the one section where I wished that more might have been included, yet this is a purely personal wish and not meant as a criticism.

A shorter third part (96 pp.) deals with interaction of dislocations and other defects such as impurities, precipitates and other crystal lattice perturbations. This section includes a survey of semi-conductors.

In this outstandingly scholarly contribution one aspect which I felt could have justly received more detailed treatment was that dealing with etch pits. But since selection there must be, inevitably some branches must be more lightly treated than they deserve, otherwise bulk becomes impossibly great.

I very much welcome this fine new text on dislocations, despite the fact that there are already perhaps half a dozen or so books on this subject. It is probably the most comprehensive of all and certainly outstandingly lucid in presentation. It will remain a main authority in the field for a long time. S. TOLANSKY

RUSSIAN RADIOPATHOLOGY

Studies in the Pathology of Radiation Disease

By N. A. Krayevskii. Translated by A. Lieberman. English translation edited by Hermann Lisco and Malcolm Walker. Pp. xv+219. (London and New York: Pergamon Press, 1965.) 100*s*. net.

AT first sight it seems pointless to translate a book on the pathology of radiation injury written in 1957 and unamended since. The justification might be that basic descriptions of morbid histology have a permanent value regardless of time, or that there are important Russian data hitherto unknown to the West.

Unhappily in this case neither claim can be upheld. The main bulk of *Studies in the Pathology of Radiation Disease* is an account, tissue by tissue, of the gross and microscopic changes induced by radiation (mostly to the whole body) in dogs, cats and rats. Both acute and chronic radiation effects have been studied using either external irradiation or internally administered isotopes. Many of the descriptions are careful and detailed but the terms used are sometimes vague or undefined. For example, what precisely is meant by "disturbed haemopoiesis . . . in the form of progressive pernicious anaemia"? and, referring to recovery from radiation, "a mobilisation of internal resources occurs which is mediated particularly through the nervous system. Following I. P. Pavlov, we may look on this process as a physiological counter-measure to the disease"?

An extraordinary omission is of any reference to blood platelets. In page after page there are descriptions of haemorrhages in various organs and all are attributed entirely to blood-vessel damage. In fact, the words 'platelet' and 'thrombocytopaenia' do not occur throughout the whole text. In compensation, perhaps, one of the longer chapters (by Prof. Portugalov) concerns the nervous system. His final comment, however, is on the 'slightness' of the radiation effects, and one wonders whether this accords with the general Russian view.

The illustrations are of mixed quality and suffer from a complete absence in the captions of any reference to staining technique or magnification.

It is in the final chapter, "Conclusions", that the lack of modernity is most evident. What is the use of quoting old speculations on radiosensitivity in different phases of cell cycle when the subject has since been studied experimentally? And the references to human radiopathology are inevitably meagre if all that has happened since 1957 is left out. Perhaps a better book could have been written in 1965; certainly this edition cannot be recommended. H. E. M. KAY

THE GRASSHOPPERS AND LOCUSTS OF AFRICA

The African Genera of Acridoidea

By V. M. Dirsh. Pp. xiii+579. (London : Cambridge University Press, 1965. Published for the Anti-Locust Research Centre.) 200*s*. net; 37.50 dollars.

WHAT is the difference between a grasshopper and a locust? The answer will not be found in *The African Genera of Acridoidea*; the reader is expected to know. Indeed, the answer has nothing to do with taxonomy, which is the subject of this book; the distinction is one of ecology and behaviour. In passing, it may be noted that in America they are all called 'grasshoppers': the name 'locust' is reserved for the cicadas. Within the British Commonwealth the grasshoppers follow a more or less 'solitary' existence ; they tend to remain throughout their lives in one locality. But certain species of Acridoidea change their habits from time to time: for long periods they may live solitary lives like the other grasshoppers; but when their numbers increase they reveal a capacity to gather together in great companies or swarms. In this 'gregarious' state they will undertake migrations, the larval stages or 'hoppers' on foot, the winged adults in the air. It is those species with the tendency to enter on such migrations that are called locusts. In certain of these species the gregarious or migratory 'phase' is strikingly different from the solitary non-migrating phase, not only in behaviour but also in coloration, morphology and physiology.

In elementary entomology those Orthoptera with enlarged hind-legs adapted for leaping (the 'Saltatoria' of Latreille) fall into three groups: the long-horned grasshoppers (Tettigoniidae), the short-horned grasshoppers (Acrididae), and the crickets (Gryllidae). Here we are concerned only with the short-horned grasshoppers which are now classified as a superfamily, the Acridoidea, of which the family Acrididae is by far the largest and is indeed subdivided into a great number of subfamilies of which no less than sixteen are represented in Dr. Dirsh's book on the African genera. The vast majority of the species included in these genera would be called 'grasshoppers'. There are only some half-dozen species belonging to the genera *Locusta* and *Locustana* (of the subfamily Acridinae), and *Anacridium*, *Nomadacris*, *Schistocerca* (of the subfamily Cyrtacanthacridinae), which are recognized as gregarious and migratory locusts.

At the present time. most of the major species of plague locusts in Africa and Asia are in a temporary-state of recession. But meanwhile the cultivator is becoming increasingly concerned about the grasshoppers, which in the past had been considered of small importance. When large-scale mechanized crop production was developed in the Anglo-Egyptian Sudan in the 1940's, about a score of local species of grasshoppers became quite serious endemic pests. Species which had normally subsisted on wild grasses found cultivated sorghum an attractive diet, and the cultivation of the soil facilitated their breeding. As mechanized cultivation spreads throughout Africa, the same problems are certain to arise elsewhere. Hence the interest in building up a systematic background to help the entomologist to recognize his potential pests. It may well be that on closer acquaintance some of these grasshopper species will be found to possess latent powers of swarming and migration which will entitle them some day to be called locusts.

The African genera of locusts and grasshoppers as described in this book number about six hundred and fifty and embrace nearly two thousand species. Many of them have been inadequately described in earlier publications. Dr. Dirsh has been faced with a most formidable task in revising, checking and collating all the information (up to 1958) available about these insects and presenting it in a compact but comprehensive form. He has accomplished this task with complete success. Some four hundred and fifty carefully drawn illustrations supplement the descriptions of the genera. There are keys and brief statements of diagnostic characters for families, subfamilies and genera, and a list of the African species in each genus. Geographical distribution is given in general terms. This book, like its companion volume already well known to workers in this field, H. B. Johnston's *Annotated Catalogue of African Grasshoppers*, is published on behalf of the Anti-Locust Research Centre, London. It will be a standard work for many years to come.

V. B. WIGGLESWORTH

MARGARINE—SCIENCE AND TECHNOLOGY

Margarine

Second revised edition. By A. J. C. Andersen and P. N. Williams. Pp. xi+420. (Oxford, London and New York: Pergamon Press, 1965.) 110*s*.

VERY little has been written about the margarine industry despite the fact that it is world-wide and that margarine is a major item in the human diet in many countries. With the first edition of *Margarine* in 1954, Andersen rendered a major service in setting out clearly, and in readable form, the basic principles on which the industry is built.

The present edition, as the preface suggests, retains virtually all the material of the first edition to which has been added the advances of the past decade. Most of the chapters are of a high standard and convey an intimate knowledge of the margarine industry.

The sections on milk and non-fatty ingredients are very comprehensive and indicate clearly the influence which these materials have on shelf-life and market acceptability. This is supplemented by a very useful chapter on storage and preservation which also underlines the importance of good plant hygiene and the correct choice of materials for plant construction.

The chapter on processing is probably the best in the book. The published and particularly the patent, literature has been dealt with in considerable detail, with the result that both chemist and engineer will find much valuable information. The flexibility in equipment and in product properties which is available to the manufacturer is clearly brought out.

A short but valuable section on physical methods of quality control has been added to this edition, signifying that the industry is moving away from the purely subjective assessment of its products.

The chapter on legislation should prove useful to those contemplating exporting or manufacturing outside their own country, although chapters such as this can quickly become out of date.

There is no doubt that this edition will rank as highly as the first. In the past ten to fifteen years the industry has undergone something of a minor revolution. Greater use than ever before is made of a wide variety of raw materials. Advances in refining and processing have improved quality in terms of texture, flavour and keepability to an unprecedented degree. A streamlined flow of crude oils and fats in at one end of the larger factories is coupled with the flow of a range of sophisticated products out at the other end. Although many of the advances are cited separately throughout the book, the total impact, to demonstrate the dynamic state of the industry, would have been greater if they had been reviewed in the introduction. The quality of the photographs also reduces this impact of the book. They are poorly defined and often create an impression of the nineteen-thirties, even though many of them depict quite modern equipment.

There are also a number of points which I feel would have added to the value of the book if they had been treated more fully. Thus, in considering the chemistry of raw materials, organic chemistry is concentrated on at the expense of physical chemistry, yet it is mainly in physical terms that the important qualities of margarine, such as melting and spreading, can be explained.

Any major work on margarine must be incomplete without a considerable discussion on hydrogenation, yet the authors in this case deal with the subject mainly by a limited number of references to other works and a general description of the properties of several hardened fats. Without increasing the size of the book, a little more detail could have been included by reducing the space devoted to some of the historical aspects of margarine manufacture.

Despite the criticisms, I regard this edition as a comprehensive standard text-book on margarine manufacture, which those with either fringe or particular interests could usefully add to their libraries. It is well presented and easily read, and for those seeking greater detail, there is a comprehensive bibliography of papers and patents.

H. Lavery

INSIDE THE BOARDROOM

Investment Proposals and Decisions

By Bruce R. Williams and W. P. Scott. Pp. 100. (London: George Allen and Unwin, Ltd., 1965.) 21*s*. net.

IN 1961 a Centre for Business Research was formed at the University of Manchester as a co-operative venture between the University and a group of business firms who agreed to provide facilities for research into some of the major problems of business. *Investment Proposals and Decisions* is based on studies of the origin and outcome of investment decisions in fourteen of the member firms. The studies were carefully chosen to show in each one capital project where, if possible, investment decisions had been concerned with innovation or expansion and not simply with replacement, involved fairly large expenditure, had been taken some time ago so that the outcome of decisions could be investigated and, finally, were of a kind which involved several departments.

It is not surprising to learn that few of the projects examined conformed to all the requirements. Some could not be studied in isolation while, with others, investigations were circumscribed by the lack of essential documents, key people or sometimes by internal politics. Nevertheless, the studies proceeded, and it is a tribute to the Centre, not only that the investigations have been made, but also that they have been so frankly reported.

The cases described include investment for a new plant embodying a new process for making an existing product; the establishment of a branch factory abroad to remain in front of competitors; the building of a new factory at home to extend a relatively new product range; the introduction of new processes to improve product quality; the wider use of management services and techniques to reduce costs; the introduction of a new product: take-over of another company to acquire a successful product; and, in a number of cases, the introduction of computers for improving data processing. So far as possible, each case is considered against four aspects of an investment decision process—recognition of potential, collection of information, evaluation of the information, and the final decision. From these detailed examinations, Prof. B. R. Williams and W. P. Scott have gleaned some useful findings about the theory and practice of investment decisions and indicate that, although the practice may be based more on 'hunch' than on substantiated principle, the 'hunch' is not necessarily a whim plucked out of thin air. "It may be the result of a subconscious sifting of ideas, information and experience." Some common pattern for investment does emerge, and few aware of boardroom practice will be surprised to learn how, in judging proposals, the status of the sponsor carries great weight. This finding would have emerged in sharper relief if the Centre had found it possible to extend its enquiry to less sophisticated firms where management decisions are arrived at even more intuitively. What matters, however, is that a start has been made and, as a guide for investment decision-making in the future, the book is significant not only for the case studies but even more for its valuable check list for investment proposals. The well-planned structure and succinct writing make for easy reading, and the only detriment in a book which would both enliven and enlighten many board meetings is that the authors seem out of love with indexes.

T. H. Hawkins

The Nuclear Reactor

By Dr. Alan Salmon. (Methuen's Monographs on Physical Subjects.) Pp. 144. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1964.) 16*s*. net.

THE Nuclear Reactor has been produced for honours physics students, postgraduate students of nuclear power and for the non-physicist whose work is associated with nuclear power.

The introductory chapter is followed by chapters covering nuclear fission, the basic theory of neutron behaviour, and the optimum use of fuel and neutron flux flattening. There is a useful chapter on heat transfer, which includes a brief account of the desirable features of reactor coolants. Reactor control and shielding are then considered, followed by a chapter on the reactor as a source of radiation. This includes a description of the basic kinds of research reactors and their characteristics. The final chapter is concerned with future developments, and discusses in some detail the future of nuclear power reactors.

Throughout the book, emphasis is placed on understanding the processes and methods involved. Each chapter has its own set of references and a useful list of suggested further reading. The graphs and drawings are distinct and adequately annotated, and fit well into the text.

For such a compact book the subject of nuclear reactors is soundly treated, though because of its size the book does suffer through its inability to spend enough time on detail. However, the aim of the book—a short succinct account of nuclear reactors—is quite well achieved, and though there are other similar books it should find a welcome place on many bookshelves. V. S. CROCKER

Progress in Medicinal Chemistry

Vol. 4. Edited by G. P. Ellis and G. B. West. Pp. ix+221. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 67*s*. 6*d*.

VOLUME 4 of *Progress in Medicinal Chemistry*, like its predecessors, is a series of review articles. Barnes and Paget, writing on drug toxicity, highlight our remarkable lack of knowledge of the nature of the majority of toxic responses. When so much effort is expended in the production of new drugs, present knowledge of the biochemistry of the toxic response seems disproportionately small. This article merits classification as compulsory reading for all those interested either in the production or in the application of new drugs. The difficult subject of drug receptor interactions is surveyed by Gill with particular emphasis on the shapes of small molecules and on their interaction with proteins. In this rapidly developing field, recent investigations on allosteric enzymes are likely to have a considerable impact. The comprehensive review by Law of recent work on biologically active peptides emphasizes the remarkable progress made during the past ten years. The volume is completed by an article on analgesics (Beckett and Casy) and by one on experimental hypersensitivity (Spencer and West). As might be expected in such a composite volume, the standard achieved varies considerably, but nevertheless there is sufficient thought in some parts of this volume to make it desirable reading. T. S. WORK

Experiments in Plant Hybridization

By G. Mendel. Pp. ix+95. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 21*s*.

THIS is a pleasing little volume appropriate both for the Mendel centenary and as a contribution to the memory of Fisher. It contains more than its title suggests, for within its covers we find Fisher's notable essay on the history of science, Fisher's notes on Mendel's paper and Bateson's biography of Mendel, in addition to Bateson's translation of Mendel's paper.

There is little for me to say except to congratulate both editor and publisher. However, a reading of the book prompts a question of some historical interest. Most outstanding science involves not only imaginative ability and painstaking work, but also an element of luck. One remark in Mendel's paper, taken together with Bateson's footnote, suggests an element of luck that has not, so far as I am aware, been noted before. This is that *Pisum* was in fact self-pollinating. Mendel states dogmatically that "It is only in the Leguminosae, like *Pisum*, *Phaseolus* and *Lens*, whose organs of fertilisation are protected by the keel", that numerous varieties maintain "a stability as great as that of species growing in the wild". Bateson comments that "Phaseolus is nevertheless insect fertilised".

Now Vilmorin[1] carried out experiments from 1856–60 with *Lupinus hirsutus* which were not dissimilar to some of Mendel's and based his interpretations on the same dogma that "comme dans beaucoup de légumineuses papilionacées, la fécondation s'y opère généralement à huis-clos et sans l'intervention d'aucun insecte". But one only has to glance at his data with hind-sight to see that he was getting dominant progeny from recessives as well as vice versa, hence that considerable outcrossing was going on.

There may therefore have been an element of luck in Mendel's choice of *Pisum* rather than *Phaseolus* for the bulk of his work. However, though Mendel nowhere throws doubt on the accepted dogma as applied to *Phaseolus*, he designed his experiments with *Pisum* so that he would know if occasional uncontrolled crossing did occur. In other words, though he may have been somewhat lucky in choosing *Pisum*, he would not have been misled had he chosen *Phaseolus*, for though he does not doubt the accepted he made sure it really did apply to his material.

These comments of mine merely illustrate the way in which this book can provoke thought about the factors involved in good experimentation. It should be widely read by biologists and historians of science, and should be in all school libraries. It is not only a document on the foundation of genetics, but a valuable education in the anatomy of experimental science. J. M. THODAY

[1] See the paper on his father's experiments by Vilmorin, H. (Société National d'Agriculture de France, Paris, 1879).

Administering Research and Development

The Behaviour of Scientists and Engineers in Organizations. By Charles D. Orth, Prof. Joseph C. Bailey and Francis W. Wolek. Pp. x+585. (London: Tavistock Publications, Ltd., 1965.) 50*s*. net.

THE organizational and human problems involved in the management and control of large-scale research and development have received considerable attention during recent years. The most thorough exposition of them in Britain, in relation to Government departments and other bodies financed wholly by the Exchequer, was afforded by the Gibb–Zuckerman report published by H.M.S.O. in 1961. This American book describes the results of a six-year investigation into the behaviour of scientists and engineers employed within industrial research and development establishments. It records case studies conducted in fourteen such establishments covering a wide spectrum of industrial activity, and contains a number of research reports dealing generally with the issues raised by these studies. It includes also a series of so-called conceptional readings which seek to illuminate, from different points of view, the factors affecting behaviour in the environment of research and development work. Perhaps inevitably, a book so composed and on a subject of such complexity is not easily digestible, and the reading and understanding of it would have been assisted had it contained an extraction of a number of common occurrences of general relevance to the situations examined, which could serve as guide-lines for those carrying senior responsibility in the research and development field. WILLIS JACKSON

PREDICTION OF FUTURE LEVELS OF LONG-LIVED FISSION PRODUCTS IN MILK

By B. O. BARTLETT and Dr. R. SCOTT RUSSELL
Agricultural Research Council Radiobiological Laboratory, Wantage, Berkshire

SINCE 1958, when the United Nations Scientific Committee on the Effects of Atomic Radiation[1] made its first comprehensive review of the consequences of world-wide fall-out, many attempts have been made to derive formulae for calculating the level of radioactivity in foodstuffs which is likely to be caused by different patterns of environmental contamination. Information has been obtained mainly by analysing relationships between measurements of fall-out and of dietary contamination made in environmental surveys. Controlled experiments on food-chain mechanisms and information on agricultural practices are, however, important supplementary sources of guidance; besides indicating whether relationships suggested by survey results are causal or fortuitous they can reveal factors which, though unimportant under conditions so far experienced, may require greater consideration in the future. Improved information can be expected as the period during which observations have been made becomes longer because both climatic factors and variations in the seasonal distribution of fall-out influence the transfer of radioactivity to diet. Conclusions previously reached in the United Kingdom[2–4] have, therefore, been reviewed in the light of the more extensive data which are now available.

Consideration has been limited to two nuclides, strontium-90 and caesium-137, in a single foodstuff, milk. These two nuclides are mainly responsible for the internal radiation doses to which long-lived fission products give rise and it is well established that the contamination in milk has provided a good guide not only to the exposure of children but also to that of adults who consume a mixed diet of the type normal in Western countries[5]. Moreover, the data on the contamination of milk are considerably fuller and more reliable than those for other foodstuffs.

The annual average levels of contamination in milk throughout the United Kingdom have alone been considered; predictions for smaller areas or shorter periods would be subject to much greater uncertainty. The country-wide average deposition of strontium-90 used in the present calculations has been derived, from the results of surveys conducted by the United Kingdom Atomic Energy Authority[6–12], by multiplying the mean concentration in rain from all sites for which quarterly determinations were made by the mean rainfall throughout the United Kingdom. The average ratio of caesium-137 : strontium-90 in fall-out is relatively constant, and the deposition of caesium-137 has therefore been calculated from that of strontium-90 taking the ratio of caesium-137 : strontium-90 as 1·7 up to 1961 and 1·5 since then[13]. The country-wide mean levels of radioactivity in milk were taken from the reports of the Agricultural Research Council Radiobiological Laboratory[14–20].

Strontium-90

It has been recognized for many years that strontium-90 may enter milk as a result of both the 'direct' contamination of pastures and other forage crops with airborne debris and the absorption of strontium-90 through plant roots from the soil. The magnitude of these two components is related, respectively, to the recent rate of fall-out and the cumulative deposit in the soil. Equations of the following type have, therefore, been widely used to relate the ratio of strontium-90 to calcium in milk to the rate of fall-out and cumulative deposit of strontium-90:

$$C = p_r F_r + p_d F_d \qquad (1)$$

where C is the 12-month mean ratio of strontium-90 to calcium in milk (pc./g), F_r is the annual deposit of strontium-90 (mc./km²/year), F_d is the cumulative deposit of strontium-90 at the middle of the year (mc./km²), and p_r, p_d are described as the 'rate' and 'soil' proportionality factors. Such equations have been shown to provide a useful basis for predicting both the average levels of contamination in milk in a number of countries and also the world-wide situation[4,5,21]. None the less, the relationship between the pattern of fall-out and the contamination of milk is considerably more complex than the equation suggests. The assumption that the soil factor is constant ignores the progressive penetration of strontium-90 deeper into the soil which will reduce its availability to pasture plants[3]. A further defect is that the equation takes no account of the fact that an appreciable, though variable, fraction of the diet of cattle consists of stored food grown in the previous year; thus the rate of fall-out at that time will influence the extent to which the current diet of cattle has been directly contaminated by airborne debris. In some areas this 'lag' effect of the rate of fall-out may be enhanced due to the absorption by plants of strontium-90 which has lodged in the basal tissues and been retained for several months without being incorporated in the soil.

The inadequacy of equation (1) from this point of view is apparent when the mean annual ratios of strontium-90 to calcium observed in milk throughout the United Kingdom between 1958 and 1964 are compared with those which would have been expected from the measured deposit of fall-out on the basis of the proportionality factors for equation (1), shown in Table 1, which were derived by a least squares analysis of the results of surveys between 1958 and 1961. The average agreement between calculated and observed values was good (Table 2) but for individual years there were appreciable divergences. The calculated values were too low for years when the lag effect was expected to be greatest because of high fall-out in the previous year (that is, 1960, 1963 and 1964); conversely, the calculated values were too high when the rate of deposition had previously been relatively low. An approximately linear relationship was found between the magnitude of these discrepancies and the extent of fall-out in the second half of the previous year. Only a small improvement was obtained when the rate factor in equation (1) was related to the deposition of fall-out in the twelve months ending in June or September instead of to that in the current year. The following equation was, however, found to give a significantly better fit to

Table 1. Estimates of Proportionality Factors for the Transfer of Strontium-90 to Milk in the United Kingdom

	Equation 1	Equation 2
Rate factor (p_r) (pc. ^{90}Sr/g Ca per mc /km²/year)	0 76*	0 70
Lag-rate factor (p_l) (pc. ^{90}Sr/g Ca per mc./km² in second half of previous year)	—	1 13
Soil factor (p_d) (pc. ^{90}Sr/g Ca per mc./km²)	0·19*	0 11

* Calculated from survey data for the period 1958 to 1961 (ref. 28).

Table 2. COMPARISON OF CALCULATED AND OBSERVED LEVELS OF STRONTIUM-90 IN MILK IN THE UNITED KINGDOM

Year	Deposition of strontium-90 (mc./km²)			Strontium-90 in milk			Percentage of strontium-90 in milk attributable to cumulative deposit in soil	
	January to June	July to December	Total	Observed (pc.^{90}Sr/g Ca)	Calculated values as percentage of observed			
					Equation 1	Equation 2	Equation 1	Equation 2
1957	1·6	1·5	3·1	—	—	—	—	—
1958	3·2	2·1	5·3	7·2	91	97	38	22
1959	6·3	1·8	8·1	9·8	104	107	40	23
1960	1·0	0·9	1·9	6·4	92	94	76	44
1961	0·7	1·4	2·1	5·9	107	90	74	53
1962	5·3	5·4	10·7	11·7	119	108	42	28
1963	9·5	9·6	19·1	25·6	90	96	37	21
1964	9·6	5·3	14·9	28·0	82	101	51	25

the data ($P < 0{\cdot}01$), the residual standard error being reduced from 16 to 6 per cent:

$$C = p_r F_r + p_l F_l + p_a F_a \qquad (2)$$

where F_l is the deposit of strontium-90 in the second half of the previous year (mc./km²) and p_l is the 'lag-rate' factor, the other symbols being defined as in equation (1). When the lag-rate factor was related to the deposit in the whole of the previous year, or in the summer months only, a poorer fit was obtained. Proportionality factors for equation (2), derived by least squares analysis, are shown in Table 1. The levels of strontium-90 in milk calculated on this basis agree closely with those observed in each year (Table 2) and it is evident that equation (2) describes the situation in past years more satisfactorily than equation (1).

The introduction of the lag-rate factor leads to a lower value for the soil factor, and a smaller fraction of strontium-90 in milk is thus attributed to absorption from cumulative deposit; it appears from equation (2) that strontium-90 from this source was responsible for from 20 to 50 per cent of the contamination of milk in past years (Table 2). The use of the lower soil factor derived by equation (2) receives some independent support from the fact that a value of 0·14 has been derived from the results of tracer experiments[3] in the United Kingdom in which strontium-89 was distributed in the soil in a manner which is broadly comparable with that observed in surveys of fall-out in permanent pasture in the recent past[19,20].

The adoption of equation (2) causes lower levels of strontium-90 in milk to be predicted when the cumulative deposit is an important source of contamination, the reverse being true when the contribution of the recent deposit, especially that in the second half of the previous year, is large. To illustrate the extent of the difference between predictions based on the two equations, calculations have been made for two hypothetical models of future fall-out, namely: Model A, the rate of fall-out will decrease by a factor of 2 annually. Model B, the rate of fall-out will remain constant. In both models it has been assumed that the annual deposit in the first year is 45 per cent of the cumulative deposit measured at mid-year (that was, the approximate situation in 1963, the year in which the highest levels of fall-out were observed) and that the rate of fall-out was similar in the second half of the previous year. The decrease in the rate of fall-out in Model A is greater than that anticipated in the absence of further weapon trials[13] while the amount of fall-out assumed in Model B would over a few years much exceed that caused by all weapon trials hitherto. Future situations resulting from weapons testing may thus be expected to lie between the two models.

Predictions made by the two equations are compared in Table 3. In considering the situation over long periods it is necessary to take account of loss of strontium-90 from the soil which arises mainly from leaching or from absorption by plants; following the United Nations Scientific Committee on the Effects of Atomic Radiation[5] an average loss of 2 per cent per annum has been assumed.

For the first year the level of contamination in milk predicted by equation (1) is about 20 per cent lower. However, under conditions of decreasing fall-out (Model A) the relative magnitude of predictions based on the two equations changes progressively, the integrated values over 10 and 50 years being about 10 and 40 per cent higher respectively when derived by equation (1). For Model B the two bases of prediction differ to a smaller extent and the difference would be still smaller if an increasing rate of fall-out were assumed.

Caesium-137

Unlike strontium-90, caesium-137 is usually subject to a considerable degree of 'fixation' in the soil on account of its entrapment in the lattice structure of clay minerals and it usually enters plant roots much less readily. It has been suggested that current fall-out is the only significant source of caesium-137 in foodstuffs, but this was disproved in 1960 and 1961. In many countries the decreasing rate of fall-out then caused smaller reductions in the levels of caesium-137 in milk than would have been expected if they were determined solely by the current fall-out[13]. An explanation of the situation seemed to be provided by the fact that caesium-137 can enter plants relatively freely from soils which contain large quantities of organic matter. The surface soil underlying permanent pastures usually shows this characteristic, and experimental investigations have indicated that caesium-137 continues to enter the edible tissues of pastures relatively freely during the 2 years after its deposition[22]. This led to the suggestion that an equation similar to (1) might be applicable except that the soil factor related only to the deposit in the previous two years[23], namely:

$$C = p_r F_r + p_a F_a \qquad (3)$$

where C is the 12-month mean concentration of caesium-137 in milk (mc./km²/year), F_r is the annual deposit of caesium-137 (mc./km²/year), and F_a is the deposit of caesium-137 accumulated over the preceding two years (mc./km²). The values of the proportionality factors (Table 4) were considerably higher than those for strontium-90 because caesium-137 is transferred from the diet of cattle to milk ten or more times as readily as strontium-90 (ref. 24). Levels of caesium-137 in milk between 1960 and 1963 calculated by equation (3) agreed reasonably with those which were observed (Table 5) and it was found that the body-burden of caesium-137 in man could be related to the pattern of fall-out in a similar manner[13,23].

Table 3. COMPARISON OF PREDICTED LEVELS OF CONTAMINATION OF MILK, BASED ON DIFFERENT EQUATIONS, FOR TWO HYPOTHETICAL MODELS OF FALL-OUT

Model	First year	Years 1–10 Integrated value		Years 1–50 Integrated value	
	Both models	*A*	*B*	*A*	*B*
Ratio of strontium-90 to calcium in milk (relative to year 1 calculated by equation 2)					
Based on equation 1	0·8	4·6	12	10	110
2	1·0	4·1	12	7	90
Caesium-137 per l. of milk (relative to year 1 calculated by equation 4)					
Based on equation 3	0·8	1·9	8	1·9	40
4	1·0	2·6	10	3·0	54

Model A, fall-out rate halved each year. Model B, fall-out rate remains constant.

The rate and cumulative total of fall-out in year 1 are taken to be the same in both models, the annual deposit being 45 per cent of the cumulative deposit at mid-year (that is, the relationship in 1963); it is further assumed that the rate was similar in the previous year.

Table 4. Estimates of Proportionality Factors for the Transfer of Caesium-137 to Milk in the United Kingdom

	Equation 3	Equation 4
Rate factor (p_r) (pc. ^{137}Cs/l. per mc./km^2/year)	3·57	3 00
Lag-rate factor (p_l) (pc. ^{137}Cs/l. per mc /km^2 in second half of previous year)	—	6 00
Soil factor (p_d) (pc. ^{137}Cs/l. per mc./km^2)	0 65 (relative to deposit in previous 2 years only)	0 03* (relative to cumulative deposit)

* Unlike the other factors, which are based on the analysis of survey data, the soil factor in equation (4) has been deduced from experimental results.

When, however, the proportionality factors were applied to fall-out data for 1964 the calculated level in milk differed appreciably from that which was observed (Table 5). As the manner in which caesium-137 is initially retained on edible herbage and removed from it by rain differs little from that of strontium-90 (ref. 25), it appeared possible that a lag-rate factor similar to that used for strontium-90 in equation (2) might be applicable. The following equation, which differs from equation (2) only in units used, was therefore applied to the survey data for caesium-137 from 1960 until 1964:

$$C = p_r F_r + p_l F_l + p_d F_d \qquad (4)$$

where C is the 12-month mean concentration of caesium-137 in milk (pc./l.), F_r is the annual deposit of caesium-137 (mc./km^2/year), F_l is the deposit of caesium-137 (mc./km^2) in the last half of the previous year, and F_d is the cumulative deposit of caesium-137 in the soil (mc./km^2). Values for the rate and lag-rate factors derived from survey data by least squares analysis are shown in Table 4; the soil factor appeared to be zero. Calculated values for the mean level of caesium-137 in milk for each year based on these rate and lag-rate factors agreed more closely with those observed than when equation (3) was used (Table 5).

Table 5. Comparison of Calculated and Observed Levels of Caesium-137 in Milk in the United Kingdom

Year	Deposition of caesium-137 (mc./km^2) January to June	July to December	Total	Caesium-137 in milk Observed (pc./l.)	Calculated values as percentage of observed Equation 3	Equation 4
1959	10·7	3·1	13·8	—	—	—
1960	1·7	1·5	3·2	26	98	106
1961	1 2	2·4	3·6	21	116	96
1962	8 0	8 0	16·0	62	100	101
1963	14 3	14·4	28·7	135	86	100
1964	14 3	8 0	22 3	153	71	100

Although the analysis of the survey data provides no evidence that the cumulative deposit of caesium-137 has contributed hitherto to the contamination of milk, experiments with tracer levels of caesium-137 indicate that this nuclide continues to enter the roots of plants, though to a small extent, for many years after its entry into the soil. Thus if equation (4) is to be used for predicting levels of caesium-137 in milk in future situations when the cumulative deposit may exceed the annual rate by much larger factors than has occurred hitherto, it is necessary to consider the possible magnitude of the soil factor. Some basis for so doing is provided by the results of experiments on several British soils; 3–5 years after they had been contaminated the ratio in which caesium-137 and strontium-90 were absorbed by plants was on average only one-fortieth of that in the soil[26]. Using this figure and assuming (*a*) that caesium-137 is transferred from the diet of cattle to milk about ten times as readily as strontium-90 (ref. 24), and (*b*) that the average calcium content in milk is about 1·1 g/l., a soil factor for caesium-137 of about 0·03 can be derived from the value for strontium-90 calculated by equation (2) (Table 1). On this basis, the cumulative deposit of caesium-137 would on average have been expected to account for less than 1 per cent of the caesium-137 which has hitherto entered milk from world-wide fall-out. The analysis of survey results for past years could not be expected to reveal this component and at the present time the soil factor can thus be estimated only from laboratory experiments.

The concentrations of caesium-137 in milk which would be predicted on the basis of equations (3) and (4) are compared in Table 3 for the two models of fall-out which have already been outlined; no allowance has been made for losses of caesium-137 from the soil by processes other than the decay of radioactivity because its movement in soil and absorption by crops are much smaller than those of strontium-90. Integrated over the first 10 years, equation (4) leads to predicted values 20–30 per cent higher than those given by equation (3) for both models; over 50 years under conditions of decreasing fall-out the integrated value given by equation (4) is 50 per cent higher. Over this period the soil would contribute about 20 per cent of the total.

Conclusions

Evidence reviewed in this article indicates that the annual average level of strontium-90 in milk in the United Kingdom is influenced to an appreciable extent by the rate of fall-out in the last half of the previous year as well as by the current rate of fall-out and cumulative deposit. An improved basis for predicting future levels of strontium-90 in milk is therefore provided by an equation which includes a proportionality factor for this 'lag-rate' effect in addition to the rate and soil factors used in former assessments, equation (2). The same type of equation is applicable to caesium-137 (equation 4) though with that nuclide the contribution from the cumulative deposit has been so small that it can be ignored under conditions of fall-out experienced hitherto.

The levels of both nuclides in milk which would be predicted under conditions of fall-out which may be expected during the next decade as a result of weapon testing are not greatly altered when allowance is made for the lag-rate effect. In any year the greatest difference is unlikely to exceed about 20 per cent and averaged over 10 years predictions for strontium-90 would be altered by a smaller amount. This degree of agreement between different methods for prediction encourages confidence in their use to forecast situations over relatively short periods of years; much greater uncertainty besets many other phases of the assessment of the effects of environmental contamination.

If, however, the integrated levels of dietary contamination are considered over long periods, especially when the rate of fall-out is declining steadily, predictions on the new basis diverge substantially from those which were made formerly. Thus, if the rate of fall-out were to decrease by a factor of two annually, the integrated levels of strontium-90 and caesium-137 in milk over 50 years would be expected on the new basis to be respectively 30 per cent lower and rather more than 50 per cent higher than those formerly predicted; this reflects the reduced soil factor for strontium-90 and the small soil factor now deduced for caesium-137. In consequence the ratio of the integrated level of caesium-137 in milk to that of strontium-90 over this period would be twice that previously calculated.

Long-term predictions of this type are subject to particular uncertainties owing to the lack of direct information on the behaviour of nuclides in soil over long periods. None the less, they deserve consideration as they form the basis for estimating the integrated radiation dose, or dose commitment, which will be received by the population; this is regarded as the best available guide to the total impact of radiation from weapon testing[5,13]. The revised proportionality factors suggest that an appreciably larger fraction of the total dose commitment is due to caesium-137 and a smaller fraction to strontium-90 than has hitherto been assumed.

In conclusion it is to be emphasized, first, that although the method of prediction here outlined appears to give a reasonable description of the average situation, appreciable divergences in individual years must be expected

because of climatic variation, and secondly, that the proportionality factors here proposed have been derived for the United Kingdom only. Moreover, variations are to be expected between countries which contrast in climate, soil type and agricultural practices. Thus a recent analysis of relationships for caesium-137 in milk in Sweden[27] gives a rate factor more than three times that calculated for the United Kingdom and a lag-rate factor (which was related to the deposit in the entire previous year) similar to the factor which relates to the deposit during 6 months only in the United Kingdom. Differences, though of lesser magnitude, are apparent between proportionality factors calculated for milk in other countries[5].

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ATMOSPHERIC CARBON-14 CONTENT DURING THE PAST THREE MILLENNIA IN RELATION TO TEMPERATURE AND SOLAR ACTIVITY

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RECENT summaries of temperature, glacial activity and solar activity[1–11] provide more exact climatological patterns than previously available against which to test changes in carbon-14 activity over the past three millennia. These patterns will be used in an attempt to reconcile contradictions in interpretations[12–15] of carbon-14 climate relationships.

Source of data. Data for carbon-14 activity were obtained from numerous investigations in which a radiocarbon date and a historic date were available and in which corrections for deviation in $^{13}C/^{12}C$ ratio had been calculated. Historic dates based on archaeological estimates were not included, but all other dates were accepted. A total of 193 measurements were obtained by these criteria, all but four of which were samples from tree boles dated by ring analysis. Data included thirty-six values from Willis *et al.*[16]; twenty-two values from Suess[17]; nine values from Broecker *et al.*[18]; thirteen values from Damon *et al.*[19,20]; fourteen values from De Vries[12] with A.D. 1840 considered as a base-line with a value of 0·0; eleven values from Münnich[21]; fifty-eight values from Ralph *et al.*[22]; and thirty values from Jansen[23]. Measurements on *Sequoia gigantea* accounted for 139 dates.

The Jansen[23] data included a series on the *Sequoia gigantea* of Willis *et al.*[16] and a series from a previous investigation[24] of *Agathis australis*, which were recalculated by Jansen[23] on the assumption that 150 more annual rings had been burnt off the specimen than had been estimated by dendrologists when the original study was made. Jansen considered this recalculation reasonable since it brought the *Agathis* data in line with trends in the northern hemisphere and since it was unlikely that there were major systematic differences in carbon-14 concentration between the two hemispheres.

The table resulting from the compiled 193 measurements showed a decreasing trend in radiocarbon activity from the earliest included date, about 1200 B.C., to an uneven trough of lower activity from the first to tenth centuries A.D. This was followed by a rise which was especially rapid after A.D. 1300 and which culminated in the fifteenth to seventeenth centuries. A subsequent decline occurred to the mid-nineteenth century, when burning of fossil fuel vitiated results of further measurement. A summation of these results by 200-year intervals (Table 1) showed a decrease from a mean radiocarbon activity of +3·5 per cent in 1203–951 B.C. to a minimum mean value of −0·7 per cent in A.D. 850–1049. This was followed by a rise to a secondary maximum of +1·5 per cent in A.D. 1450–1649, with a subsequent decrease to a mean of +0·5 per cent in 1650–1860.

Carbon-14 and Climate A.D. 1200–1860

Willis *et al.*[16] suggested the most promising line of enquiry into climate–carbon-14 relationships might be to look for evidence of climatic change around the period of rapid increase in carbon-14 activity which their study indicated had occurred during the thirteenth century A.D. (mean carbon-14 activity from Willis *et al.*[16] plus Jansen[23]: 1209 = −0·6, 1259 = +0·2, 1309 = +0·4).

Table 1. Carbon-14 Activity 1203 B.C. to A.D. 1860

Period	*n* Observations	*n* Laboratories	Carbon-14 Mean	Min.	Max.
B.C. 1203–951 B.C.	10	3	+3·1	+1·9	+4·8
950–751	5	3	+2·6	+2·3	+3·0
750–551	6	3	+1·9	+1·4	+2·2
550–351	4	2	+1·3	+0 9	+1·8
350–151	4	2	+0·8	+0·1	+1·7
150–49 A.D.	9	4	+0·1	−0·7	+1·1
A.D. 50–249	7	5	−0·0	−1·0	+1·0
250–449	9	3	−0·0	−1·0	+2·0
450–649	10	3	−0·4	−2·6	+0·4
650–849	16	8	−0·2	−3·0	+1·3
850–1049	18	7	−0·7	−2·3	+1·8
1050–1249	14	8	−0·2	−1·4	+0·9
1250–1449	17	9	+0·7	−0·4	+2·1
1450–1649	26	11	+1·5	−0·4	+3·4
1650–1860	38	11	+0·5	−1·8	+3·5

Mean carbon-14 activity for all data summarized in Table 1 was −0·4 in the thirteenth century and +0·5 in the fourteenth century. This period of rapid increase in carbon-14 activity was contemporaneous with a world-wide trend of declining temperature as indicated by extensive evidence summarized from Greenland[3], Iceland[2,5], Europe[2–4,25,26] and New Zealand[27], which included information on glacial activity and sea-ice, plant growth and distribution, agriculture, mining, and human exploration and settlement.

The appearance of lower temperatures during the thirteenth and fourteenth centuries marked the beginning of a generally cooler period which extended to around the end of the nineteenth century[2,4,5]. During this period, temperatures in England, especially during the summer months, were from 1° to 2° C lower than in the warmer centuries preceding A.D. 1200 (ref. 2), with the interval A.D. 1600–1900 generally cooler than A.D. 1200–1600 (ref. 6). Several intervals of noteworthy low temperatures occurred during the 1300–1900 period. The first was around early to mid-fifteenth century as shown by records of cooler summers in Germany during 1428–1460 (ref. 2), severe winters in France and Germany in the 1430–1440 decade[4] and a recession towards a damper climate near Stockholm in the region of 1450 (ref. 2). The next period of lower temperature occurred towards the end of the sixteenth century as indicated by: (1) resumption of glacial advance in north-west North America at the beginning of the seventeenth century which was dependent on several decades of colder temperature at the close of the sixteenth century[8]; (2) rapid increase of glaciation in west Greenland[7]; (3) complete abandonment of cereal and grain cultivation in Iceland[5]; (4) rapid increase in sea-ice off the coast of Iceland between 1550 and 1570 (refs. 2 and 5); (5) cold winters and wet summers throughout Europe from historic descriptions[3]; (6) abandonment of high alpine mine workings about 1570 (ref. 2). The greatest temperature minimum may have occurred in the late seventeenth century during the latter part of the sunspot dearth period from around 1656–1723 (ref. 8) with especially cold years in the 1690's (refs. 1 and 4) as documented by: (1) historic temperature measurements[2,11] and climatic descriptions[3] in Europe; (2) glacial advance in Europe from 1690–1720 (ref. 11); (3) major glacial expansion in north-west North America from 1700 until 1732 (ref. 9). The early nineteenth century also had cooler temperatures as shown by temperature records[2,11,28] and by glacial advance in north-west North America from about 1811 until 1853 (ref. 9).

The period A.D. 1300–1900 can be summarized as one of generally cooler temperatures and expanding glaciers with parts of the fifteenth, sixteenth, seventeenth and nineteenth centuries especially severe. During this period, carbon-14 activity increased from the minimum levels which characterized A.D. 0–1300 to reach maxima from the fifteenth to the end of the seventeenth centuries. No similar level of maximum carbon-14 activity occurred in the cooler intervals of the nineteenth century, probably because of the increasing release of fossil carbon dioxide.

Carbon-14 and Climate—1200 B.C.–A.D. 1200

Prior to A.D. 1200, there was a milder climate period which extended back to the latter part of the first millennium B.C.[2]. In this period there was little glacial activity and many glaciers which expanded during the A.D. 1500–1900 interval were non-existent or reduced in volume and cover[7,9]. Towards the end of this milder period there was a climatic optimum which occurred around A.D. 950–1200 (refs. 2 and 3) in which there was freedom from sea-ice in the Iceland–Greenland region[2,5], grain cultivation in Iceland[5] and a rise in the altitudinal limits of vineyards in Baden[2]. The long period of lower carbon-14 activity from around the first century B.C. to the thirteenth century A.D. may reflect the milder temperatures which apparently predominated in the middle north latitudes at that time.

The decrease in relative carbon-14 content from around 1200 B.C. to the period of low relative carbon-14 during 100 B.C. to A.D. 1200 may be the result of climatic amelioration following a period of renewed glacial activity. A period of deterioration to a colder climate occurred in Europe in the period 1000–500 B.C.[15], perhaps reaching a climax around 850 B.C.[29]. During this interval there was (1) bog regeneration and (2) increased glaciation in the Alps termed the Larstig and dated by lichenometry as being about 800–500 B.C.[7]. This period was apparently contemporaneous with an episode of glacial advance in Alaska[2].

Carbon-14 and Temperature

The two periods of high carbon-14 activity, 1200–500 B.C. and A.D. 1300–1860, were coincident with the two major periods of lower temperatures and glacial advance since the Hypsithermal. The long period of lower carbon-14 activity from about A.D. 0 to 1200 was coincident with a well-documented period of glacial retreat or stagnation which culminated in the "Little Climatic Optimum". From these long-term trends it seems probable that a lower carbon-14 activity occurred in periods of higher temperature and higher carbon-14 activity in cooler periods. These conclusions support those of Suess[14] and Lamb[15].

An estimate of temperature was made for each century from A.D. 800–1860 based on summaries of temperature[1–6,26,28] and glacial activity[7–9] in Europe and North America. The interval 1600–1860 was divided into solar activity periods as outlined by Bray[9]. The resulting alignment (Table 2) showed a decrease in carbon-14 activity from the estimated minimum temperature intervals in A.D. 1656–1723, 1600–1655 and 1500–1599 to the maximum temperature intervals from A.D. 800–1299. Exclusion of data from the indefinitely dated *Agathis australis* (column 7, Table 2) did not alter the predominant trend.

A comparison was made of radiocarbon activity with mean yearly temperatures conjectured for central England (Table II of Lamb[4]) for the period A.D. 800–1850. Because of the variable amounts of data on radiocarbon activity for the different temporal intervals in Table II, a non-parametric test[30] was applied to these data. This test gave a χ^2 of 4·7 and a probability of $< 0{\cdot}03$, which indicates a significant negative relationship between radiocarbon activity and yearly temperatures in Central England. Analysis of imperfect temperature readings in the London region in the late seventeenth and early eighteenth centuries[1] indicate there was a cool period from around 1688–1702 and a warm period from 1723–1739. Radiocarbon activity was markedly high at the close of the seventeenth century (mean activity = +1·1, 1688–1702), but there are only two values (+0·9, +0·4) from 1723–1739, which are insufficient to determine if a lower activity occurred during this warmer period. The last third of the eighteenth century was notably warm with glacial

Table 2. Variation in Carbon-14 Activity, Temperature and Solar Activity, 800–1860 A.D.

Period A.D.	Estimated temperature	Mean length sunspot cycle (yr.)	Mean yearly sunspot number	Years with sunspots or aurorae (from ref. 33)	Carbon-14 activity n	Mean	Mean‡
1656–1723	− − −*	11·4	22†	1	14	+1·3	+1·3
1600–1655	− − −	11·0	—	4	8	+1·5	+1·3
1500–1599	− − −	—	—	4	15	+1·4	+1·3
1400–1499	− −	—	—	3	9	+1·5	+1·5
1799–1833	−	11·9	26	0	6	+0·6	+0·6
1300–1399	+	—	—	7	7	+0·5	+0·6
1834–1860	+	11·1	56	0	11	−0·2	−0·2
1724–1798	+ +	10·7	54	4	7	−0·1	−0·1
800–899	+ +	10·0	—	9	8	−0·7	−0·4
900–999	+ +	—	—	5	9	−0·4	−0·1
1200–1299	+ +	10·8	—	9	8	−0·4	−0·3
1000–1099	+ + +	—	—	6	9	−0·2	−0·0
1100–1199	+ + +	10·2	—	16	5	−0·4	−0·4

* Ordered from lowest (− − −) to highest (+ + +) estimated values
† Data for 1699–1723 only.
‡ *Agathis australis* data excluded.

recession and higher temperatures during 1770–1795 comparable with those from 1925–1950, a period of similar glacial recession[38]. Tree growth over the period 1656–1964 reached a maximum of 9·8 cm²/yr. for *Picea engelmannii* boles in forests in British Columbia, Canada, during the interval 1756–1798 (ref. 9). The next highest growth interval was 1914–1964 with a value of 9·3 cm²/yr. The probable correlation of this growth with temperature[8] gives additional evidence for a warm period in mid- to late-eighteenth century. A maximum tree growth interval in the eighteenth century was also shown for the polar Ural Mountains[31] with a secondary maximum from the 1920's. If the foregoing evidence is considered together with the annual temperature trends for central England[1], a generally cooler period from about 1688–1722 and a generally warmer period from around 1723–1800 can be summarized. The mean carbon-14 activity for the period 1688–1722 was +1·4 compared with −0·1 for the warmer one 1723–1800. This again suggests an inverse relationship between carbon-14 activity and temperature.

The first two decades of the nineteenth century were very cool[1,28] and there was widespread glacial advance in the first half of the century[8,9,28]. Forest tree growth in British Columbia, Canada, for 1799–1833 was 8·7 cm²/yr., a minimum value for the period 1656–1964 (ref. 9). Mean carbon-14 activity for the period 1800–1860 was +0·0, which was slightly higher than the value of −0·6 for the preceding warmer period 1723–1800. Fossil fuel contamination may have appreciably reduced this value, otherwise carbon-14 activity-levels comparable with those in the late seventeenth century might have occurred in the early nineteenth century.

Carbon-14 and Solar Activity

Variation in cosmic-ray flux due to changes in solar activity was suggested as a mechanism for changes in the $^{14}C/^{12}C$ ratio by Suess[17]. Stuiver[32] noted that measurements over the past two sunspot cycles showed a decrease in cosmic-ray flux during sunspot maxima, and he found a similarity between the inverse curve for carbon-14 activity over the past 1300 years of Willis *et al.*[16] and the sunspot activity curve of Schove[10].

Variation in carbon-14 activity in relation to strength of the sunspot maxima per cycle from Schove[10] is shown in Table 3. There is an increase in carbon-14 activity with decreasing strength of sunspot maxima especially at the weaker sunspot maxima levels. Differences in carbon-14 activity between levels were tested with the median test for two samples[30]. The results of these tests showed a significant decrease in carbon-14 activity with increasing solar activity, but the two highest sunspot-levels (106–>136) did not have a significantly lower carbon-14 activity than the two higher intermediate levels (76–105).

Table 3. VARIATION IN CARBON-14 ACTIVITY AND STRENGTH OF SUNSPOT MAXIMA 200 B.C. TO A.D. 1860

Solar activity	Carbon-14 activity	
Sunspot No. for year of maximum*	n	Mean
>136	26	−0·0
106–135	30	−0·1
96–105	15	+0·1
76–95	20	−0·2
66–75	35	+0·4
56–65	17	+1·0
46–55	17	+1·0
<45	5	+1·4

* Data from ref. 10.

Additional demonstration of the tendency for carbon-14 activity to decrease with increasing solar activity is shown in Table 2. The lack of any further decrease in carbon-14 activity at the higher solar activity levels is again apparent. A possible positive correlation of solar activity and terrestrial temperature[8,9] is given indirect support by the interrelationship of these factors with carbon-14 activity.

I thank Dr. E. K. Ralph and Mr. H. S. Jansen for supplying me with copies of their results in advance of publication.

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HISTORICAL STUDY OF THE SEASON OF BIRTH IN THE CITY OF YORK, ENGLAND

By URSULA M. COWGILL

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AN examination of the contemporary season of birth in man in various parts of the world[1] suggested that a historical study of the matter might reveal some interesting facts. A study of the ten existing parish records of the City of York[2] were considered and one rural parish, namely Gilling[3], which has an excellent record, has also been included. The births recorded in the *Dictionary of National Biography*[4] (*DNB*) covering the period from 1200 to 1900 were also tabulated by hand. This collection was thought to provide good comparative material; though it gives a highly selected body of data, it does not limit its biographical sketches only to those who had attained financial, intellectual and/or religious success. Births were tabulated from *Debrett's Peerage*[5] for the eighteenth and nineteenth centuries and *Who's Who*[6] was used for added material including the latter half of the past century.

All dates before September 2, 1752, were converted to the new-style calendar in order that they should bear a constant relationship to the solar year, since there is evidence[1] from the clear contemporary inversion, seen when Britain is compared with New Zealand, that a basic

cycle determined by the physical progress of the seasons exists. Since, however, the social calendar as well as the meteorological is also probably involved, the position of Christmas as celebrated on the old calendar is indicated by an arrow on the time scales.

The parish records from the City of York largely confine their tabulation to baptism rather than birth. For every half-century a number of births were recorded together with the baptismal date. In the limited number of cases in which data for both events were given, prior to 1750, the average waiting time between birth and baptism is about 3 days. After 1750, with the exception of adults who are being admitted to the Established Church, the waiting time is about 5 days. In most cases the difference is so small that, for the purposes of this study, the baptismal and the birth date can be considered to be essentially the same. While the York data are based entirely on baptismal time the material gathered from the *DNB*, *Debrett's Peerage* and *Who's Who* refers to date of birth.

The tabulated data were normalized for the varying number of days of the month. A monthly mean for the whole year was then obtained and the ratio of the total number of births or baptisms per normalized month to this mean was calculated.

Huntington[7] used the *DNB* data, but with a different end in view, as he hoped to show a difference in the birth seasons of the most eminent, with the longest biographical essays, when compared with those more shortly noticed. This procedure has its dangers, and he perhaps underestimated the *Dictionary* as a compilation of the lives of the extraordinary, rather than necessarily those of political, religious and intellectual leaders. He made no attempt to consider any secular changes in birth season in the data, and his treatment, though worth mentioning, is of no further direct concern to the present study. The same is true of his treatment of Ellis's[8] compilation of one thousand of the most eminent British personalities. It is perhaps desirable to point out that it is not clear how far Huntington normalized his data for varying lengths of the calendar months or corrected for the change from the old style to the new style calendar.

In considering parish records, which are coming to be regarded as major sources of demographic data, Drake[9] has pointed out that the records prior to the eighteenth century are admirable when compared with the eighteenth-century ones, since the latter suffered the effects of a somewhat lackadaisical clergy coupled with the spread of non-conformity. This becomes more apparent when it is observed how long it took some parishes to adjust to the new-style calendar. A problem arises particularly in the seventeenth century, namely that many children died without benefit of baptism, and as such were never included in the record or only under burial headings.

Although it would appear safe to assume that the population of the City of York was not markedly migratory, moving mainly from one parish to another within the city limits, Laslett and Harrison[10] have suggested some caution about such assumptions as a result of their study of Clayworth and Cogenhoe, where an unexpected turnover of population occurred in the seventeenth century. None of these possible sources of error in the records is likely to have an effect on the present demographic study, since they do not seem to introduce seasonal bias. Temporary migrations of labour, regarded by Huntington[7] as of prime importance in determining birth seasons in some communities, are probably of much less importance in an urban population of the kind inhabiting York.

The most striking observation that can be made by examining the season of birth in York (Fig. 1) over the three centuries for which there are data is the initial bimodality, from which it appears that chances for successful conception were equally good in July and January. The pattern then gradually evolved into that now observed where the peak in conceptions occurs early in the summer. After 1752 it appears that an essentially modern pattern has developed.

It is interesting to note that the June minimum in births persists for about the first century for which there are data, gradually shifts to August and finally attains the characteristic November minimum which occurs throughout Europe[1].

The births of people recorded from the *DNB* show, prior to 1700, the same bimodality as that of the York population, with equal chances of conception in the summer and the winter. Prior to 1800, the bimodality is still exhibited by the *DNB* group and the June minimum is still in effect, though the York population shifted to an August one by 1652. The *DNB* people are approaching a modern distribution by 1900 while the York population attained this periodicity by 1752. Somewhat in opposition to a hypothesis proposed by Frazer Roberts[11], where he suggests that winter conceptions are more likely in intelligent families, the people of the *DNB* before 1700 show a slightly higher incidence of babies conceived in the summer than those conceived in the winter. The effect is no doubt somewhat diluted by the recording of the extraordinary that are not intellectually gifted, nor from

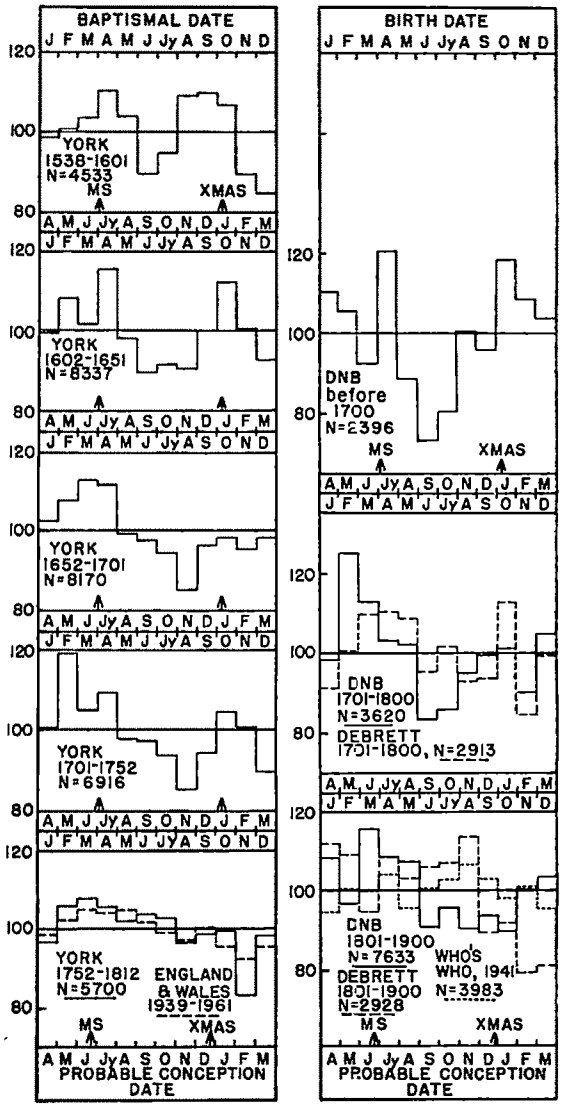


Fig. 1. The season of baptism in York, England, from 1538 to 1812 with the modern histogram of England and Wales (1939–1961) included. The season of birth listed in the *DNB* from 1200 to 1900, those listed in *Debrett's Peerage* in the eighteenth and nineteenth centuries and those in *Who's Who*. The old style dates for Christmas and Midsummer's Day are indicated by arrows. *N* refers to the total number of individuals involved

intellectual families. It is possible, however, that this effect is a modern one. There appears to be an indication in the data from *Who's Who* in which the peak in conceptions is in November.

The peerage for the eighteenth century shows a marked incidence of winter conceptions, though a bimodality is present and the modern November minimum in births is much in evidence. By 1800 the bimodal effect has decreased showing the major peak in conceptions in November with the contemporary November minimum in births. The data, however, are very irregular and no valid conclusions can yet be drawn.

Parenthetically, it may be noted that the sex ratio prior to 1701 in the York population is 104·6 (N = 21,051) while that after this period is 106·9 (N = 12,616) which is about the present English value.

Pasamanick *et al.* have recently shown[12] in a study of a segment of the population of Baltimore that the seasonality, or the amplitude between maximum and minimum, is greater among the poorer classes. The amplitude shown by the population of York is a little over twice as high in the sixteenth century as is now shown for all of England (Table 1). It gradually increases to a peak in the first half of the eighteenth century and then declines somewhat below what it was in the sixteenth century. The *DNB* group shows an extremely high seasonality for all people prior to 1801, at which point it is essentially the same as the York group was for the previous half-century. The peerage shows an increase in seasonality in the nineteenth century which emulates that of the York group during the first half of the eighteenth century. The modern data, both from *Who's Who* and the present material from the British *Census*, indicate essentially the same amplitude in the season of birth. The data are scarcely consistent with the type of effect noted by Pasamanick *et al.* having operated in England throughout the past four centuries. If the dependence of seasonality on economic conditions is due to the more affluent being more protected from the environmental variables by adequate artificial heat and light, the existence of the correlation before the nineteenth century perhaps would not be expected.

Table 1. Seasonality shown by various populations over the period of time studied

Time	*York*	*DNB*	*Debrett*	*Who's Who*	Modern
1538–1601	25·9				
1602–1651	26·1				
1652–1701	27·2				
1702–1751	34·3				
1752–1812	24·2				
Prior to 1700		40·8			
1701–1800		40·4	28·0		
1801–1900		24·6	34·0		
1851–1900				11·0	
1939–1961					11·9

In 1600 the number of baptisms exceeded the number of burials, but from this time onward[13] the number of deaths exceeded the number of births until 1760. After this period the reverse remained true with the exception of data presented for 1780. Many influences were in effect to prevent the natural growth of the population, such as the plague epidemics of 1604 and 1631, the continuous infection of small-pox, measles, influenza and other diseases which brought about one epidemic after another during the early portion of the eighteenth century. Severe weather conditions throughout this time also helped to increase mortality. All such influences may have had a bearing on the well-being of the population and so perhaps on the seasonality. Until further work is done on the matter nothing more can be stated.

The main result of the present investigation is to show that both in the population represented by the York registers and in that from which the *DNB* is drawn there has been a change in the annual incidence of births. Initially there were two major maxima corresponding to maxima in conceptions in July and December or January (N.S.) and minima in September and March. This condition is clearly apparent in York up to 1652 and in the grouped data for the *DNB* before 1700. After this there is a transition, which in York appears to have been completed by the middle of the eighteenth century, to the contemporary pattern with a maximum of conceptions in June, a minimum in November and an extremely small secondary maximum around Christmas. The two original peaks in births, initially of about the same amplitude, thus have become extremely uneven, while the one which has persisted has moved a little earlier into the year. There can be little doubt that the modern pattern is largely meteorologically controlled, as it is well known[1] that it is displaced by six months in the contemporary populations of the southern hemisphere. The most reasonable explanation of the observed historical facts is that superimposed on the climatically determined variation is a cycle of initially great amplitude determined by calendary festivities, notably the celebrations around and after Christmas. This is shown by the seasonal (Fig. 2) distribution of marriages, which in the first period investigated exhibits a dramatic minimum in Lent and dramatic maximum in November, presumably mainly just before Advent. Evidently there was a tradition for being married shortly before Christmas which persists feebly in later half-centuries. The decline in the cycle is, in a general way, historically reasonable. In the medieval period long working days were relieved primarily by religious festivals, which must have set the rhythm of the year almost as much as the physical seasons, to which of course the religious calendar was in effect geared. After the Reformation, and particularly during the times of Puritan ascendancy, the religious calendar became less significant. Celebration of Christmas was in particular anathema to the Puritans, who believed it should be a fast.

Even though it is perhaps straining the evidence to insist that the maximum in Christmas conceptions is lower in the period 1652–1701 than in the subsequent half-century, it is reasonable to suppose the data indicate a gradual break-up of a medieval pattern as the religious practice and secular need for calendary celebrations became less pressing. The physiological and psychological

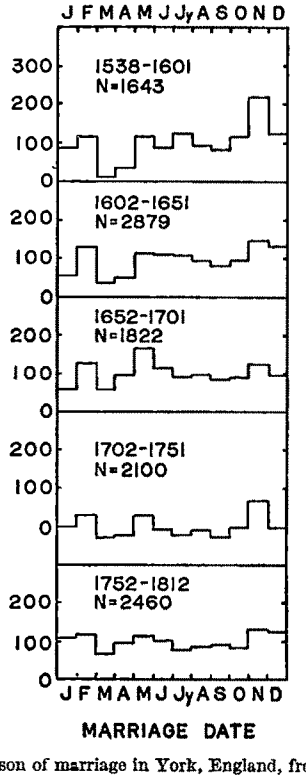


Fig. 2. The season of marriage in York, England, from 1538 to 1812

mechanisms by which the meteorological and social cycles are incorporated into reproductive behaviour remain open questions. It may, however, be pointed out that in the sixteenth century, if the excess of marriages in November resulted in conception within two months of marriage in about 58 per cent of the cases, and live birth, the maximum in births around September would be explained. A good deal of the bimodality, therefore, may be due to customs relating to marriage time combined with a disproportionate significance of primiparous births in a society with a fairly high adult death rate.

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NEWS and VIEWS

Nuclear Physics Division, Science Research Council : Mr. John Clemow

Mr. John Clemow has been appointed head of the Nuclear Physics Division of the Science Research Council in succession to Dr. A. C. W. Clarke. Mr. Clemow has been a director of G.E.C. (Electronics) since 1961. Previously he was chief engineer (weapons) and a special director of the Vickers-Armstrong (Aircraft) Co., Ltd., Weybridge. Educated at St. John's College, Cambridge, where he read mathematics, Mr. Clemow served with the Royal Artillery and was on the staff of the Military College of Science during the Second World War. He is a Fellow of the Institute of Mathematics and its Applications and a Member of the British Nuclear Energy Society. In 1948 he was seconded to the Royal Aircraft Establishment, Farnborough, and later joined the former Ministry of Supply as director of Guided Weapons Projects.

Applied Mathematics in Monash University : Prof. B. R. Morton

Dr. Morton, senior lecturer in mathematics in the University of Manchester, has been appointed to a chair of applied mathematics in Monash University, Melbourne. Dr. Morton graduated in physics and mathematics in 1948 in the then Auckland University College. He took the mathematical tripos at the University of Cambridge in 1952, and went on to carry out research in fluid mechanics under Dr. G. K. Batchelor and Sir Geoffrey Taylor, qualifying for the Ph.D. degree in 1956. During 1949–50 he was junior lecturer in physics at Auckland University College, and during 1955–56 assistant lecturer in mathematics, University College, London. He joined the University of Manchester as a lecturer in 1956 and became senior lecturer in 1965. In the past few years he has been especially concerned with the organization of postgraduate courses in fluid mechanics at Manchester. Dr. Morton's present research interests are centred mainly on geophysical fluid dynamics and low-speed fluid mechanics. Research projects in which he is at present engaged include: dynamics of rotating fluids, including buoyant jets in a rotating environment and applications to vorticity amplification in the atmosphere, and termination of concentrated vortex cores at boundaries with applications to tornadoes and waterspouts; dynamics of stratified fluids, including motion of vortex parts in a stratified environment and double convection of hot salt systems; flame and fire problems, including turbulent diffusion flames, pool fires, and the ascent of very hot masses of gas; cumulus dynamics, including the development of time-dependent models for growth of cumulus clouds; and a class of pipe flows in which buoyant and centrifugal forces produce important flow rearrangement with interior stagnation and flow reversal. He is a member of a subcommittee of the Meteorological Research Committee of the United Kingdom Ministry of Defence.

Research in Education

In a written answer in the House of Commons on February 15, the Secretary of State for Education and Science, Mr. A. Crosland, stated that the support of the Department for educational research in 1965–66 included about £250,000 for projects related to the schools and schoolchildren, about £55,000 of which would be included in payments to the National Foundation for Educational Research. None of the Department research funds were allocated through the local education authorities, but those authorities, charitable foundations and universities also supported such research.

Directorate of Research and Information

In a written answer in the House of Commons on February 14, the Parliamentary Secretary to the Ministry of Public Building and Works, Mr. J. Boyden, stated that the work of the Directorate of Research and Information included advising on research policy and specific projects, commissioning research work, activities concerned with the dissemination of technical information, and investigation of problems connected with the maintenance of buildings and with the use of computers in the construction industry. The Directorate also dealt with training and education for that industry. The Directorate was headed by a Director, who was a deputy chief scientific officer, and an assistant secretary, supported by twenty-one professional and scientific officers, ten technical, nine executive and twelve clerical officers. The direct cost of the Directorate for 1965–66 was about £165,000, and overheads were estimated at £42,000.

University Building Programme

In a written answer in the House of Commons on February 14, the Secretary of State for Education and Science, Mr. A. Crosland, stated that some 25 per cent of the building programme for universities, including the colleges of advanced technology, for 1965–66 would now be started in 1966–67. The building programme of £120 million for the four years 1966–67 to 1969–70 was not affected by the revised arrangements for dealing with deferment announced by the Chancellor of the Exchequer on February 8.

Man-power in Aviation Research

In a written answer in the House of Commons on February 16, the Minister of Aviation, Mr. F. Mulley, stated that on December 1, 1963, the employees in the scientific officer class in the research establishments controlled by the Ministry numbered 997. For 1964 and 1965 the corresponding figures were 1,022 and 1,037, respectively. The 1965–66 estimates provided £11·75 million for the European Launcher Development Organization.

Morecambe Bay Reservoir Scheme

An alternative proposal to the Morecambe Bay Project is outlined by Prof. J. R. D. Francis and Mr. S. H. Wearne in a memorandum from the Faculty of Technology, University of Manchester. The scheme proposes a marshland reservoir, created by and completely surrounded by embankments on a higher part of the present estuary, the top water-level being about 30 ft. above marsh-level. The reservoir would have a system of aqueducts from rivers and streams and a road link allowing tidal movements into the upper estuary and would thus be permeable. It offers advantages over the barrage scheme in greater depth, outward pressure gradient, lower capital costs and marginally smaller running costs, easier avoidance of waterlogging of nearby land and freedom from obstruction to migrating fish. There are, however, no subsidiary benefits in amenity, cooling water provision or transport links.

Aid to Developing Countries

In a short paper, "Developing Countries in Debt", in the *Westminster Bank Review* for February 1966, Mr. S. E. Mynott points out that besides their continuing need for development finance, the developing countries need measures to enable them to reduce their burden of debt. Taking as developing countries those of Latin America, Africa (except South Africa), non-Communist Asia (excluding Japan) and Oceania (except Australia and New Zealand) and the Mediterranean States of Turkey, Cyprus, Greece, Yugoslavia, Malta and Spain, the total aid given in 1964 is estimated at £2,360 million, net of capital repayments arising from previous aid, more than 80 per cent of which came from the United States (53·5 per cent), France (12·7 per cent), the United Kingdom (7·4 per cent) and West Germany (7 per cent). The total, however, is scarcely increasing even in monetary terms, partly due to difficulties experienced by several donors, such as the United Kingdom, with their balances of payment. Debt servicing is now the main problem for all recipients of aid, and Mr. Mynott discusses the various methods of assistance which could be used. One way is to provide aid on soft terms. Another of more immediate importance is to agree on how debt now maturing can be repaid without excessive strain and without resorting to *ad hoc* arrangements like The Hague and Paris re-finance clubs. Mr. Mynott suggests adoption of the United Nations Conference on Trade and Development experts' liquidity plan.

Employment of Graduates in Biological Subjects

Dr. S. J. Pirt, head of the Department of Microbiology, Queen Elizabeth College, London, writes: "The abstract of information from the University Grants Committee on the numbers of graduates in biological subjects in 1962–63 and their employment (*Nature*, 209, 356; 1966) is a sad reflexion of the paucity of data about the demand and supply of different types of biologist. It does not show the rapid changes which have been occurring in the pattern of demand for biologists. There is no mention of microbiologists, whom it appears are now as much sought after as any other type of biologist. In the U.G.C. data on numbers of graduates for 1962 (*J. Inst. Biol.*, October 1963) we see that there were twenty-eight graduates in microbiology (including bacteriology). Since that time, any increase in the total number would appear to be slight, yet information accumulated in this Department shows that in 1965 more than 140 posts for microbiologists in Britain were advertised. Compare, for example, the zoologists, of whom 170 were available for employment and 138 gained employment. Increasingly one hears complaints from universities and research laboratories that they are forced to work permanently below their establishment for microbiologists. Many of the vacancies in microbiology have to be filled by graduates in some other discipline and who, for many years, at great cost to the progress of their work, learn 'on the job'. This points to another feature veiled by the U.G.C. data, namely, that many biological graduates at the end of their training have to be re-trained in another biological discipline which they could have learned in the first place. Postgraduate training of microbiologists is in an equally parlous state, and this seriously restricts the possibility for growth of university teaching in microbiology. The average number of graduates per year in each school of microbiology is minute (about five). To use staff efficiently, this number should at least be quadrupled. From such a base a steady expansion in the number of graduates could be aimed at which in 10 years might catch up with the demand. Finally, it is interesting to ask, by what means does the U.G.C. propose to avoid a similar dearth of graduates from arising in other rapidly developing sciences ?"

National Museum of Wales

From April 1, 1965, the National Museum of Wales came under the Welsh Office instead of being sponsored by the Treasury. The fifty-eighth annual report of the National Museum of Wales for 1964–65 also records that the great majority of the local authorities in Wales have agreed to contribute to the Council of Museums in Wales (Pp. 91+4 plates. Cardiff: National Museum of Wales, 1965). In the Department of Geology, the compilation of adequate biographies and source-books of Welsh geology (1897–1958) has been completed, while in the Department of Botany the construction of exhibits for the new cryptogamic gallery has continued throughout the year. Two exhibits showing photomicrographs of the anatomy and development of various invertebrates and wild-life conservation were shown in zoology, and in archaeology work was concentrated on the preparation of exhibits for the new Prehistoric Gallery. The new Department of Industry was engaged in the occupation of the galleries and offices allocated, and the Department of Art showed several new exhibitions. In the Welsh Folk Museum work has continued on the re-erection of the tannery removed from Rhayader, Radnorshire. The report also records the usual extensive list of publications by members of the staff.

City Museums and Art Gallery, Leicester

For the year 1964–65, the Museums and Art Gallery of Leicester report a period of consolidation rather than spectacular achievement (Fifty-ninth Report, April 1, 1964–March 31, 1965. Pp. 48+4 plates. Leicester: City of Leicester Museums and Art Gallery, 1965). During the year, the archaeological collections have been removed from the Museum at New Walk to the new Jewry Wall Museum. This has involved transferring many thousands of specimens; some, the tesselated pavements and the Roman milestone, weighing several tons, and almost all, especially the fragile Roman glass, needing great care in handling. Excavations were undertaken north and south of Thornton Lane and a considerable area near the presumed forum was cleared. Labels have been found for the order beds at Belgrave Hall Gardens which promise to be permanent, legible and relatively vandal- and weather-proof. They consist of engraved plastic strips set in metal holders. A list of several hundred accessions

indicates that registration is maintained and is up to date.

Smithsonian Institution

The annual report of the Smithsonian Institution for the year ended June 30, 1964, is, as usual, a large volume and includes several original papers as well as the formal routine reports of the various departments (Pp. xiii + 553 + 71 plates. Washington, D.C.: Government Printing Office, 1965. 4 dollars). During the period under review, the Institution made a determined effort to carry its international activities beyond traditional overseas field expeditions and research, which primarily benefit the Smithsonian, to co-operation with other Government agencies and private institutions in the development of exchange and international exhibits programmes, to the benefit of others. Among the original papers is one in which the computer is brought to bear on Stonehenge from the astronomical angle, and another on recent theories regarding the origin of mountains.

Gems in the Smithsonian Institution

Books and papers on gemstones are numerous, and the Smithsonian Institution has recently issued an excellent handbook to the principal stones in their collections (*Gems in the Smithsonian Institution*. By Paul E. Desautels. Pp. 74. Publication No. 4608. Washington, D.C.: Smithsonian Institution, 1965). An introductory chapter on the characteristics of gems is followed by notes on their shaping, substitutes, lore and a description of the chief gem species illustrated by accurate colour plates. The booklet concludes with a list of the notable gems in the collection at the Institution.

Digital Searching Service for Spectra

The Scientific Documentation Centre, Ltd., has announced a new digital searching service for the identification of unknown substances. The Centre has a library of approximately 64,000 infra-red spectra and about 18,000 ultra-violet spectra. These are coded on eighty-column punched cards. The library can be searched by spectrum (with an accuracy of 0·1μ), by chemical structure, by empirical formula (for carbon, oxygen, nitrogen and sulphur) or, for the less common elements, by elements. Any combination of these methods of search can also be used. The data available consist of spectra from sources in the literature and from eight published spectra collections. These are: American Petroleum Institute Research Project 44, Sadtler Catalogue of Spectra, Sadtler Catalogue of Commercial Spectra, National Research Council–National Bureau of Standards File, Documentation of Molecular Spectroscopy, Coblentz Society Spectra, Manufacturing Chemists Association Spectra and the Infra-red Data Committee of Japan. Computer tapes for infra-red spectra are available for *IBM* 7090, 7094 and 1401, Honeywell 400 and Burroughs 5,000 computers. It is hoped that tapes for *IBM* 7070 and 1460 will be available soon. Further details can be obtained from the Scientific Documentation Centre, Ltd., Halbeath House, Dunfermline, Scotland.

Gallenkamp and Company Ltd.

The name Gallenkamp has for many years past been a household word in the commercial field of scientific laboratory furnishing, equipment, and apparatus; from relatively small beginnings, this firm has grown into an organization of world-wide repute, with headquarters in London, sales and distribution centres at Widnes, Lancashire, and Stockton, Co. Durham, and similar facilities throughout forty-three countries overseas available via appointed agents. Apart from the well-known comprehensive catalogue, occasional leaflets and brochures describing newly designed, specialized instruments and apparatus, Gallenkamp also publish a bi-annual magazine *News and Review* (A. Gallenkamp and Co., Ltd., Technico House, Christopher Street, London, E.C.2. Pp. 24. No. 12, Autumn Issue; October 1965). *News and Review* first appeared as a spring number in February 1960, the motive being then, as simply stated, " . . . we aspired to produce a readable reference to our current affairs", the *raison d'être* of most industrial house-journals to-day. In that first number, the aims of the enterprise were succinctly defined: ". . . the desire to draw attention to apparatus and equipment recently introduced, and to invite enquiries and orders concerning them . . . to further the personal contact between instrument maker and instrument user . . . to pay tribute to those universities, research and industrial laboratories proffering information on the changing methods and new techniques which are constantly being developed . . . news to be offered concerning policy changes, future planning and expansion, price variations . . . news of our Agents (who) look after the interests of overseas customers . . . and other efforts for improving service both at Home and Abroad." These ambitions have been fully realized, so far as the reader is concerned, in the later issues of this publication, and the present number is no exception; it is equally no mere catalogue; much background information on the design, construction, and advantages of modern (often new) apparatus, well illustrated, is included. The introductory article on Magna Carta (1965 was the seven hundred and fiftieth anniversary of its signing) quotes two relevant excerpts; one is of interest to overseas customers: "All merchants are to be safe and secure in leaving and entering England, and in staying and travelling in England, both by land and by water, to buy and sell free all maletotes by the ancient and rightful customs." The other: "Let there be one measure of wine throughout our kingdom, and one measure of ale, and one measure of corn, namely the London quarter, and one width of cloth whether dyed, russet or halberjet, namely two ells within the selvedges. Let it be the same with weights as with measures." This editorial note concludes with the comment: "Was this the first attempt to lay down a British Standard?" For the rest, *News and Review* announces, among many other items, a new orbital incubator, designed in consultation with Dr. S. J. Pirt, Queen Elizabeth College, London; a Mark 2 autoflash Pensky-Martens closed cup flash point apparatus; a new Gallenkamp colorimeter Mark 3, with transistorized stabilizer circuit; a remarkably compact flame analyser, primarily for sodium and potassium determinations (with accessory interference filter for calcium); the Lloyd gas analyser (Gallenkamp hold exclusive licence to manufacture and sell this apparatus); a tube furnace with Crusilite (silicon carbide) elements (manual or auto-control) for temperatures up to 1,400° C; a new international model B-20 high-speed, refrigerated centrifuge; and a well-designed Abbé refractometer with extended range up to R.I.1·740. Available also is the latest development in diamond polishing techniques, 'Microsol', an aerosol application of 'Microfin' diamond polishing compound for metallurgical work.

Fluorocarbon Resins

'Teflon' is the registered trademark of E. I. du Pont de Nemours and Co. for a series of fluorocarbon resins, fibres and films manufactured by them, including polytetrafluoroethylene (PTFE) and fluorinated ethylene propylene (FEB) resins. 'Teflon' was discovered by Du Pont more than twenty-five years ago, and has been available in European markets since the early 1950s. Its unique combination of properties has in recent years benefited an increasing number of industries, particularly in connexion with designing plants demanding material for high-temperature service possessing excellent mechanical, electrical, and chemical properties. An outstanding feature of these 'Teflon' resins is their very high heat

resistance; the PTFE fluorocarbons are capable of continuous service at 260° C (500° F); they can withstand much higher temperatures for limited periods. So important are these 'Teflon' products that they modestly fill a periodical magazine, *The Journal of Teflon*, in their own right, among other Du Pont publications, and a recent issue contains much technical information concerned with their performance in many different circumstances, but notably under high temperature conditions (Du Pont de Nemours International S.A., Case postale, CH-1211 Geneva 24, Switzerland (Du Pont Co. (U.K.), Ltd., Fetter Lane, London, E.C.4). 6, No. 5; October–December 1965).

Among the many successful applications of these resins are: wire insulation rated for continuous service at 260° C; fuel hose in aircraft and missiles at temperatures to 260° C; pump diaphragms rated for service up to 149° C; valve packings handling corrosive chemicals at temperatures as high as 260° C; and ball seals in aircraft valves, to give a leakproof seal at temperatures up to 204° C. It is stated that few, if any, other resins or elastomers can show such an impressive record. In the article describing these properties, information is also given on the behaviour of PTFE resins at temperatures ranging from 149° to 427° C; these should be valuable to the designer in predicting performance and life expectancy of components of these resins at such temperatures. Many of their applications impose severe combinations of mechanical, thermal, environmental, and electrical stresses simultaneously; relevant data for these conditions are given under sub-headings: strength at high temperatures, stiffness and deformation, expansion and friction, electrical properties, special design considerations, and safety in design.

Building in Warm Climates

THE ultimate success of a building should not be gauged solely in terms of its appearance or investment potential; too little consideration is given generally to its functional design. So argues J. F. van Straaten in a paper entitled "Warm Climates and Building" (National Building Research Institute, South African Council for Scientific and Industrial Research, Pretoria. Reprinted from *CIB Bulletin No.* 4, 1964. CSIR ref. *R/BOU* 149. Pp. 5.). The author is chief research officer to the Research Institute, and although his remarks are directed critically to building in warm climates, much of what he says regarding functional design is of much wider significance. Buildings should satisfy minimum requirements in respect of thermal performance, ventilation, lighting, acoustics, structural stability, durability of materials, overall resistance to rain penetration, and all other factors consistent with the functioning of good community and family life. Admittedly some of these requirements are not controlled by climate, but this certainly does play a major part in any concept of functional design. It is claimed that in warm climates, particularly, more research is necessary into effects of asymmetric radiation conditions on sensations of warmth, selection of rational design weather data in terms of probability of concurrence of relevant weather elements, and degree of control of indoor environment by building procedure. Special attention is given in this paper to the thermal performance of heavy-weight and light-weight buildings; it is shown that heavy-weight structures are thermally superior to light-weight structures in warm–arid areas, the opposite applying in warm–humid areas. In considering the thermal environment of buildings, the important factors to take into account are heat-storing capacity of the structure as a whole, insulation properties of the various elements, control of direct and indirect solar heat gains, and rate of ventilation of the building. Some preliminary results of tests now in progress on test structures have so far proved that uninsulated single-skin light-weight construction, even with a concrete floor, is not acceptable for permanent housing; partial insulation of structures serves very little useful purpose; addition of insulation to walls and roof considerably improves indoor living conditions; and if exposed surfaces are painted white, the thermal performance of light-weight structures improved significantly; their ventilation, however, is of prime importance.

Protochordata

THE protochordate section of *The Zoological Record* affords some measure of the sheer enormity of the task of compiling and presenting information of this kind, and particularly so when it is realized that this section is but one of twenty in the present volume of the *Record* (100, section 14; 1963. *Protochordata, together with Pogonophora, Enteropneusta, Graptolithina, Pterobranchia and Phoronidea.* Compiled by Dr. D. B. Carlisle. Pp. 50. London: The Zoological Society of London, 1965. 12*s.* 6*d.*; 1.80 dollars). Section 14 deals with published literature on the Tunicata and Cephalochordata, together with the Hemichordata, Phoronidea and Pogonophora. The information is treated under three headings: a list of titles arranged according to authors; a subject index in which each of the five taxa is dealt with separately; and a systematic index. It is beyond the scope of this review to attempt an evaluation of the entries, but three seem worthy of special mention. The year 1963 saw the publication of Dr. Carlisle's translation of the monograph by Ivanov on the Pogonophora, and in that same year Poulsen recorded pogonophores from the Cambrian of Sweden, thereby giving the phylum a history at least as long as the Phanerozoic Era; in 1963 also, A. H. Müller's text on the Stomochorda appeared. The number of entries concerned with the extinct hemichordate class, the Graptolithina, is impressive, and if the services of a palaeontologist had been enlisted during compilation it is likely that a number of errors would have been avoided. Thus, for example, Llandeilian would not have appeared as 'Llandillian', nor would a reference to *Mesograptus* have been included in the Monograptidae instead of the Diplograptidae. These are minor defects, however, and detract little from the immense value of the publication.

Boarding Schools for Maladjusted Children

THE Department of Education and Science *Building Bulletin* No. 27 is not concerned with the theories of the treatment and cure of maladjustment, and only discusses the personal relationships which play such a large part in therapy where they appear to affect the design of boarding-school buildings (*Boarding Schools for Maladjusted Children.* Pp. v+16. London: H.M.S.O., 1965. 4*s.* net). Its purpose is to analyse the requirements of maladjusted children and to advise the architect on how to produce the sympathetic physical environment which they need. The ideas set out in the *Bulletin* have been assembled in the hope that they may assist local authorities and others concerned with planning new boarding schools for maladjusted children to help the child so that he can return to his normal place in family life as soon as possible. Although the *Bulletin* develops in some detail one particular approach in design, it leaves room for a variety of solutions.

University News: Aberdeen

THE following appointments have been made: *Lectureships*, Dr. P. S. Ashton and Mr. K. Jong (botany); Dr. I. Parsons (geology); *Research Fellowships*, Dr. I. M. Brattsten and Mr. H. Kramer (chemistry).

Belfast

DR. P. W. KENT has been appointed to the chair of biochemistry in the Queen's University of Belfast. The

following lecturers have also been appointed: Dr. M. A. McKervey (organic chemistry); Mr. D. B. McAlister (pure mathematics).

Bristol

The following lecturers have been appointed: Dr. P. Lloyd (physics); Dr. H. E. Rose (mathematics).

Leeds

Dr. J. P. Roberts has been appointed professor of ceramics in the Houldsworth School of Applied Science. Mr. L. T. Hildyard has been appointed lecturer in the Department of Mathematics.

Liverpool

Dr. J. R. Holt has been appointed to the newly established chair of experimental physics. The following lecturers have also been appointed: Mr. T. R. Williams (zoology); Dr. P. A. Chatterton and Mr. B. W. Hogg (electrical engineering and electronics).

Reading

Dr. I. M. Mills has been appointed professor of chemical spectroscopy. The following lecturers have also been appointed: Mr. A. E. Canham (soil science); Dr. C. E. Linderholm (mathematics); Dr. J. R. Milford (meteorology); Mr. H. A. Moisley (geography); Dr. A. P. Musgrave and Mr. G. Rice (applied physical sciences); Miss B. Pickersgill (agricultural botany).

Announcements

Prof. W. Q. Kennedy, professor of geology in the University of Leeds, has been awarded the Clough Medal of the Edinburgh Geological Society for the period 1965–66. Prof. Kennedy has made many important contributions to the understanding of the geology of Scotland in the fields of igneous petrology, tectonics, metamorphism, and Pre-Cambrian stratigraphy.

Dr. H. R. Ambler, who has been scientific adviser to the British High Commissioner in India since 1959, is retiring from his post and leaving India in March 1966. He was chief inspector of military explosives under the Government of India during 1940–47 and director of the Ministry of Supply Tropical Testing Establishment in Nigeria during 1947–54, after which he was assistant director of chemical inspection, Woolwich, before taking up his present post.

Dr. N. Miller, at present James Rowland Angell professor of psychology in Yale University, has been appointed professor of psychology in the Rockefeller University as from July 1.

Mr. H. E. Collins, Mr. L. R. Milligan and Prof. P. R. Owen have been reappointed members of the Safety in Mines Research Advisory Board.

The British and Irish Group for Co-ordinating Human Tumour Investigations, inaugurated in 1963 for the purpose of encouraging the widest experimental investigation of human material, proposes to issue a second bulletin listing and classifying research at present being carried out. Anyone interested in this field of study who wishes to contribute to the *Bulletin* is invited to contact the secretary, Dr. R. W. Baldwin, Cancer Research Department, The University, Nottingham.

The Institute of Biology is arranging a dinner on March 21, 1966, to celebrate the eightieth birthday of Dr. Edward Hindle, founder president of the Institute. Information about the arrangements can be obtained from the Institute of Biology, 41 Queen's Gate, London, S.W.7.

A one-day symposium on "Colour Vision" and "Colour Measurement", arranged by the Colour Group (Great Britain), will be held in the University of Edinburgh on April 1. Further information can be obtained from Mr. R. S. Sinclair, Paisley College of Technology, High Street, Paisley.

A meeting of the Scottish Branch of the Institute of Mathematics and its Applications will be held in Edinburgh on March 18. Prof. E. C. Zeeman will lecture on "A Mathematical Model of the Brain". Further information can be obtained from the Institute of Mathematics and its Applications, c/o the Mathematical Association, Gordon House, 29 Gordon Square, London, W.C.1.

A meeting of the Yorkshire Branch of the Institute of Mathematics and its Applications will be held in the University of Sheffield on March 24. Prof. K. Stewartson will lecture on "The Boundary Layer". Further information can be obtained from the Institute of Mathematics and its Application, c/o the Mathematical Association, Gordon House, 29 Gordon Square, London, W.C.1.

A meeting of the Bristol Branch of the Institute of Mathematics and its Applications will be held in Bristol on March 22. Mr. J. S. Sawyer will lecture on "Mathematics Applied to the Large-scale Dynamics of the Atmosphere". Further information can be obtained from the Institute of Mathematics and its Applications, c/o the Mathematical Association, Gordon House, 22 Gordon Square, London, W.C.1.

The Night Sky in April

All times are in Universal Time

Moon

New Moon	20d 21h
Full Moon	5d 11h

Conjunctions with the Moon

Venus	16d 13h, 6° N.
Mars	—
Jupiter	25d 08h, 3° S.
Saturn	17d 20h, 3° N.

Planets

Times of Rising (*R*) and Setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_φ (10^8 miles)	Zodiacal position
Mercury	—	Unfavourable for observation			+0·7	75	Pisces
Venus	*R*	4h 10m	3h 45m	3h 20m	−3·9	70	Aquarius
Mars	—	Unfavourable for observation			—	226	Pisces
Jupiter	*S*	1h 20m	0h 30m	23h 40m	−1·6	517	Taurus
Saturn	*R*	Unfavourable for observation		3h 30m	+1·4	973	Aquarius

D_φ is the distance of planet from the Earth on the 15th of the month

Occultations of Stars Brighter than Magnitude +6 at Greenwich

Star	*R*/*D*	Time	Mag.
γ Vir *m.*	*D*	5d 01h 46·7m	+2·9
γ Vir *m.*	*R*	5d 02h 51·0m	+2·9
$\varkappa$ Tau	*D*	23d 19h 23·5m	+4·4
67 Tau	*D*	23d 19h 25·6m	+5·4
118 Tau *f.*	*D*	24d 22h 15·9m	+5·9

(*D*, disappearance; *R*, reappearance)

MEDICAL EDUCATION IN BRITAIN

THE terms of the Rock Carling Fellowship, which was founded by the Governing Trustees of the Nuffield Provincial Hospitals Trust, provide that the holder shall make a review, by way of a monograph, of one of the fields in which Sir Ernest Rock Carling had been interested. Medical education was one of these, and in the preface to his monograph*, Prof. R. Milnes Walker, emeritus professor of surgery in the University of Bristol, mentioned some of Sir Ernest's activities in this field.

The establishment of a university degree as the only legal qualification to practise medicine was introduced in England as long ago as 1421. During the subsequent centuries, other means of obtaining the right to practise were permitted. For a time, only a minority of doctors held university degrees, and even up to 1938 less than half the medical students obtained degrees; to-day all doctors obtain admission to the Medical Register by a university degree.

The major steps in the evolution of medical education in Britain were the Apothecaries Act of 1815 and the Medical Act of 1858. In the interval between these dates the customary way into medical practice in England was by taking the Membership of the Royal College of Surgeons as a qualification in surgery, and the Licence of the Society of Apothecaries as a qualification in medicine. For those who aspired to Membership of the Royal College of Physicians, for which a university degree was necessary, it was customary to go to Scotland or to the Continent, where a doctorate in medicine was easier to obtain than at Oxford or Cambridge.

The impact of Abraham Flexner's survey of medical education in Europe in 1912 and his further investigation of medical education in general, published in 1925, cannot be exaggerated. It is to be regretted that Britain was so long in heeding his advice.

Many of the recommendations of the Goodenough Committee have now been implemented, however, and they were, in the main, adopted in establishing the relationships between the medical schools and the teaching hospitals when the National Health Service was brought into effect in 1948. Its report made no recommendations about medical man-power but, in an appendix, the Government Actuary estimated that if the number of practising British doctors were to be increased from the then figure of 45,400 to 55,000 in 15 years' time, the medical school entry would have to be raised from the pre-war figure of 1,800 to 2,630. This allowed for a net emigration of only one in every nine doctors. The Committee considered that the increase should be brought about by raising the existing schools to their full capacity before embarking on the establishment of new schools, but it criticized the concentration of medical schools in London and the relatively excessive number of students in the Scottish schools.

Owing to the slow rate of hospital building since the introduction of the National Health Service, the facilities for clinical teaching which the Committee recommended have fallen short of the mark. Their recommendations regarding staffing of pre-clinical and clinical departments have, however, been implemented to a considerable extent. Almost every school now has full-time professors in general medicine and in general surgery, and obstetrics is moving fairly fast in the same direction. Some departments are still deplorably housed and, where this affects pathology and the clinical departments, this has in part been held up by the scarcity of new hospital building. No unit in an undergraduate school in Britain has the control of 100 beds, which was suggested in the Haldane Report as long ago as 1913.

Much progress has been made in implementing the proposal of the Goodenough Committee for the teaching of social medicine, child health and psychiatry, but the maternity training is often unsatisfactory in that students may have to make use of facilities far removed from their teaching hospitals.

The compulsory pre-registration hospital appointments for twelve months were introduced in 1954 and have become an accepted part of the British educational and hospital structure, but the suggested reduction of the undergraduate course to 4·5 years has not come about. That Britain is still short of its needs in medical education has been emphasized in many places, but particularly in the *Review of the Medical Services in Great Britain*, which was published in 1963.

Prof. Milnes Walker referred to some of the changing aspects of medical practice which must influence medical education. In Britain, the increasing average age of the population raises problems as the elderly require an extra share of medical attention. It is wrong to assume that the Welfare State, with its better standards of living, will reduce the demand for medical care. When people have greater means they demand a better standard of medical attention, but they tend to be less inclined to look after their sick relatives at home, and, in fact, conditions often make this quite impossible. So the demand, and the cost, of a National Health Service continue to rise, and as it is unable to meet the demand an increasing number of people take out insurance to cover the cost of private medical attention outside the State Service.

The other important factor is the increased body of medical knowledge, which inevitably leads to increasing specialization. This widens the gap between general practitioners and specialists, and even among specialists themselves. The provision of group practices working from adequately staffed premises would lead to greater efficiency, and provide some means of improving the association between general practitioners and specialists.

Britain is going to be extremely short of doctors. Until recently, there has been one for every 930 of the population, but present figures indicate that there will be a proportion of one to 1,100 in a few years. This figure can be compared with one in about 600 in some European countries, and puts the ratio below that of Australia, Canada and the United States. Doctors are leaving Britain permanently at the rate of 400 a year, and this figure may well rise unless conditions in the Health Service are radically altered. It is important that enough doctors be trained for home needs and for emigration. An annual intake into medical education of about 3,500, which is almost a 50 per cent increase on the present number, should be the ideal.

In discussing courses and syllabuses, Prof. Milnes Walker advises the abolition of the First M.B. examinations; the medical schools would cease to teach the pre-medical subjects in their present form to medical students.

The pre-clinical course should be increased to 2·5 years, or 3 years if a science degree is taken, but provided there was a second year of pre-registration appointments, the clinical period could be reduced to 2 years.

**Medical Education in Britain.* Pp. 101. (London: The Nuffield Provincial Hospitals Trust, 1965.) 5*s*.

Of the 2 years of compulsory pre-registration appointments, the second year need not be one of residence in hospital, and, for many students, part of it should be spent in general practice, approved for the purpose and working from a health centre.

The aim should be to increase the intake of medical students to 3,500 a year as quickly as possible. Pre-clinical schools could be expanded to an intake of up to 200, but clinical schools should not normally have an intake of more than 100. Thus, more clinical schools would be required, and at present there are suitable hospitals in some of our provincial cities with medical schools which could be adapted as second clinical schools without great expense, and would share the university departments. The number of pre-clinical schools in London could thus be reduced.

In teaching hospitals the distinction between general medical and surgical wards should be abolished, but the teams in charge would comprise both physicians and surgeons, and the leaders of the teams might belong to either discipline.

In postgraduate training the increase in the organized teaching in district hospitals is welcome, and will need further expansion if a second pre-registration year is introduced. In London another look should be taken at the grouping of the special hospitals and institutes and some of them might be associated with or even incorporated in general hospitals.

For the better organization of postgraduate training of overseas doctors, an increase in the contacts with these countries and their medical schools is desirable, and regional boards might play a part in this.

SYMMETRY PRINCIPLES AT HIGH ENERGY

IN so rapidly developing a subject as elementary particle physics frequent conferences play an important part in the exchange of knowledge. The doyen of such meetings is the 'Rochester' Conference, affectionately called after its place of origin even though to-day its meetings are as likely to be held in Geneva or Dubna. It is a gathering of several hundred theorists and experimenters, and when it comes together every two years its sessions are concerned with all aspects of the subject. A necessary complement to so large and infrequent an assembly is the holding of regular small conferences on specific topics. The Coral Gables Conferences on Symmetry Principles, organized by the Center for Theoretical Studies of the University of Miami, have come to occupy an honourable place among such meetings.

The first two Conferences were held during a hectic and euphoric time for the practitioners of symmetry principles: the rapid discovery by experimentalists of more and more particles was matched by the invention by theorists of higher and higher symmetries with more extensive multiplets in which to locate them. Such boom conditions could not last for ever and if the atmosphere at the third Conference in January 1966 was less feverish this simply reflected a return to normal after the heady days of the immediate past. There were, nevertheless, many contributions of sober interest.

One of the most powerful techniques in symmetry physics has been the use of the current algebras invented several years ago by Gell-Mann and exploited by him to give many results in agreement with experiment. The method takes as its basic assumption the equal-time commutation relations of the integrated components of certain currents associated with weak, electromagnetic, and (by means of another assumption called the partially conserved axial current) strong interactions. During the past year Fubini and Furlan devised an important way of using dispersion theory in these calculations and this was employed by Adler and Weisberger to calculate the ratio of the Fermi coupling to Gamow–Teller coupling in β-decay, in excellent agreement with experiment. However, a useful assumption in many calculations, namely, that the commutation relations are saturated by a small number of neighbouring states, encountered certain theoretical difficulties which were pointed out by Coleman. In his address to the Conference, Gell-Mann explained how these difficulties could be avoided by working with matrix elements of states corresponding to infinite momentum and using only that sub-algebra of currents which has finite matrix elements to states of finite mass in this limit. It is known that the octet and decimet baryons of $SU(3)$ do not saturate these relations. Gell-Mann and Dashen are engaged on a calculation using various low-energy experimentally determined parameters as the input by which they hope to determine what further states are needed for saturation and so to arrive at a prediction of the nature of the higher-lying baryon resonances.

The current algebra technique does not commit its users to the belief that the algebra involved is a symmetry of the Hamiltonian applying to the whole of physics. It is only necessarily relevant for the matrix elements of the particular currents involved. Current algebras are thus essentially statements about the dynamical workings of the theory rather than about general invariance requirements. The relation between broken symmetries and dynamics is one that is little understood. Many people believe that the answer lies in a bootstrap theory which pictures elementary particles as all being bound states of each other, held together by forces due to the exchange of the particles themselves. In this way no particle is more elementary than any other and they can all be regarded as being composite. Simple models suggest that such theories might lead to symmetries, and Capps presented a contribution at the Conference on this question. However, realistic dynamical calculations are extremely difficult, not least because of a lack of understanding of the effect of multiparticle states on the structure of the theory.

The second Coral Gables Conference had heralded the entry of non-compact groups into elementary particle physics by the construction of relativistic versions of $SU(6)$. The unitary representations of these groups, which are needed in quantum mechanics, are infinite-dimensional and considerable ingenuity was expended in breaking the symmetry in elegant ways so that finite multiplets corresponding to sets of elementary particles could be obtained. However, ever since hole theory, elementary particle physicists have enjoyed making a virtue of vice, so that increasing numbers of people have thought that the presumably infinite sets of all elementary bosons and fermions, discovered and yet to come, might correspond to two or more infinite-dimensional representations of a non-compact group. This idea of killing infinitely many birds with one stone gained some encouragement from the fact which had long been known that the bound states of the hydrogen atom correspond to a single representation of $O(4,1)$. Fronsdal and Rühl gave details of some investigations they had made along these lines. Some initial difficulties in the satisfactory coupling of representations have been overcome and an unexpected feature is that the Born approximation already gives non-trivial momentum-dependent form factors. Another worker in this field is Salam, and his absence from the Conference owing to illness was much regretted both for

this reason and for the fact that his enthusiasm for symmetry physics is one of the expected pleasures of such occasions.

Non-compact groups are the mathematically opulent approach to elementary particles. A more mathematically economical, if physically rather opaque, line of attack is afforded by the use of quark models. Indeed, so economical is this latter approach that it has been noticeably successful in getting many of the $SU(6)$ results without actually assuming $SU(6)$. An investigation of this was reported by Lipkin. Characteristically he finds an interesting new sub-group as the cause.

An elusive particle (if it exists at all) is the magnetic monopole suggested by Dirac in 1931. As he pointed out, it is theoretically desirable since it leads to the otherwise unexplained quantization of electric charge. Schwinger gave a new account of the theory which leads to a different, more restrictive, quantization condition.

The final arbiters of all symmetry schemes are the experimentalists, whose findings can sometimes confirm and often destroy a theoretically elegant possibility. Excellent surveys of the present situation were given by Samios and Frisch. However, occasionally the theorists can get their revenge. Another experimentalist gave a talk on an experiment which he was planning, but a very quick-witted theorist pointed out in a spontaneous comment that the effect sought was identical with, and thus not capable of being disentangled from, another interaction also present. As someone else pointed out, the saving as a result of this observation more than paid for the whole Conference.

J. C. POLKINGHORNE

EARTHING OF ELECTRICAL SYSTEMS

THE safety and integrity of electrical power supply systems are almost universally dependent on some form of earthing which aims at limiting, under fault conditions, the potential difference between the general body of earth and certain parts of the system. The British Standards Institution has recently published a Code of Practice (*CP* 1013: 1965—*Earthing*) dealing with the subject*.

System earthing, as distinct from the earthing of electrical apparatus, began in the 1890's when the supply was utilized almost wholly for lighting and the need for equipment earthing on consumers' premises was relatively slight. Practice in the matter of earthing consumers' installations developed relatively slowly. While there is no mention of earthing in the first edition of the Institution of Electrical Engineer's *Wiring Regulations* issued in 1885, the third edition of 1897 recommends the earthing of the frames of dynamos and motors and of transformers. The eighth edition published in 1924 contains a substantial section dealing with consumers' installations and mentioning the provision of earth terminals.

Although the safety of electrical installations is a subject which has received much attention in recent years, it is probably not generally sufficiently realized that satisfactory protection by means of simple earthing, together with fuses or circuit breakers, becomes progressively more difficult of achievement as the load taken by the consumer increases.

While, in general, the question of how an electrical system shall be earthed is governed by legislation, the regulations are so worded as to permit any type of earthing provided that it is as safe as is practicable and that it is unlikely to interfere with telecommunication.

The new code of practice deals comprehensively with general considerations and with specific practices relating to the earthing of supply systems and of consumers' installations. There are six main sections, two of which, entitled "General" and "Design Considerations", deal with principles and practice concerning power stations, transmission and distribution systems, consumers' premises, traction and lightning protection. Sections 3, 4 and 5 deal respectively with the temporary safety earthing of high-voltage apparatus or mains, inspection and testing and maintenance. Section 6, which completes the work and is entitled "Miscellaneous", is concerned mainly with a number of specific legislative provisions.

Opening with a statement of scope, followed by a list of definitions, the subject is developed clearly and concisely and with an adequate amount of explanatory material. The Code constitutes an admirably comprehensive guide to earthing practice which will be a work of day-to-day reference for supply engineers, electrical contractors, maintenance engineers and, indeed, for all who have responsibility for electrical installations.

JAMES GREIG

* The Council for Codes of Practice, British Standards Institution British Standard Code of Practice, *CP* 1013: 1965—*Earthing*. Pp. 129. (London: British Standards Institution) 30*s*. net.

VOLCANOLOGY RESEARCH IN NEW ZEALAND

IN 1963, the Royal Society of New Zealand and the New Zealand Geological Survey (Department of Scientific and Industrial Research) invited the International Association of Volcanology to hold its 1965 symposium in New Zealand, to mark the centenary of the New Zealand Geological Survey. This symposium, held during November 22–December 3 at Auckland, Rotorua, Taupo and Wellington, was attended by more than one hundred delegates from twenty-three countries. The two main subjects were acid volcanism (including ignimbrites) and geothermal resources.

The problem of the genesis of acid volcanic rocks is particularly acute in an area such as the central volcanic zone of the North Island of New Zealand, where the volume of these highly silicic rocks greatly exceeds that of basic and intermediate types, and where individual eruptive units of acid rock are of such enormous volume. The problem hinges on whether this acid magma was produced by the large-scale fusion of crustal material or is a fractionation product of basic magma, itself generated originally within the upper mantle. A further possibility is that the acid magma may even come directly from the mantle.

In New Zealand, the basement which is believed to underlie the volcanic rocks includes a great thickness of Mesozoic to Tertiary greywackes. The chemical composition of these greywackes is such that their partial fusion could produce a melt similar in composition to the observed acid volcanic rocks. This origin is at present favoured by New Zealand geologists; basic and intermediate volcanic material is not sufficiently abundant to be quantitatively acceptable as a parental magma. Geochemical studies which have a bearing on this problem were discussed

during the symposium from other circum-Pacific areas, and it was shown how the $^{87}Sr/^{86}Sr$ and $^{18}O/^{16}O$ ratios supported the concept of crustal fusion for the origin of certain extensive acid volcanic accumulations in Guatemala.

It is now known that in volcanic fields such as the central zone of New Zealand, where acid volcanic rocks are present in enormous bulk, a high proportion of the acid material was ejected in fragmental condition by great explosive eruptions. The resulting pyroclastic sheets have a total volume in the Taupo–Rotorua district of New Zealand of the order of 20,000 km³. Particularly noteworthy are the deposits called ignimbrite ('shower-of-fire rock') by the New Zealand geologist, Marshall, in 1935. These deposits are the products of glowing cloud-type eruptions several orders of magnitude greater than has ever been observed in historic eruptions. Often the disrupted particles of magma were still so hot when they came to rest, even at a distance of several tens of kilometres from the vent, as to weld together. Similar deposits have since been found to be widespread in many parts of the world, and the term 'ignimbrite' is now internationally used (although its usage has been varyingly interpreted).

Outstanding contributions to the understanding of the nature and origin of ignimbrites have in recent years come from Japan, New Zealand and the United States, and the recent findings were discussed at the symposium. The relationship of many of these ignimbrites to large collapse calderas, about which they are distributed, is particularly striking; the formation of these calderas seems to be consequent on the rapid emission of huge quantities of fragmented rhyolitic magma.

The central volcanic zone of New Zealand, wherein virtually all the active volcanoes lie, includes, at either end, large andesitic volcanoes—Ruapehu, Ngauruhoe, Tongariro and White Island—which are quite frequently in eruption, although they seldom produce large volumes of new rock. In contrast, the central part of the zone, which includes Lakes Taupo and Rotorua, is characterized by occasional rhyolitic eruptions of extreme magnitude, and the geological record indicates that such eruptions recur at intervals of the order of several thousand years. In this connexion, the problem of the definition of 'active volcano' is particularly pertinent; although the last great eruption was nearly 2,000 years ago, the Taupo–Rotorua area must be regarded as one of high volcanic potential.

To offset the volcanic risk, New Zealand is fortunate in having geothermal fields of great economic value in the central volcanic zone. Since 1952 a power station has been producing more than 100 MW of electrical power from natural steam tapped by boreholes at Wairakei. Drilling programmes in other parts of the zone are at present investigating the further exploitation of this natural asset. Scientific papers presented during the symposium discussed such problems as the economic extraction of the geothermal energy, and theoretical aspects, such as the manner of transfer of heat within a geothermal system. Data were presented from geothermal fields in Iceland, Italy, Japan and the United States as well as from New Zealand.

New Zealand, a leader in the field of geothermal power, was among the first to take steps in the harnessing of the great energy output of volcanoes. Many nations are now following this lead.

I. G. Gass
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RESONANT SPIN STATES IN THE SOLAR SYSTEM

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TWO explanations have recently been proposed to account for non-synchronous rotation of Mercury[1]. In the first, Peale and Gold[2] showed that the vanishing of the time-averaged tidal torque on Mercury would imply a rotation rate which is intermediate between the planet's instantaneous orbital angular velocity at perihelion and its mean orbital angular velocity. The asymptotic spin angular velocity is determined by the amplitude and frequency dependence of the planet's 'Q' ($1/Q$ is the specific dissipation function[3]). For some reasonable Q's the final spin rate is near the observed 59 days. More recently, Colombo[4] has proposed that the rotation period of Mercury may be precisely 2/3 of its orbital period of 58·65 days. He suggested that this commensurate rotation rate might be stable if the figure of Mercury possessed a sufficiently large permanent deformation from axial symmetry with the long axis oriented toward the Sun at perihelion. Our investigation of such commensurate spin rates indicates that Mercury may indeed have a stable spin velocity which is 3/2 the orbital angular velocity but in one sense almost despite its permanent deformation rather than because of it.

We consider a planet the spin axis of which is normal to its orbital plane. The principal moments of inertia of the planet are denoted by A, B, and C; C being the moment about the spin axis, and B and A being the larger and smaller of the moments in the equatorial plane. The planet moves in an orbit with semi-major axis a, eccentricity e and instantaneous radius r. In Fig. 1 we illustrate the angles we shall use. Line IF is fixed in inertial space, whereas line PS is the planet–Sun centre line. The angle between the long axis of the planet and the centre line is Ψ, f is the true anomaly and θ gives the angular position of the long axis relative to the inertial line IF. In the absence of tidal torques the equation of motion for θ is (ref. 5):

$$C\ddot{\theta} + \frac{3}{2}(B-A)\frac{GM}{r^3}\sin 2\Psi = 0 \qquad (1)$$

where G is the gravitational constant and M is the mass of the Sun. From Fig. 1 we find $\theta = f + \Psi$. Suppose we wish to determine the stability of a planetary spin angular velocity near $pn/2$, where n is the mean motion of the planet and p is any integer. Defining a new angle $\gamma = \theta$

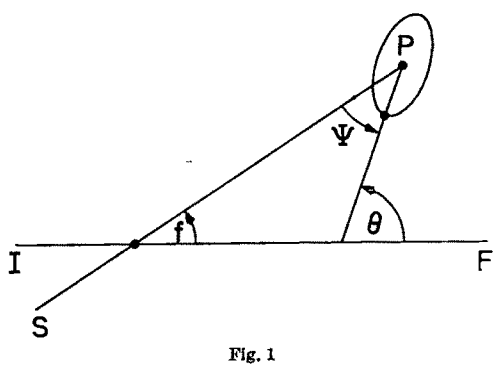


Fig. 1

$-pnt/2$ (where $t=0$ at $f=0$), we may average equation (1) over an orbit period keeping γ fixed since it only varies slightly during this period. Including terms up to second order in e, we have:

$$C\ddot{\gamma}+\frac{3}{2}(B-A)n^2 \sin 2\gamma\left[-\frac{e}{2}\delta_{p1}+(1-\frac{5}{2}e^2)\delta_{p2}+\frac{7}{2}e\,\delta_{p3}+\frac{17}{2}e^2\,\delta_{p4}\right]=0 \quad (2)$$

where δ_{pq} is the Kronecker delta. From equation (2), which is the ordinary pendulum equation, we observe that in the absence of tidal torques a planet will exhibit stable librations at values of $\dot{\theta}=n/2,\ n,\ 3n/2,\ 2n$. (Higher order commensurabilities are also stable, but they involve higher powers of e.) For the first case ($\dot{\theta}=n/2$) stability occurs for oscillations about $\gamma=\pi/2$, or in other words, at perihelion the short axis of the planet points approximately towards the Sun. In the other cases oscillations occur about $\gamma=0$, and the long axis is approximately pointing toward the Sun at perihelion. The synchronous rotation corresponds to $\dot{\theta}=n$, whereas $\dot{\theta}=3n/2$ is the rotation state which Colombo has proposed for Mercury.

It is natural to question the stability of these commensurate spin states when tidal torques are present. Now the averaged tidal torque ($-T$) must be added to the right-hand side of equation (2). For a given p we have:

$$C\ddot{\gamma}+\frac{3}{2}(B-A)\,f(p,e)\,n^2 \sin 2\gamma=-T \quad (3)$$

when $f(p,e)$ is the coefficient of the appropriate Kronecker delta in equation (2). ($f(p,e)$ is either 0 or of the order $e^{|p-2|}$.) In cases where Q is frequency independent and $\dot{\theta}$ is greater than the instantaneous orbital angular velocity at perihelion, T is constant (that is, T is independent of γ, $\dot{\gamma}$ and t). Equation (3) is now the pendulum equation with an additional applied constant torque. For stability the applied torque must not exceed the maximum possible restoring torque which occurs at γ displaced by $\pi/4$ from its equilibrium value. Thus the stability criterion is:

$$T<\frac{3}{2}(B-A)\,f(p,e)n^2 \quad (4)$$

We can apply this condition to examples provided by the solar system.

For the Moon in its present orbit with the known value of $(B-A)/C=2\times10^{-4}$, all the commensurabilities we have discussed as well as some depending on terms of higher order in e are stable. The commensurabilities occur for both faster and slower rotations than the present synchronous rotation of the Moon. Even if the Moon were much closer to the Earth, with a smaller orbital eccentricity, some of the higher order commensurabilities would still be stable. For Mercury the value of $(B-A)/C$ is unknown. However, for values as small as 10^{-8} and probably even 10^{-9} the $\dot{\theta}=n/2$ and $\dot{\theta}=2n$ commensurabilities would be stable. With such an exceedingly small deviation from axial symmetry which is necessary for stability in the $n/2$ and $2n$ modes, the question arises as to how Mercury and the Moon could have passed through either of these states to reach their present ones. It seems exceedingly likely that Mercury and the Moon must have passed through one or the other of the adjacent stable commensurate spin states to attain their present spins. Similar strong indications that satellites may pass through these resonances are provided by the observed synchronous rotations of several satellites of the major planets. Of special interest is Iapetus because of its weak tidal torque, relatively high orbital eccentricity, and well-confirmed synchronous rotation[6]. Equation (3) provides a probable explanation of this behaviour.

If we multiply equation (3) by $\dot{\gamma}$, the left-hand side is dE/dt, where E is the energy integral of the pendulum equation. Since:

$$\frac{dE}{dt}=-T\dot{\gamma} \quad (5)$$

we see that a constant tidal torque will not damp the amplitude of a libration about the minimum energy value of γ. Thus, while the commensurate spin rates are stable for sufficiently weak tidal torques, any initial libration about the stable value of γ will persist indefinitely. On the other hand, if any term in T is proportional to $\dot{\gamma}$ we see that these librations will be damped.

Now consider the rotation of a non-axisymmetric planet as tidal friction slows its spin from a rate which is several times n. As the spin of the planet slows it must approach one of these stable commensurabilities. We cannot determine definitely from our approximate analysis whether libration about the stable value of γ will ensue or whether the planet will pass through this commensurability and continue to 'de-spin'. However, as the amplitude of any libration about a stable commensurate spin is not damped from its initial value of $\pi/2$ by a constant tidal torque, this marginally stable condition cannot persist, and the planet must eventually escape from this commensurability and continue to 'de-spin'. However, a term in T proportional to $\dot{\gamma}$ would tend to damp the libration and trap the planet in a high-order commensurability. Even in this case it may be possible for the planet to escape from the commensurability before the damping has been sufficient to trap it. This has actually occurred in some numerical calculations performed by one of us[7].

From the examples provided by the Solar System it seems that Mercury, the Moon and several other satellites known to be synchronous have successfully avoided being trapped in higher order commensurabilities in spite of the small permanent asymmetry required to stabilize these states. This conclusion holds even for initially retrograde spins, since stable commensurabilities exist for retrograde rotations as well. Mercury may be in the $3n/2$ resonance. Because of its large orbital eccentricity, the spin angular velocity corresponding to the 59-day period is actually slower than the instantaneous orbital angular velocity at perihelion. Thus the tidal torque on Mercury reverses twice during each orbit which leads to a term equal to a positive constant times $\dot{\gamma}$ in the average tidal torque (even if the torque is constant between reversals). As explained previously this term produces a damping of the librations in γ and hence may stabilize the rotation of Mercury in the $3n/2$ commensurability. Thus the reversal of the tidal torque discussed by Peale and Gold[2] seems to be a necessary condition for the trapping of Mercury's spin into the 3/2 commensurability. In other words Mercury can be in the 3/2 commensurability but only because the tidal friction tends to bring it there. Otherwise it would have passed through this commensurability as it has apparently escaped others. It should be emphasized that no strong limit on the permanent deformation or rigidity of Mercury is required by either the final state of $\dot{\theta}=3n/2$ or the final state determined by the gravitational tidal friction alone. Barring trapping in any of the higher order commensurabilities (which seems possible only for Mercury), we find that the planet will end up spinning either synchronously or at the slightly faster rate discussed by Peale and Gold (see ref. 8). Because of the aforementioned reversal of tidal torque, librations about synchronous rotation are damped regardless of eccentricity.

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IONIZATION RELAXATION IN SLIGHTLY IMPURE ARGON

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In recent years there have been several experimental investigations of the growth of ionization behind strong shock waves in the noble gases: all these have suggested that impurities in the gas can significantly influence the time taken to reach equilibrium ionization. At the time of writing, however, there was no satisfactory quantitative theory existing assessing the role of these impurities.

The most widely quoted investigation of this problem is that of Petschek and Byron[1]. They suggested that the change of the degree of ionization α with time t in the initial ionization stage where the first free electrons are produced was entirely due to the presence of impurities and given by:

$$\frac{d\alpha}{dt} = (1 - \alpha)^2 A N_I \exp\left(-\frac{T_{ACT}}{T}\right) \quad (1)$$

where A and T_{ACT} are constants to be found from experiment, while N_I is the impurity species number density and T is the relaxing translational temperature. When equation (1) is combined with their proposed final ionization stage rate then they obtain an ionization time τ given by:

$$\tau = \int_0^{\alpha^*} \frac{d\alpha}{(1-\alpha)^2 A N_I \exp\left(-\frac{T_{ACT}}{T}\right) + N_A B \alpha^2 \ln\left(\frac{\alpha_{EQ}}{\alpha}\right)} \quad (2)$$

Here N_A is the neutral atom number density, B is given in graphical form in the reference cited while α_{EQ} is the equilibrium degree of ionization. It should be noted that τ is not an ionization relaxation time since we cannot in general define a true exponential approach to equilibrium throughout the entire relaxation zone (see Clarke and McChesney, ref. 2). Petschek and Byron found $A = 8 \times 10^{-8}$ cm^3/sec and $T_{ACT} = 11{\cdot}5$ eV for argon.

Several criticisms of this investigation can be made: (i) Their (modified) frequency factor A is too high; this follows, since simple kinetic theory permits us to write:

$$A = \left(\frac{2kT}{\pi\mu}\right)^{\frac{1}{2}} Q_{IA}\left(1 + \frac{T_{ACT}}{T}\right) \quad (3)$$

where μ is the reduced mass of the argon atom–impurity species collision complex while Q_{IA} is the cross-section for the collision process the activation 'temperature' of which is T_{ACT}. The Petschek–Byron value for T_{ACT} when combined with a cross-section as large as 10^{-17} cm^2 (see below) still yields an A value three orders of magnitude smaller than the Petschek–Byron value; no sensible juggling with the variables of equation (3) can be made to yield an A value anywhere near 10^{-8} cm^3/sec. (ii) A value of $T_{ACT} = 11{\cdot}5$ eV is that corresponding to excitation of an argon atom whereas it should be the activation energy of an easily excited impurity. It should be noted that Petschek and Byron do not actually state that the impurities react through a cumulative mechanism as is commonly implied. (iii) They neglect the possibility of concurrent argon atom–argon atom excitation collisions. (iv) They claim that impurities are important over all shock Mach numbers and obtain an ionization time always significantly dependent on the impurity level; however, their experimental results at a shock fore-pressure of 10 torr using argon containing an impurity of 1 part in 10^3 are identical with their results for an impurity-level of 5 parts in 10^5.

The experimental investigations of Johnston and Kornegay[3] and Harwell and Jahn[4] show that initial ionization in a pure gas will proceed cumulatively and not in a single step as proposed by Bond[5]. This has been supported by Weymann[6], who proposed a two-step mechanism for ionization, namely, impact excitation and then impact ionization. It is likely that this proposed two-step mechanism is correct, but the simple kinetic considerations used by Weymann are incorrect. Neither Harwell and Jahn nor Johnston and Kornegay quantitatively discuss impurities while Weymann incorrectly assesses their role in the same way as did Petschek and Byron. We shall use the two-step ionization mechanism for the following concurrent reactions in the initial stage of ionization:

$$Ar + Ar \xrightarrow{k_A^*} Ar^* + Ar \longrightarrow Ar^+ + e^- + Ar \quad (4)$$

$$I + Ar \xrightarrow{k_I^*} I^* + Ar \longrightarrow I^+ + e^- + Ar \quad (5)$$

$$I^+ + Ar \rightleftharpoons I + Ar^+ \quad (6)$$

We shall utilize the final stage ionization mechanism used by Petschek and Byron. Their corresponding ionization rate expression is inaccurate and more detailed calculations are in hand by one of us (M. Mc.) on the electron energy balance in this regime. These coupled processes yield an ionization time:

$$\tau = \int_0^{\alpha^*} \frac{d\alpha}{(1-\alpha^2) N_A\left(k_A^* + \frac{N_I}{N_A} k_I^*\right) + N_A B \alpha^2 \ln\left(\frac{\alpha_{EQ}}{\alpha}\right)} \quad (7)$$

which should be compared with equation (2). The significant difference between our equation (7) and the Petschek–Byron equation (2) is that the former considers concurrent excitation processes: as we shall note, this results in the ionization time being considerably less sensitive to the impurity level at high Mach numbers than suggested by Petschek and Byron.

The intermediate excited level of the noble gas is taken as the first metastable level: any further cumulative excitation will not be rate controlling; photo-excitation is inadmissible to this level—as is spontaneous de-excitation. Due to the high inter-particle collision frequency and very low radiation density in the near-visible radiation field, photo-ionization of the metastable state is negligible as will be induced emission processes. Furthermore, calculations not discussed here show that due to the relatively low concentration of excited levels, collisions between two excited atoms are negligible. We are obliged to ignore cascade transitions due to paucity of oscillator strength data for ArI. Following Petschek and Byron we ignore all recombination processes since the analysis is only applied up to a degree of ionization α^* where recombination is still secondary.

In equation (7) the k^* factors are reaction rate constants given by:

$$\frac{1}{2}\left(\frac{2\pi kT}{\mu}\right)^{-\frac{3}{2}} \int_{g_0}^{\infty} \exp\left(-\frac{\mu g^2}{2kT}\right) Q(g) 4\pi g^3 dg$$

where apart from standard symbols, g is the relative velocity of the colliding species, $Q(g)$ is the velocity dependent inelastic cross-section and $\frac{1}{2}\mu g_0^2 = \frac{1}{2}kT_{ACT}$ which for a step-function form of cross-section yields the right-hand side of equation (3): this is a better form for heavy particle inelastic collisions near threshold than the ramp function suggested by Harwell and Jahn (see following). We can ignore the ionizing collisions given by the second part of the reactions described in equations (4) and (5); this steady-state approximation is justifiable

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from the experimental investigations to be reported here as well as on theoretical grounds since it is known that the cross-sections for the ionizing collisions are large and of order of the momentum transfer cross-sections (see Hirschfelder and Eliason, ref. 7).

The experimental values of the ionization time were measured in a combustion-driven shock tube using a Mach–Zehnder interferometer. The difficulties attached to time-resolved optical interferometry due to the small overall fringe shifts occurring and the speed at which they change together with the complications due to the self-luminosity of the shock-heated gas were largely overcome by utilizing the photo-electric monochromator technique for recording the fringes described by Jones and McChesney[8]. Fig. 1 shows values of $p_1\tau^*$ (where p_1 is the shock fore-pressure and τ^* is the total time taken to reach equilibrium) plotted versus T_{AO}, the reciprocal of the post-shock unrelaxed translational temperature together with the values predicted by Petschek–Byron theory. A complete description of the diagnostic techniques used together with the method of analysis of the results is given by Jones[9]. It will be seen that Petschek–Byron theory overestimates the role of impurities at all Mach numbers M_s and also that the total time to reach equilibrium ionization is insensitive to the impurity level (within sensible limits) above Mach 12. Analysis yielded the following results: the excitation cross-section for the first part of the reaction given by equation (4) is $2{\cdot}5 \times 10^{-18}$ cm^2 for a known activation energy of 11·6 eV. Noble gas excitation cross-sections of this size have been reported by Harwell and Jahn and also Johnston and Kornegay without comment: however, their large size merits comment.

Bates and Massey[10] have made Born approximation calculations of heavy particle impact excitation and ionization collisions and found them to be of order 10^{-22} cm^{-2} or less at threshold: of course, the Born approximation is likely invalid at very low energies since it makes all inelastic collisions near adiabatic. The low-energy beam experimental investigations of Amme and Hayden (private communication) yield an ionization cross-section for direct impact ionization of argon by another argon atom that rises like a step function from a value of 5×10^{-20} cm^2 at a relative kinetic energy of 28 eV to a (approximately) constant value of about 10^{-16} at 105 eV. There are no corresponding excitation cross-sections (either emission or absolute) in this energy range reported in the literature: the only neutral particle excitation data of any relevance are those of van Eck *et al.*[11], whose results suggest that the large excitation cross-sections are possible if intermediate molecule formation occurs in the collision complex with its corresponding multiplicity of potential energy surfaces allowing an excitation jump.

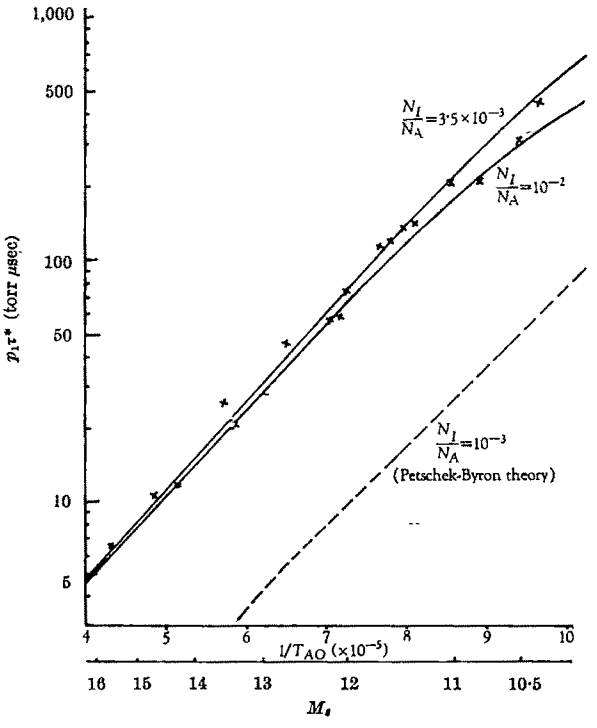


Fig. 1. Equilibrium ionization times as a function of shock fore-pressure, p_1, shock Mach number, M_s and reciprocal post-shock unrelaxed translational temperature, $1/T_{AO}$. Solid lines are given by equation (7); ×, experimental points

A full discussion of the excitation and charge exchange cross-sections together with the nature of the impurity atoms and the possible role of chemi-ionization reactions in the ionization process will appear elsewhere.

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MANGANESE-54: FRACTIONAL DISTRIBUTION IN WHEAT AND OCCURRENCE IN OTHER FOODS

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MANGANESE-54 (^{54}Mn), a neutron activation product, was detected in fall-out samples from Eniwetok testing grounds in 1956 (ref. 1). The distribution pattern of manganese-54 during that period and of other radioactive non-fission products such as iron-55, iron-59, cobalt-57, cobalt-58 and cobalt-60, and zinc-65, was estimated from measurements on whole plants and animals by means of γ-ray spectrometry. Generally, these radionuclides were found in sea water and some marine animals, but were undetected in marine plants. Few or none were detected on the ground, in plants growing in the soil, or in herbivorous field animals[2]. In 1961 and 1962, large amounts of manganese-54 were found in debris from a series of atmospheric nuclear weapons tests. From these tests, measurements of manganese-54 by γ-ray spectrometry have been reported in samples of grain and food[3].

Stable manganese was detected in many parts of plants and in several organs of animals and humans more than 50 years ago, and was found, with few exceptions[4], in all substances analysed. The concentrations of stable man-

ganese reported in animals and humans were appreciably less than those observed in plants. In experiments with wheat seedlings, Burstrom[5] found manganese salts to be unique when compared with salts of the alkali, alkaline earth, and heavy metals, in that assimilation or conversion of nitrate to plant protein was effected only in the presence of this element. This observation and later findings confirmed that stable manganese was an essential minor element for the normal growth of plants. In studies of other natural elements that are minor but essential and important in crop production, namely, iron, zinc, copper, and cobalt, Robinson[6] found that there was little variation in the accumulation in plants of any of these except manganese. Besides investigations of plant nutrition and crop production in relation to stable manganese, a considerable amount of work has been done towards establishing the chemical forms and reactions of manganese in soil. It is generally agreed that stable manganese exists in the soil in several chemical forms. Briefly these forms are: (1) manganous manganese (Mn^{+2}), which usually exists in an exchangeable form between soil and plants; (2) manganic manganese (Mn^{+3}), which is easily reduced by bacterial action within the soil; (3) the manganese oxides, which include various structural types and oxidation states. The availability of these various forms of manganese to plants is dependent on several environmental factors, such as soil type, hydrogen ion and anion concentration, organic constituents, bacterial action and seasonal variation[7–11].

The specific chemical form of radio-manganese in fallout is unknown but is generally assumed to occur mainly as inert manganese dioxide. No evidence is available as to whether atmospheric weathering or existing soil conditions provide an environment favourable to reducing this oxide to forms available for plant uptake. Thus, during the work on two fission products, cerium-144 and caesium-137, in 10 g of ash from samples of the 1963 United States wheat crop[12], we separated manganese for detailed investigation using radiochemical and instrumental methods.

The radiochemical procedure and the instrumental techniques used for the measurement of manganese-54 and stable manganese were as follows. Equilibration of stable manganese and radiomanganese was accomplished by extracting the sample ash with a mixture of hydrochloric acid and hydrogen peroxide. Manganese dioxide was separated from the sample matrix with strong nitric acid and sodium chlorate. The precipitate was dissolved, transferred to a small vial, and manganese-54 measured by γ-ray counting with a single-channel analyser[13]. Under standard conditions, the counting efficiency for manganese-54 was 20 per cent. The error due to counting for the tabulated values was within 10 per cent. The disintegration rate of manganese-54 was determined from the counting rate and corrections were made for background, efficiency, and decay. Purity was established by counting the samples in groups, using a 256-channel γ-ray analyser with an 8 × 4 in. sodium iodide (Tl) crystal over a range from zero to 3 MeV. Only the characteristic 0·84-MeV peak of manganese-54 was observed from the spectrum of each group, with a small contribution from the 1·40-MeV peak of potassium-40, which was inherent to the crystal. As a further means of establishing the purity of the samples, the group γ-ray spectra were resolved graphically, using a method based on the use of an integral spectrum. The latter technique, using the integral spectrum rather than the differential spectrum, offered the advantage that the resolved spectra are linear with unit slope when plotted on logarithmic paper[14]. Graphs of each of the group spectra indicated a single-component system.

Stable manganese was determined spectrophotometrically in the original sample as permanganate after periodate oxidation[15]. Measurements were made using a model '*DK*-2' Beckman spectrophotometer with maximum absorption at 525 mμ. The actual amount of stable manganese present in each sample was determined separately by taking a 1-g portion of the sample ash through the radiochemical procedure after adding a known amount of manganese-54. The tracer was used to determine the overall chemical recovery, and a sample portion was then analysed spectrophotometrically. The latter value was used to correct the yield of the 10 g of ash from the original sample. Average chemical recoveries were 95 and 80 per cent, respectively, for the 1-g and 10-g portions of ash.

The amounts of manganese-54 and stable manganese found in samples of the 1963 United States wheat crop from eleven states are shown in Table 1. These states produced about 75 per cent of all the wheat grown in the United States during 1962 with Kansas producing 20 per cent of this. The value for each State is reported at a time considered to be the mid-point of the harvest.

The concentrations of manganese-54 and stable manganese, and consequently the ratio of picocuries of manganese-54 to milligrams of stable manganese, were fairly constant with three exceptions. The samples from Missouri and South Dakota accumulated a relatively larger amount of stable manganese than was found for other samples and the Ohio sample showed the smallest uptake of stable manganese. Neither of these apparent extremes is unusual for stable manganese accumulation in healthy plants, and this variation may be the result of prevailing soil conditions.

For an investigation of the balance of distribution of the radionuclide, a Kansas wheat sample was separated into its milling fractions. As shown in Table 2, the distributions of the measured manganese-54 and stable manganese are remarkably similar for all fractions. The concentration of this stable element and its radionuclide was larger in the inner layer shorts fraction (55 and 52 per cent respectively) than in the bran fraction which forms the outermost layers of the wheat kernel (35 and 39 per cent respectively). The patent flour fraction which is used for human consumption contained 4 per cent manganese-54 and stable manganese. From this distribution pattern it seems that rapid exchange occurred between radio-manganese and natural manganese and that uptake has been through the root of the plant rather than as a result of physical adherence of inert radio-manganese.

In order to estimate the occurrence of manganese-54 in the 1964 diet, ten food samples were selected from the open market in May 1964 in New York City and measured for manganese-54 and stable manganese. The values are reported as of the middle of the sampling month in Table 3. Manganese-54 was detected in small amounts in fresh

Table 1. Results of Analysis of Manganese-54 and Stable Manganese in Samples of Wheat Grown in the United States during 1963

Location	Harvest mid-point	Content of original material pc. ^{54}Mn/kg	mg Mn/kg	pc. ^{54}Mn / mg Mn
Indiana	July 15	800	40·5	19·8
Kansas I	June 15	678	38·1	17·3
Kansas II*	June 15	696	40·7	17·1
Missouri	June 30	557	60·3	9·2
Montana	July 31	793	34·7	22·9
Nebraska	June 30	974	39·6	24·6
North Dakota	July 14	551	24·6	22·4
Ohio	July 15	931	20·0	46·6
Oklahoma	May 25	634	26 8	23·7
South Dakota	July 31	209	56 8	3·7
Texas	May 15	855	36·8	23·2
Washington	July 31	462	28·8	16·0

* Duplicate sample of Kansas (I), which is a composite of eight varieties of wheat.

Table 2. Material Balance of Manganese-54 and Stable Manganese in Kansas Wheat and Milling Products

Fraction / Wheat	Percentage of total	Content of original material pc. ^{54}Mn/kg	mg Mn/kg	Fractional contribution to kg of total pc. ^{54}Mn	mg Mn	Percentage in fraction ^{54}Mn	Mn
(Kansas II)*	100	696	41	696	41		
Patent flour	52	53	3·3	28	1·7	4	4
First clear flour	17	111	7·7	19	1·3	3	3
Second clear flour	6	278	20	17	1·2	2	3
Shorts	14	2,713	182	380	25	52	55
Bran	11	2,542	147	280	16	39	35
Total				724	45	100	100

* Duplicate sample of Kansas (I), which is a composite of eight varieties of wheat.

Table 3. RESULTS OF MANGANESE-54 AND STABLE MANGANESE IN FOOD SAMPLES OBTAINED IN NEW YORK CITY IN MAY 1964

Sample type	Contents of original material (per kg) pc. ^{54}Mn	mg Mn
Fresh fruit	5·4	3·2
Fresh vegetables	10·2	3·3
Root vegetables	≦0·81*	3·5
Potatoes	≦0·83*	3·4
Rice	≦0 79*	12·9
Rice (water washed)	≦0·69*	9·2
Dry beans	58	16·3
Flour	62	5·7
White bread	12·8	3·1
Whole wheatbread	132	28·5

* Less than or equal to single Poisson counting error.

fruit and fresh vegetables. It was not detected in a sample of root vegetables that contained carrots, onions, and turnips, or in a separate sample of potatoes. Neither was it detected in rice. It was found in almost equal amounts, 60 pc./kg, in dry beans and white flour. The amounts of manganese-54 and stable manganese measured in whole wheat bread were approximately ten times higher than the amounts found in white bread. The ratio of picocuries of manganese-54 to milligrams of stable manganese for whole wheat bread and white bread was 4·6 and 4·1, respectively. Since the specific activity of manganese in the 1964 bread samples was much lower than can be accounted for by radioactive decay, assuming a 310-day half-life, in the representative 1963 wheat sample, it can only be assumed that the 1963 crop was not the sole origin of wheat for the 1964 diet. The fact that the specific activity showed good agreement for whole wheat and white bread further substantiates exchange between manganese-54 and stable manganese.

The ten food samples shown in Table 3 represent approximately 35 per cent of the total yearly diet[16]. Assuming a corresponding amount of manganese-54 for the entire diet, though it is believed that this estimate is high, an intake of about 160 pc. manganese-54 per week may be postulated for the general population in 1964. Since the maximum permissible intake for chronic exposure to manganese-54 is approximately 4,000 pc. per week for the general population[17], the amounts found in the 1964 foods are not considered to constitute a health hazard.

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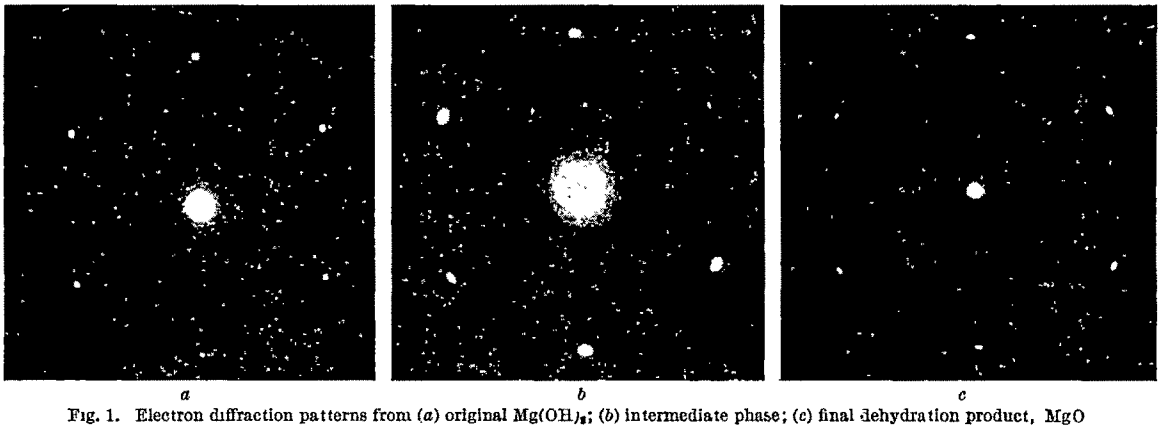
TOPOTACTIC DEHYDRATION OF BRUCITE

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SINGLE crystals of brucite, naturally occurring $Mg(OH)_2$, may be dehydrated topotaxially[1] to periclase, MgO. $Mg(OH)_2$ has a layered structure of the CdI_2 type and may be described as consisting of hexagonally close-packed hydroxide ions with the small Mg^{++} ions occupying octahedral interstices between alternate sheets, . . . *AmB AmB* . . . . Since no hydrogen bonds exist in the structure[2] we may consider the lattice arrangement in terms of close-packed oxygen atoms. The pseudomorphic dehydration product, MgO, is based ideally on a cubic close-packed oxygen framework with the small cations occupying all the octahedral interstices, . . . *AmBmCm* . . . . From a knowledge of the initial and final structures it has been possible to discuss plausible mechanisms for the dehydration process[3].

In a recent kinetic investigation of this transformation[4] it was suggested that certain differences may exist between thermal decomposition and the decomposition observed by one of us, using the beam of an electron microscope[5]. Accordingly, we have examined the purely thermal decomposition of brucite *R* (ref. 4) (kindly supplied by Dr. P. J. Anderson) using electron microscopy and selected-area electron diffraction. Specimens were prepared from crystallites, scraped from a cleavage face, which were deposited on a carbon support film. The dehydration was performed at 320° C in an electric furnace at a pressure less than 10^{-4} mm of mercury. At various stages during the 3–4 h required for complete reaction, specimens were slowly cooled to room temperature and examined in a Siemens 'Elmiskop 1'. Electron diffraction patterns taken with the electron beam parallel to the [001] direction of brucite revealed that under normal operating conditions the original $Mg(OH)_2$ showed no decomposition in the electron beam (Fig. 1*a*). The crystallographic and



a *b* *c*

Fig. 1. Electron diffraction patterns from (*a*) original $Mg(OH)_2$; (*b*) intermediate phase; (*c*) final dehydration product, MgO

morphological changes occurring during the thermal decomposition were the same as those observed previously by decomposition in the electron beam[5]. It seems reasonable to conclude that the decomposition in the electron beam is caused primarily by the rise in temperature of the specimen, irradiation effects apparently being of only minor importance.

After heating for 15 min, a well-defined spot pattern due to the previously reported intermediate phase was obtained (Fig. 1*b*). Thus, by controlled heating the intermediate structure can be recognized as a separate phase, which under normal conditions does not revert to brucite. The nature of this intermediate structure provides an important clue to the decomposition mechanism. Since decomposition temperatures greater than 800° C may ultimately produce traces of a spinel-type by-product it has been tentatively proposed[6] that heating causes a migration of cations within the close-packed oxygen lattice. This idea of cation migration can readily explain the present electron diffraction data. As the filling of tetrahedral interstices is regarded as highly unlikely at moderate temperatures because of the unfavourable radius ratio parameter, the migrating Mg^{++} ions must enter the unfilled octahedral holes which exist between alternate sheets in the ideal $Mg(OH)_2$ structure. Local electrical neutrality within the new structure will then be preserved by protons diffusing into those regions from which there has been a loss of Mg^{++} ions. Whether discrete water molecules are formed within the structure at this stage is uncertain but seems probable. What is certain is that, at this primary stage, kinetic experiments reveal no loss of water[4]. The previously observed lattice shrinkage[5] of about 5 per cent relative to brucite was observed in the (*hk*.0) plane of the hexagonally symmetric intermediate phase. Like the isostructural $Ca(OH)_2$ (ref. 7) it seems probable that in $Mg(OH)_2$ the octahedra of oxygen atoms surrounding each cation are compressed along the hexagonal axis, the compressional force presumably arising from the asymmetry of the electrostatic environment in the layered lattice rather than from any steric factor. The migration of Mg^{++} ions into the previously unfilled interlayer interstices in the hexagonally close-packed oxygen lattice will relieve the original electrostatic compression and result in the observed compensating lateral contraction within the (*hk*.0) plane. In descriptive terms, the intermediate phase may be considered to possess a highly defective NiAs type structure with only half the total available octahedral sites occupied by Mg^{++} ions, that is, a cation-deficient structure somewhat analogous to that possessed by the $CoTe_x$ system ($1 \leqslant x \leqslant 2$) (ref. 2). The discrete but diffuse nature of the spots in the (*hk*.0) single crystal diffraction pattern of the intermediate phase (Fig. 1*b*) is characteristic of a structure possessing interstitial disorder and possibly also stacking faults. Direct evidence for the existence of stacking faults in the intermediate structure is provided by the occurrence in some of the diffraction patterns of a weak hexagonal network of diffuse streaks joining the reciprocal lattice nodes.

In the second, slow stage of the decomposition, which occurs on further heating, the hexagonal pattern of the intermediate phase gradually fades, leaving only those strong but diffuse spots due to the cubic MgO structure (Fig. 1*c*). During this stage there is (i) the transition from a predominantly hexagonal to cubic close-packing of oxygen atoms via dislocations and their resulting stacking faults, and (ii) the loss of half the total number of oxygens as water vapour. The diffusion-controlled loss of water from the structure along dislocations[8] is envisaged as occurring from those regions of the intermediate phase which are deficient in Mg^{++} ions. The final structure will be highly porous, which explains the unusually high specific surface areas found for dehydrated $Mg(OH)_2$ crystals by gas adsorption studies[4]. The discrete but diffuse spots in the diffraction pattern for MgO (Fig. 1*c*) indicates that the overall final structure may be considered a highly imperfect single crystal.

To determine the *c*-crystallographic dimension of the hexagonal intermediate structure, X-ray powder photographs were obtained from partially decomposed $Mg(OH)_2$. The resulting diffraction patterns were complex, containing sharp rings due to the unchanged $Mg(OH)_2$ ($a = 3{\cdot}15$ Å, $c = 4{\cdot}74$ Å), diffuse rings due to the porous decomposition product MgO ($a = 4{\cdot}19$ Å), and weak, arced lines which can be ascribed to small equilibrium amounts of the intermediate phase ($a = 2{\cdot}98$ Å, $c = 4{\cdot}83$ Å). A few additional, very weak, arced lines were observed in the 2–5 Å region which do not correspond to reflexions from the ideal hexagonal lattice of the intermediate phase. All but one of these subsidiary reflexions can be ascribed indices (00.*l*) having half integral *l* values indicating that the length of the unit cell in the *c*-direction is double that of the fundamental spacing. This observation is compatible with the presence of stacking sequences in the close-packed oxygen layers of the type . . . *ABAC* . . . . The remaining 'extra' line corresponds to a (00.*l*) reflexion with *l* one-third integral, indicating the presence of domain regions with a layer stacking of the type . . . *ABCACB* . . . (ref. 9). The existence of such stacking faults is consistent with our proposed decomposition mechanism. Thus, as in the analogous γFeO(OH) system[10], the proposed decomposition mechanism clearly demonstrates that the transformation does not involve any *de novo* crystallization of the reaction product but is controlled solely by the close-packed anion arrangement of the initial structure.

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TETRAMISOLE (*R* 8299), A NEW, POTENT BROAD SPECTRUM ANTHELMINTIC

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THIS article reports the discovery of tetramisole (*R* 8299 (I)), a new, potent broad spectrum anthelmintic, which is at present undergoing large-scale clinical trials in several countries.

In the course of our routine screening programme for anthelmintic activity the aminothiazol derivative (II) (thiazothienol or *R* 6438) was found to be active against *Heterakis*, *Ascaridia* and *Capillaria* in the chicken.

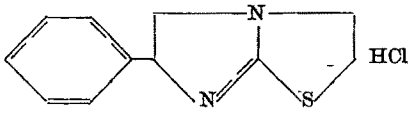
The major metabolite of (II) was isolated from the faeces, and tentatively identified as the imidazothiazole-derivative (III) (*R* 8025). Its structure (III) was confirmed by chemical synthesis and found to be fully active

in removing *Heterakis*, *Ascaridia* and *Capillaria* in the chicken as well as a variety of gastrointestinal and pulmonary nematodes in sheep—and at significantly lower dose-levels than its parent compound (II).

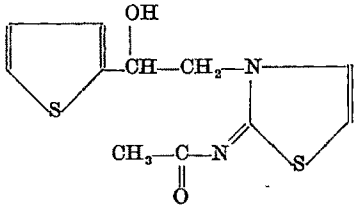
An extensive series of chemically related compounds was later synthesized and screened for anthelmintic, pharmacological and toxicological activity.

As a result of these investigations tetramisole (I) (*R* 8299), the white, stable and water-soluble (21 per cent at 20° C) crystalline hydrochloride of 2,3,5,6-tetrahydro-6-phenyl-imidazo[2,1-*b*]thiazole, emerged as the most promising substance for detailed investigation as a potential anthelmintic.

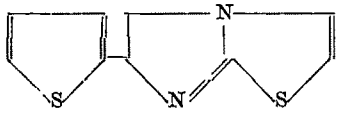
These investigations showed tetramisole (I) to be active at low, atoxic dose-levels against adult and immature gastrointestinal and pulmonary nematodes tested in this laboratory (Table 1), both by the oral route (drench, capsule, in drinking-water or in feed) and by subcutaneous, intramuscular or intraperitoneal injection.



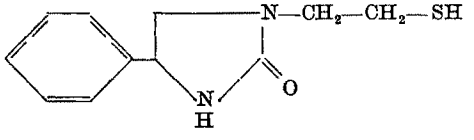
(I)



(II)



(III)



(IV)

(I) *R* 8299, tetramisole, $C_{11}H_{12}N_2S.HCl$, m.p. 264° C. 2,3,5,6-tetrahydro-6-phenyl-imidazo[2,1-*b*]thiazole hydrochloride.

(II) *R* 6438, thiazothienol, $C_{11}H_{12}N_2O_2S_2$, m.p. 133° C, 2-(acetylimino)-3-[2-hydroxy-2-(2-thienyl)-ethyl]-thiazoline.

(III) *R* 8025, $C_9H_8N_2S_2$, m.p. 62° C, 5,6-dihydro-6-(2-thienyl)-imidazo[2, 1-*b*]thiazole.

(IV) *R* 9280, $C_{11}H_{14}N_2OS$, m.p. 88° C, 2-oxo-3-(2-mercaptoethyl)-5-phenyl-imidazolidine.

Depending on the worm species and on the type of host, the optimal therapeutic dose of tetramisole varies from 2 to 40 mg per kg body-weight, orally or parenterally, most nematodes being removed with one single dose. (In this article all doses of tetramisole are expressed in mg of the base per kg body-weight. One mg of the base is the equivalent of 1·18 mg of the hydrochloride salt.)

Low concentrations of tetramisole exert a rapid paralysing action *in vitro* on a variety of nematodes such as *Heterakis*, *Ascaridia*, trichostrongylids, *Trichuris*, *Chabertia* and *Bunostomum*.

Critical tests and large-scale field experiments in chickens have shown that a single oral dose of 40 mg tetramisole per kg body-weight (in capsule, drinking-water or feed) expels 97–100 per cent of all immature and adult forms of *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria obsignata* within 24 h. No undesirable effects were observed after oral administration of up to 640 mg/kg. There is no depression of egg production at therapeutic dose-levels. Full activity against the three common nematodes in the chicken has not yet been described with any other drug.

Critical tests in naturally infected lambs and sheep showed tetramisole (5–20 mg/kg orally, subcutaneously or intramuscularly) to be fully effective in a large majority of the animals against adult and immature forms of *Trichostrongylus*, *Ostertagia*, *Cooperia*, *Haemoncus*, *Nematodirus*, *Strongyloides*, *Oesophagostomum*, *Bunostomum*, *Chabertia*, *Trichuris* and *Dictyocaulus*. The bulk of the

Table 1. GASTROINTESTINAL AND PULMONARY NEMATODES AGAINST WHICH TETRAMISOLE (*R* 8299) IS ACTIVE

	Authority†
(1) Chicken:	
Ascaridia galli (Schrank, 1788)	2,4,9,*
Heterakis gallinarum (Schrank, 1788)	2,*
Capillaria obsignata (Madsen, 1945)	2,4,*
(2) Sheep:	
Trichostrongylus spp	3,4,6,8,9,*
Ostertagia spp	3,4,6,8,9,*
Haemoncus contortus (Rud, 1809)	1,3,4,6,8,9,*
Cooperia spp	3,6,*
Nematodirus spp	3,6,8,9,*
Strongyloides papillosus (Wedl, 1856)	1,3,6,8,*
Oesophagostomum spp	1,3,4,6,*
Chabertia ovina (Fabricius, 1794)	3,6,*
Bunostomum sp	3,6,8,*
Gargeria pachyscelis (Railliet et Henry, 1910)	1
Trichuris ovis (Abildg, 1795)	1,3,6,8,*
Dictyocaulus filaria (Rud, 1809)	3,6,*
Protostrongylus rufescens (Leuckart, 1865)	6
(3) Cattle:	
Trichostrongylus spp	3,9,*
Ostertagia spp	3,4,9,*
Haemoncus contortus (Rud, 1809)	1,3,9
Cooperia spp	1,3,4,9,*
Nematodirus spp	3,9,*
Bosicola (Oesophagostomum) radiatum (Rud, 1809)	1,9
Bunostomum phlebotomum (Railliet, 1900)	1,9
Trichuris sp	3,9,*
Dictyocaulus viviparus (Bloch, 1782)	3,4,7,*
(4) Dog:	
Toxocara canis (Werner, 1782)	3,4,9,*
Toxascaris leonina (Linstow, 1902)	3,4,9,*
Ancylostoma caninum (Ercolani, 1859)	1,4
Uncinaria stenocephala (Railliet, 1884)	4,*
Trichuris vulpis (Froel, 1789)	3,*
(5) Pig:	
Ascaris suum (Goeze, 1782)	3,6,9,*
Oesophagostomum dentatum (Rud, 1803)	3,6,*
Hyostrongylus rubidus (Hassall et Stiles, 1892)	3
Trichuris sp	3,*
Metastrongylus spp	3,9
(6) Horse:	
Parascaris equorum (Goeze, 1782)	3,*
Oxyuris equi (Schrank, 1788)	3,*
Strongylus vulgaris (Railliet, 1923)	3
Trichonema sp	3,*
Dictyocaulus arnfieldi (Cobbold, 1884)	3
(7) Cat, tiger:	
Toxocara mystax (Zeder, 1800)	*
(8) Pigeon:	
Ascaridia columbae (Gmell, 1790)	*
Capillaria obsignata (Madsen, 1945)	*
(9) Duck:	
Syngamus bronchialis (Mühl, 1884)	*
(10) Peacock, pheasant:	
Heterakis sp	*
Pseudoaspidodera sp	*
Capillaria sp	*
(11) Rat:	
Syphacia obvelata (Rud, 1802)	*
Nippostrongylus muris (Travassos, 1914)	4
Strongyloides ratti (Sandground, 1925)	4
Trichosomoides crassicauda (Bellingham, 1840)	*
(12) Mouse:	
Syphacia obvelata (Rud, 1802)	4
Nippostrongylus muris (Travassos, 1914)	1
Aspiculuris tetraptera (Nitzsch, 1821)	4
Nematospiroides dubius (Baylis, 1926)	1,4
Trichinella spiralis (Owen, 1835)	4
(13) Man:	
Ascaris lumbricoides (Linnaeus, 1758)	5
Enterobius vermicularis (Lin, 1758)	5

* Data from this laboratory.

† Information communicated by: 1, Benazet, F., Graber, M. 2, Bruynooghe, D. 3, Davey, D. G., Forsyth, B. A., Froyd, G., and Walley, J. K. 4, Goennert, R., Federmann, M., and Meister, G. 5, Martins Campos J, V., and Viterito, I. A. 6, Nilsson, O. 7 Pouplard, L 8, Roveda, R. J , and Galofré, E. J 9, Waletzky, E.

gastrointestinal worm burden is expelled within the first 24 h and most of the lungworms within the first 12 h after administration.

Within the first 6 h after treatment, the most rapidly expelled worms, for example *Dictyocaulus*, *Chabertia* and *Bunostomum*, are often detected alive, whereas nematodes expelled later are dead and partially decomposed. No side-effects or evidence of local irritation were observed after administration of therapeutic doses of up to 40 mg/kg orally or up to 10 mg/kg parenterally. With higher doses (40 mg/kg subcutaneously or 80 mg/kg orally) transient side-effects, including hyperpnoea, salivation and lacrimation, occurred in a few animals and subsided within 1 or 2 h. Clonic seizures and mortality were observed with much higher doses. Therapeutic doses were well tolerated in pregnancy. There were no abortions or malformations.

Very similar results were obtained in cattle, tetramisole showing high activity against all gastrointestinal and pulmonary nematodes tested, that is, *Trichostrongylus*, *Ostertagia*, *Cooperia*, *Nematodirus*, *Trichuris* and *Dictyocaulus viviparus*. There were no undesirable effects with a single dose of 5 or 10 mg/kg orally or parenterally.

In mongrel dogs and in beagles, a single oral dose (capsule) of 20 mg/kg of tetramisole was fully effective against *Toxascaris* and *Toxocara*, highly active against *Uncinaria* and marginally active against *Trichuris*. A 3 per cent incidence of vomiting was noted at this dose-level. With 40 mg/kg orally or 20 mg/kg subcutaneously emesis occurred in about half of the animals. Administration of 20 mg/kg daily during 8 weeks failed to produce evidence of toxicity in beagles.

The anthelmintic spectrum of tetramisole seems to be limited to the various species of nematodes. No activity was detected against cestodes (for example *Moniezia* in sheep) or trematodes (for example *Fasciola hepatica* in sheep).

Tetramisole was also inactive against a series of fungi *in vitro* (*Mucor* sp, *Sporotrichum schenckii*, *Phialophora verrucosum*, *Saprolegnia* sp, *Aspergillus fumigatus*, *Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans*, *Microsporum canis*, *Trichophyton mentagrophites* and *Trichophyton rubrum*). The compound was equally inactive *in vivo* against *Microsporum canis* and *Trichophyton mentagrophites* (guinea-pig). Tetramisole is devoid of antibacterial activity *in vivo* (*Streptococcus pyogenes*, *Erysipelothrix rhusiopathiae*, *Salmonella pullorum gallinarum*). *In vivo* no activity was observed against *Trichomonas vaginalis* (mice), *Trichomonas columbae* (pigeons) and *Eimeria* infections (sheep, chicken).

Tetramisole is furthermore devoid of antihistaminic, anticholinergic, adrenolytic or any other classical pharmacological property.

In laboratory animals, clonic seizures are observed at sub-lethal and lethal dose-levels. The following acute $L.D._{50}$'s (in mg/kg; $P < 0{\cdot}05$ confidence limits) were obtained by probit analysis :

Route	Albino mouse	Wistar rat
Intravenous	22 (16– 29)	24 (19–29)
Subcutaneous	84 (58–123)	130 (94–181)
Oral	210 (156–284)	480 (361–638)

The daily administration in feed of an average of 9 and 35 mg/kg of tetramisole for 120 consecutive days in Wistar rats of both sexes had no significant effect on food consumption, growth rate, a number of parameters of blood and urine biochemistry, haemograms, organ weights or tissue histology. Depression of food consumption and of growth rate was seen at a daily dose-level of 140 mg/kg.

In alkaline conditions, tetramisole slowly hydrolyses to form 2-oxo-3-(2-mercaptoethyl)-5-phenyl-imidazolidine (IV), a water-insoluble neutral crystalline solid. (IV) also appears to be one of the major metabolites of tetramisole in several species. It is devoid of anthelmintic activity and almost atoxic.

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AUTORADIOGRAPHIC EVIDENCE SUGGESTING *in vivo* TRANSFORMATION OF SOME BLOOD MONONUCLEARS IN REPAIR AND FIBROSIS

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MAXIMOW'S well-known views on the pluripotentiality of lymphocytes scarcely require documentation here[1]. Until 3 or 4 years ago this opinion of Maximow's was held by few pathologists[2–5] and discredited by many[6,7]. However, in recent years, many investigators have established, beyond reasonable doubt, that, at least *in vitro*, some lymphocytes can be stimulated either by phytohaemagglutinin (PHA) or by suitable antigens, to transform into large and possibly pluripotential-looking 'blast' cells which may later mitose[8]. Moreover, Hall and Morris[9] have described apparently similar cells in efferent ducts from lymph nodes stimulated antigenically *in vivo*, and even in afferent lymph draining peripheral tissues into which antigens had been injected. This latter finding, and the increase in lymph-borne antibody associated with the appearance of such 'blast-like' and later also 'plasma-like' cells in lymph[9] leave little doubt that activation, transformation and mitosis of small lymphocytes (and possibly of other cells, too) is a regular response of these cells *in vivo* as well, at least after antigenic stimulation.

However, the finding of these notable changes in lymphocytes by immunologists seems to have led to the impression that such activation and transformation of lymphocytes is essentially and solely to be found in immune responses. Maximow's broader claims for the potentialities of lymphocytes have still to be substantiated by modern objective methods—as opposed to the more usual subjective cytodiagnostic evidence pressed forward to Maximow's support by many investigators—including one of us[2].

However, almost a decade ago, a careful re-examination of the histogenesis of healing wounds in man and animals[10] led one of us to conclude that: ". . . All the available evidence . . . indicates that the new fibroblasts, which develop during the healing of connective tissues in cutaneous wounds, are derived primarily from cells which have migrated from the circulation, or which have been derived from the peripheral tissue surrounding blood vessels and the *residual* portions of the stratum papillari of the dermis about hair follicles . . . ". This view was, in general, later confirmed by Grillo[11] using tracer techniques.

As part of a series of investigations into the factors determining and regulating wound healing and fibrogenic processes in general, it was thought desirable to examin-

once more, and with modern techniques, the timing of the cellular changes preceding the onset of fibrogenesis in wounds and around implanted foreign bodies.

Several authors[11,12] have, in recent years, reported that when cells were labelled with tritiated thymidine, injected just prior to killing their previously wounded animals, the isotope was taken up first in cells cuffing small vessels in sites of injuries. It is around these vessels, too, that, in healing wounds, mitotic figures have often been reported to appear first[13]. Such mitoses have been stated to occur in perithelial cells or local fibrocytes but not in 'round cells'[11,12]. No significance and certainly no subsequent fibroblastic role were attributed by these investigators to the numerous 'round cells' (lymphocytes and monocytes) generally recognized to appear regularly during one phase of the inflammatory reaction which consistently precedes fibrogenesis in healing wounds or around foreign bodies.

Recently, Volkman and Gowans[14,15] reported that monocytic cells, named macrophages by them (whether or not they contained ingested material—see Gillman and Gillman[2] for criteria for naming 'macrophages' and other connective tissue cells), were present in large numbers 24 h after making Rebuck-type skin windows. These authors clearly demonstrated that a far greater percentage of mononuclears were labelled if the isotope was injected 18 to 24 h before making the 'windows' and that very few cells were labelled 24 h post-operatively if rats were injected only post-operatively. They concluded that these labelled 'macrophages' were derived from circulating monocytes which, they claimed, originated from cells previously labelled in bone marrow and which were then transported by the blood to the skin windows.

We have recently confirmed Volkman and Gowans's findings that a far greater number of round cells (lymphocytes and monocytes), which accumulate in sites of injury, carry label if isotope is injected 18 h prior to inflicting the injury than if the isotope is given only post-operatively (see Fig. 1 and legends to Figs. 2 and 3).

However, Volkman and Gowans did not follow, beyond 24 h post-operatively, the fate of the many labelled blood-derived mononuclears which appear perivascularly in injured tissues within a day or two of trauma. Their observations were confined to the first 24 h after preparing skin windows in rats that had received tritiated thymidine at various times pre- and post-operatively.

Our experiments were designed to determine what relationship, if any, exists between injury, the invariable post-traumatic inflammation, and the subsequent genesis of new fibrous tissue—a subject which, in our view, has hitherto received too little attention. This aspect of pathology seems to be of fundamental importance for our understanding, in any tissue or organ, of both inflammation in general and of fibrogenesis in particular. For, while acute inflammatory processes often resolve, those following trauma may, and frequently do, herald the onset of repair and thus often precede fibrogenesis (see Gillman, 1963, for distinction between repair and regeneration)[16]. Thus, post-traumatic inflammation is very commonly followed by the early supervention of features generally described as 'sub-acute' or 'chronic' inflammation and characterized by fibroplasia.

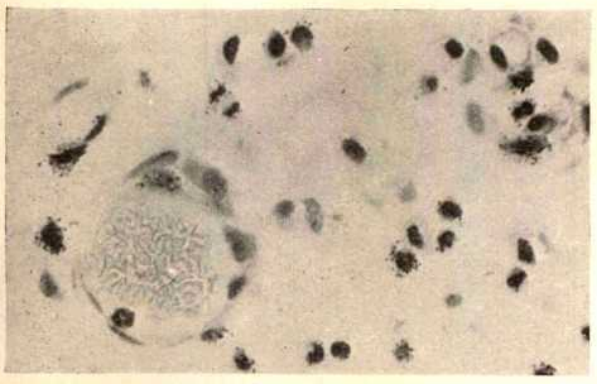


Fig. 1. Autoradiograph of dilated vessels at base of a full thickness excised wound in a pre-operatively labelled rat 24 h after wounding, showing distribution of labelled extravascular round cells, presumably blood-borne mononuclears that have emigrated from dilated venules. One of these labelled round cells, well away from the perithelium, is in mitosis. Very few equivalent round cells in post-operatively labelled rats contain label at this time. Haematoxylin and eosin counterstain on autoradiograph. Exposed for 61 days (×*c.* 550)

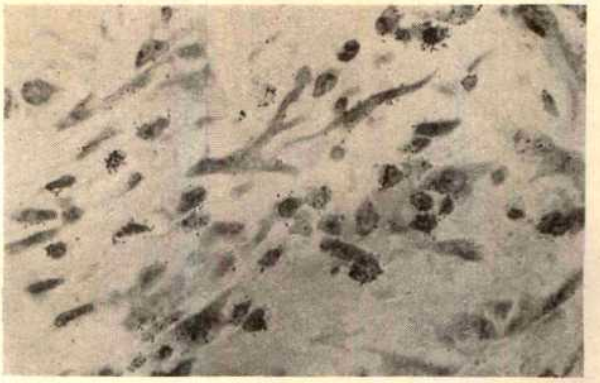


Fig. 2. Autoradiograph of granulations in 3-day-old wound from a pre-operatively labelled rat. Note here numbers of labelled mononuclears and, near top centre, two clearly labelled fibroblastic-like cells, one spindle-shaped and one stellate. Stained with haematoxylin and eosin, and exposed for 61 days (×*c.* 550)

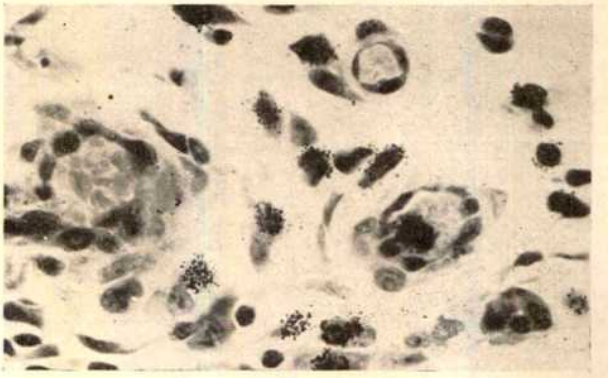


Fig. 3. Autoradiograph of base of granulations in 3-day-old wound from post-operatively labelled rat. Note location and morphology of heavily labelled cells—some clearly mononuclears and others (top) stubby and spindle-shaped. Compare distribution and morphology of labelled cells here with those in Figs. 1 and 2. Perithelial cells are not notably labelled. Stained with haematoxylin and eosin and exposed for 61 days (×*c.* 550)

As part of our efforts to elucidate what determines the invariable transition of inflammation to fibrogenesis, in healing wounds and around foreign bodies, we conducted two sets of experiments. In both types of experiments tritiated thymidine was used as the tracer because: (*a*) it is incorporated into nuclear DNA just before mitosis; (*b*) it has, therefore, been taken generally (and we think in some situations incorrectly) to be an excellent indicator of cells about to enter mitosis; (*c*) being intranuclear, it is a 'tight' label; re-utilization of the isotopic thymidine for DNA synthesis being, at most, very slight and always very slow. In both types of experiments 1 cm diameter excised, full thickness wounds were made in the back skin (through panniculus carnosus to deep fascia) and, in each of the same rats, small polyvinyl sponges and 'Millipore' filters were implanted in a distant site, subcutaneously and/or intraperitoneally.

The experiments differed in one important detail. In one series of rats the tritiated thymidine was injected pre-operatively, intravenously, either once, 18 h, or twice,

namely 18 and 5 h pre-operatively. The rats received no further isotope post-operatively and were killed at intervals from 1 to 25 days after the operations. It was hoped that such pre-operative labelling would provide some indications of the types of cells appearing at different times after injury, the movements, and also the ultimate fate of those cells which must have been labelled themselves, or were descendants of cells labelled before inflammatory and fibrogenic changes had been initiated.

The second group of previously unlabelled rats were operated on and then only one intravenous injection of the same dose of isotope (1 μc./g body-weight) was given post-operatively just 2 h before killing the rats. Thus, in this second group of rats, labelling was post-operative, being confined to the 2 h before death, because it is widely accepted that such 'flash' or 'pulse' labelling with thymidine would mark only those cells which had just synthesized new DNA immediately before dividing. However, as will emerge later, it is reasonable to expect some label also in the daughter cells derived from progenitors that had utilized the tritiated thymidine before mitosis and which, by subsequent mitosis, had given rise, just before death, to two very new, albeit less heavily labelled descendants.

Imprint or touch preparations were made of portions of the granulation tissue which formed in the excised wounds, and of the sponges, filters, spleen, lymph nodes, and bone marrow of all experimental animals. These imprints were made, on specially cleaned slides, according to the method previously described[2]. The slides were fixed in C.P. methanol for at least 24 h (to eliminate the possibility of autochemograms) covered with '*NTB* 2' emulsion by dipping and, after exposure for periods varying from 14 to 114 days, were developed and stained by the Giemsa method fully described by Speirs[17] with minor modifications. Autoradiographs were similarly prepared of serial paraffin sections of all the aforementioned tissues, the sections being stained through the emulsion with haematoxylin and eosin after developing the autoradiographs.

In rats injected with isotope only post-operatively, that is, 2 h before killing, notable labelling of cells in the lesions studied was first seen only between 24 and 48 h after operation. By the third day label was most obvious in lymphocytes and monocytes cuffing small venules in sites of injury and in some of the endothelial cells of these same vessels. However, at this time, too, labelled mononuclears were occasionally seen even within the lumina of arterioles or venules in the injured areas (Fig. 3). This is not totally unexpected since Volkman and Gowans, and we too, have found that 0·5 to, at the most, 1 per cent of blood mononuclears are labelled even within 1–2 h after such isotope injections. This finding is important, however, since it strongly suggests that the labelled perivascular cells, shown in Fig. 3, may well be a mixed population comprising local cells entering mitosis but also of already labelled emigrated blood-borne cells—recent arrivals on the scene. However, the number of labelled round cells in injured areas, in post-operatively labelled rats, became very much greater between the third and fourth post-operative days, but comprised maximally only about 15 per cent–20 per cent of all cells in imprints or sections. Thus, in animals killed 3–7 days post-operatively, but injected only 2 h before death, large numbers of cells were labelled—including, in addition to those already mentioned, spindle-shaped fibroblast- and fibrocyte-like cells (Figs. 3, 5 and 7). These results would seem to confirm, in general, those of MacDonald[12] and of Grillo[11], who, however, considered the majority of their labelled fibroblasts to be locally derived.

In contrast, rats injected with isotope once, or better twice, pre-operatively only, when examined even at 24 h post-operatively, manifested label in 40–80 per cent of perivascular round cells within and/or around the wounds, sponges or filters (Fig. 1). It was easily possible, on Giemsa-stained imprints, to compare these cells with those in the smears of blood and haemapoietic organs from the same animals, and hence, in the initial stages of the inflammatory reaction, to classify them, on haematological criteria, as lymphocytes or monocytes. Very occasionally on the first day (Fig. 1), but more consistently and frequently by the second and third days post-operatively (and, incidentally, therefore, more than 2–3 days after the last dose of isotope) these labelled mononuclears, well removed from perithelium, could be seen to be in mitosis (Fig. 1) and to acquire morphological features seemingly indistinguishable from those described as typical of small lymphocytes activated *in vitro* (see foregoing).

It might be added, in passing, that a small but regular percentage of cells, generally acceptable on morphological grounds to be fibroblasts and fibrocytes, with label in their nuclei, were consistently identified in these rats killed 3, 7 or even up to 14 days after the last dose of pre-operatively injected label (Figs. 2, 4 and 6). The same applied also to the multinucleated giant cells[18].

Since the round cells, in the one group of rats, had obviously been labelled well before the inflammatory and fibrogenic processes were initiated, they were clearly brought to the injured tissues by the blood. Emigration of blood mononuclears and granulocytes is, of course, an accepted aspect of inflammation. Such pre-operatively labelled mononuclears, or their descendants, appeared to arrive in cohorts, each at increasing times after isotope injections and subsequent wounding, and, therefore, with the expected progressively decreasing amounts of nuclear label resulting from repeated 'dilution' in their daughter cells after mitosis (compare Figs. 1, 2 and 4). Thus, we have found the bone marrow to be virtually depleted of

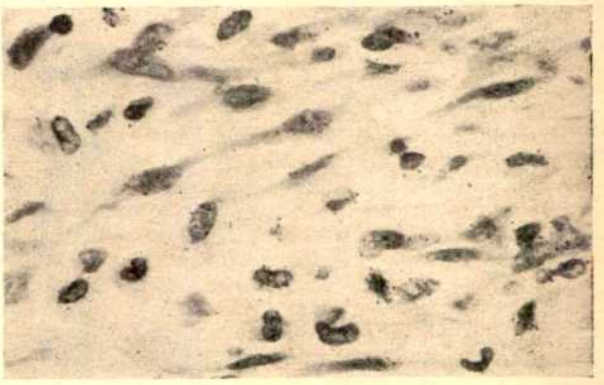


Fig. 4. Fibroblast-like and mononuclear cells in base of granulations in 7-day-old wound in pre-operatively labelled rat. Label intensity is less than at earlier times presumably due to 'dilution' of label by repeated mitosis of cells labelled pre-operatively, that is, 7 days and 5 h previously. Stained with haematoxylin and eosin, and exposed for 61 days (×*c.* 550)



Fig. 5. Labelled spindle-shaped cells, taken to be fibroblasts, at surface of granulations immediately below new epithelium (seen, in part, at top). At this stage, 7-day-old wounds, labelled fibroblasts are predominantly near surface in post-operatively labelled animals and near base after pre-operative labelling. Stained with haematoxylin and eosin, and exposed for 61 days (×*c.* 550)

labelled nuclei by the sixth or eighth day after the last dose of isotope—a finding in conformity with Volkman and Gowans's results. It could, therefore, not be expected that the ultimate labelling of 7-day fibroblasts in pre-operatively labelled rats would be as intense as in rats injected with isotope only 2 h before death.

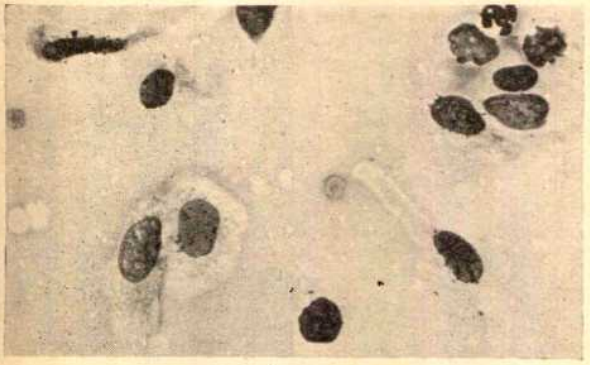


Fig. 6. Two fields from an imprint preparation of the capsule of a 7-day-old subcutaneous sponge from an animal last injected with label 7 days and 18 h previously. At left, top, a heavily labelled nucleus in a spindle-shaped fibrocyte with below, right, a lightly labelled large histiocytic cell with vesicular nucleus. At right, centre, another moderately labelled spindle-shaped fibrocyte with, above, a labelled mononuclear (? lymphocyte) nucleus. Differences in intensity of nuclear label attributed to mitotic 'dilution' of lighter labelled cells. Stained with Giemsa. Autoradiograph prepared by exposure for 28 days (× *c.* 550)

Many of these pre-operatively labelled round cells, after emigrating into sites of injury, became phagocytic; many apparently moved away from the inflammatory sites—both acceptable findings confirming recent observations[19]. But many also remained in the injured sites for days and even weeks (Fig. 5), and some of these clearly became transformed first into blast-like or mitosing cells (Fig. 1) and afterwards even into cells indistinguishable from fibroblasts and fibrocytes (Figs. 2, 4 and 6).

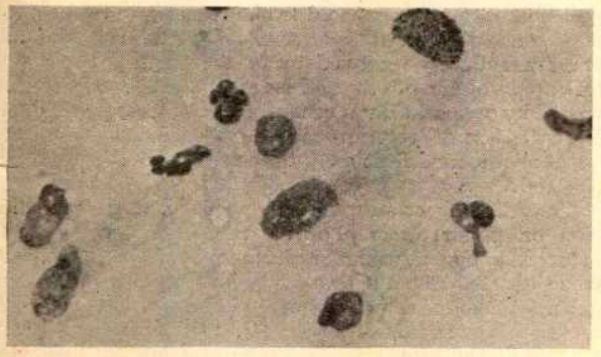


Fig. 7. Two heavily labelled fibroblasts (one top, one centre) from a 7-day-old wound of a post-operatively labelled rat. Compare with Figs. 4, 5 and 6. Since fibroblasts near the surface are most heavily labelled with post-operative tritiated thymidine injections (see Fig. 5) they come off readily in imprints. Not so with pre-operatively labelled rats in which basal fibroblasts, as in Fig. 4, are labelled and are surrounded by collagen. Stained with Giemsa and exposed for 42 days (× *c.* 550)

The connective tissues, in sites other than those specifically injured, were always sampled by us at the time of the operation and were found to be virtually unlabelled, except for a relatively small number of cells around hair follicles and occasionally in the panniculus carnosus. However, we are not concerned here with either of the latter since, in our excisions, both the entire dermis and the panniculus were resected, while implants were always deep to the panniculus carnosus.

It is clear, therefore, that some of the blood mononuclears, which are consistently borne by the blood to injured sites, 24–48 h after operation, remain in the zones of fibrogenesis and apparently later undergo notable morphological changes. That these cells also prepare to mitose soon after they arrive is indicated, at 48–72 h post-operatively, by the frequent and notable uptake of label when tritiated thymidine is first given only shortly before death (compare Figs. 1 and 3).

This seems to be strong evidence to show that, after their emigration, activation of circulating blood mononuclears and their transformation into blast-like and mitosing cells are common in the acute inflammation which regularly precedes fibrogenesis in wounds and around foreign bodies. Of course, it is quite possible that such transformation, in tissues, of emigrated blood monocytes (apparently primarily) and perhaps also of blood lymphocytes, may be in response to antigen inadvertently introduced with the foreign bodies at the time of operation, or derived from damaged tissues. This possibility cannot be overlooked in view of Hall and Morris's[9] findings already noted. However, the persistence, in fibrosing areas, of pre-operatively labelled blood mononuclears, and the subsequent frequency of labelled fibrocyte-like cells, to be described in detail in future investigations, leaves little doubt that activation and transformation, extravascularly, of emigrated blood mononuclears, that is, monocytes and/or lymphocytes, is an integral aspect of fibrogenesis and not solely the prerogative of uncomplicated immunological responses.

These findings, therefore, lead us to suggest that blood-borne mononuclear cells can become activated *in vivo* and undergo morphological changes, similar to lymphocytes *in vitro*, at least in sites of fibrogenesis. It is clearly shown that a population of cells can be labelled pre-operatively, somewhere in the body (according to Volkman and Gowans in bone marrow), which afterwards becomes borne by the blood to sites of injury. Once they have emigrated into the injured tissues these cells may: (1) become phagocytes and later leave the reaction sites; (2) remain in the area, become activated and mitose; (3) be found in the injury up to 14 days following the trauma (and 15 days after the last isotope injection) when some of them become morphologically indistinguishable from classical stellate fibroblasts or spindle-shaped mature fibrocytes; (4) fuse to form multi-nucleated foreign-body-type giant cells[18].

It should be added here that no unequivocal evidence emerged from our investigations of preferential labelling or mitotic activity in perithelial cells. Our evidence indicates that emigrating labelled blood mononuclears, which may mitose when still near the perithelium (Fig. 1) or which, while motile and hence elongated, resemble perithelium and become arranged concentrically around vessels (Fig. 3), have perhaps been mistaken by other workers to be mitosing perithelium.

The evidence here presented thus seems to provide objective support for Maximow's contention that at least one pluripotential cell does indeed exist in circulating blood. Whether this pluripotential blood-borne cell is the monocyte or lymphocyte (or both) is not established by our experiments; the findings of Volkman and Gowans at least seem to indicate that their monocytic cells come from bone marrow rather than from spleen or lymph nodes. Our results certainly do not indicate that fibroblasts are derived only from circulating mononuclears; the latter, however, do seem to give rise to a proportion of fibrocytes in sites of repair.

These findings suggest that blood-borne mononuclears may, in appropriate circumstances, 'switch on' either antibody or collagen synthesis. If so, these observations may have considerable implications for further investigations of repair and sclerosis of many organs and tissues and perhaps of the relationships between immune responses and fibrosis.

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In summary then it can be stated that:

(1) Using autoradiographs of serial sections and imprints of tissues, a comparison has been made of the localization, numbers, morphology and intensity of nuclear labelling of cells in healing excised wounds and implanted sponges or 'Millipore' filters in rats injected, intravenously, with tritiated thymidine either only pre-operatively or only immediately before killing on various days post-operatively.

(2) It has been shown that, during the first 2 days, a far greater number of labelled blood leucocytes appear in sites of injury and fibrosis in rats receiving isotope pre-operatively only. Pre-operatively labelled mononuclears (that is, lymphocytes and monocytes) or their descendants emigrate from the local vessels, migrate into the local fluid exudates, mitose and undergo transformations into macrophages and other cell forms.

(3) No evidence emerged to support the widely held views that perithelial cells play any significant part in fibrosis.

(4) Blood mononuclears, labelled pre-operatively only, or their descendants (less heavily labelled by virtue of isotope 'dilution' following mitosis) have been shown to be present in fibrosing sites even up to 14 days post-operatively. The frequent existence of cells, morphologically indistinguishable from fibroblasts and fibrocytes, with labelled nuclei, many days after the last isotope injection, taken with the localization, cytology, movements and apparent transformation of mononuclears labelled pre-operatively, is considered to indicate that at least some fibroblasts, in healing wounds and chronic inflammatory processes, may be derived from some blood-borne pluripotential cell(s).

(5) Activation, transformation and mitosis of blood mononuclears has, in recent years, been described and generally taken to be a primarily immunological reaction. The data reported here indicate that similar changes in blood mononuclears occur *in vivo* in those inflammatory responses which precede fibrosis. The implications of these findings for understanding the pathogenesis of 'subacute' and 'chronic' inflammation and the fibrogenesis related thereto, in many organs and tissues, are briefly discussed.

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FORMATION OF ADENOSINE TRIPHOSPHATE BY HUMAN ERYTHROCYTE GHOSTS

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It was previously reported from this laboratory[1,2] that small amounts of phosphorus-32 labelled adenosine triphosphate ($AT^{32}P$) were formed by human erythrocyte ghosts on incubation for a short time with radioactive orthophosphate ($^{32}P_i$). It has been suggested[3,4] that a glyceraldehydephosphate dehydrogenase–phosphoglycerate kinase enzyme complex may be the mediator of active phosphate transport into the human erythrocyte. This implies that the $AT^{32}P$ formation would be enhanced if ADP and other co-factors were added to the medium. In the present article, some experiments are described which indicate that the ATP is formed in ghosts in the phosphoglyceraldehyde dehydrogenase and phosphoglycerate kinase reactions.

A soluble fraction was obtained from red cell ghosts, which had been washed six times with water saturated with carbon dioxide, by extracting them six times with a 0·016 M *tris*-glycylglycine buffer, *p*H 8·2. The pooled extracts were purified by 'Sephadex' filtration[1,2] and used for the following experiments. The ghost residues remaining after extraction were also tested for their ability to form $AT^{32}P$. The soluble membrane fraction or ghost residues (ghosts) from 100 ml. to 150 ml. whole blood were incubated in 0·05 M *tris*-acetic acid buffer, *p*H 7·5, for different times with 2 μmoles of $^{32}P_i$ having a radioactivity of 2 mc. The final volume was 20 ml. Unless otherwise stated, the following substances were added in addition to $^{32}P_i$: 25 μmoles magnesium chloride, 10 μmoles nicotine amide, 10 μmoles cysteine, 5·9 μmoles glyceraldehyde 3 phosphoric acid (3-GAP), 3·5 μmoles nicotinamide-adenine dinucleotide (NAD), 35 μmoles of ADP and 200 μmoles of *tris*-acetic acid buffer, *p*H 7·5. All incubations were carried out at 37° C. The reaction was stopped by addition of 50 per cent cold trichloroacetic acid (w/v) to a final concentration of 5 per cent. The trichloroacetic acid was immediately removed by extracting five times with an equal volume of ethyl ether. The solution was concentrated by evaporation *in vacuo*, and chromatographed on a 'Dowex 1 × 2' column according to Hurlbert *et al.*[5], with slight modifications[6] to enable the $AT^{32}P$ to be isolated.

The $AT^{32}P$ fraction was further identified after purification on paper electrophoresis in 0·1 M pyridine-acetic acid, *p*H 5, by analysing for phosphorus[7], ribose[8], and base[9] constituents. Fig. 1 shows a typical 'Dowex 1' chromatogram of the trichloroacetic acid extract from a $^{32}P_i$-incubated membrane fraction (only the phosphorus compounds eluted after $^{32}P_i$ are shown). The ATP formed in the experiments was estimated radio-chemically by measuring the incorporation of $^{32}P_i$ into ATP and by analysing the total amount of phosphorus in the ATP fraction and finally, by measuring the ultra-violet absorbancy at 260 mμ. The molar absorbancy[10] was assumed to be $14·9 \times 10^3$. The nitrogen content of the membrane fraction, ghosts and haemolysate was determined by a micro-Kjeldahl method.

Evidence for the presence of the enzymes glyceraldehydephosphate dehydrogenase and phosphoglycerate kinase in erythrocyte ghosts was obtained in the following experiments. Ghosts were incubated with and without 3-GAP, in addition to the other substances in the afore mentioned medium. In a control experiment, ghosts were incubated with only $^{32}P_i$. $31·9 \times 10^{-5}$ μmoles of phosphate/mg nitrogen was incorporated into the AT

when 3-GAP was also present. When a similar incubation was performed without 3-GAP in the medium, $1{\cdot}2 \times 10^{-5}$ μmoles of phosphate/mg nitrogen was incorporated into the ATP. The corresponding figure for the control incubated with only $^{32}P_i$ was $0{\cdot}95 \times 10^{-5}$ μmoles/mg nitrogen. The addition of 3-GAP thus increased the synthesis of ATP twenty-six times.

For comparison, another series of experiments was performed with ghosts which had been washed six times with carbon dioxide–water and those which, in addition, had been extracted six times with the alkaline *tris*-glycylglycine buffer. Haemolysate, filtered through 'Sephadex *G* 25' to remove low molecular weight compounds, was also included. The haemolysate was concentrated by ultrafiltration to about a tenth of its original volume by the same technique as that used for concentrating the membrane fraction[1]. The ATP synthesized, expressed as the phosphate incorporation into ATP in μmoles of $^{32}P_i$/mg nitrogen, was lowest for the haemolysate (Table 1). The highest incorporating activity, however, was recorded in the haemolysate fraction.

Table 1. Incubation of Ghosts (see text) and Haemolysate

ATP synthesis expressed as the phosphate incorporation into ATP in μmoles $^{32}P_i \times 10^5$/mg nitrogen.

A	*B*	*C*
118	85	17

Total incorporation of phosphate into ATP in μmoles $^{32}P_i \times 10^5$.

A	*B*	*C*
2,124	1,235	4,930

Each of the three fractions corresponds to 15 ml. of packed red cells. Incubation time, 6 min. *A*, Ghosts only washed six times with carbon dioxide–water. *B*. Ghosts washed six times with carbon dioxide–water, as well as six times with the *tris* glycylglycine buffer. *C*, Haemolysate (after haemolysis 1 : 10) eluted through 'Sephadex *G* 25' with 0 05 M *tris*-acetic acid buffer, *p*H 7 5, and concentrated about ten times.

Table 2 shows the formation of ATP expressed as the incorporation of $^{32}P_i$ into ATP in μmoles of $^{32}P_i$/mg nitrogen. It is seen that the membrane fraction content of ATP increased progressively with time during the first 4 h, whereas in the ghosts there was a rise up to 6 min, followed by a decline. It is possible that this difference was due to the presence of ATPase in the ghosts[11] and its absence in membrane fraction[1,2].

de Verdier[12] has shown that there may be a rapid exchange of phosphate groups between inorganic phosphate and the terminal phosphate group of ATP in haemolysates of human red blood cells. The following experiments were performed to provide additional evidence of a *de novo*

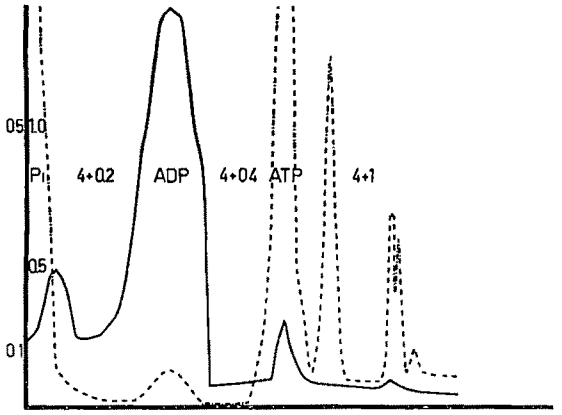


Fig. 1. 'Dowex 1' chromatogram of the trichloroacetic acid extract of the incubated membrane fraction. 20 ml. membrane fraction incubated for 4 min with 2 μmoles of $^{32}P_i$, 25 μmoles of magnesium chloride, 10 μmoles of nicotinamide, 10 μmoles of cysteine, 5 9 μmoles of 3-GAP, 3·5 μmoles of NAD, 35 μmoles of ADP and 200 μmoles of *tris*-acetic acid buffer, *p*H 7·5. The phosphorus compounds were eluted stepwise as follows: 4 N formic acid, 0·2 M ammonium formate + 4 N formic acid, 0 4 M ammonium formate + 4 N formic acid, 1 M ammonium formate + 4 N formic acid. ·····, Radioactivity; ———, ultra-violet absorbancy at 260 mμ. Numbers on the left of the *y* axis are c p m./ml. × 10^{-3} and those on the right the ultra-violet absorbancy at 260 mμ. The *x* axis represents the tube number

Table 2. Comparison between Membrane Fraction and Ghosts

	Membrane fraction	Ghosts
10 sec	2·0	13·7
30 sec	1·0	10 5
1 min	1·15	31·9
3 min	11·8	35·4
6 min	25·0	85·0
8 min		52·6
30 min		23·3
1 h	33·3	
4 h	92·3	
16 h	91·9	

Membrane fraction and ghosts incubated for various times with orthophosphate and co-factors (see text). ATP synthesized, expressed as the phosphate incorporation into ATP in μmoles $^{32}P_i \times 10^5$ mg nitrogen

synthesis of ATP in red cell ghosts. 80 ml. of the membrane fraction was incubated with 8 μmoles of $^{32}P_i$, 100 μmoles of magnesium chloride, 40 μmoles of nicotinamide, 40 μmoles of cysteine, 23·6 μmoles of 3-GAP, 14 μmoles of NAD, 158 μmoles of ADP and 1,000 μmoles of *tris*-acetic acid buffer, *p*H 7·5. After 4 min, 20 ml. of the fraction was removed and precipitated at once with trichloroacetic acid. At the same time, 2 μmoles of P_i was added to the remaining solution (60 ml.). The operation was repeated twice at 4-min intervals with the addition of 1·3 μmoles of P_i and 0·6 μmoles of P_i to the remaining membrane fraction in the first and second repetitions, respectively. The final 20-ml. portion was precipitated with trichloroacetic acid after a 4-min incubation period. The trichloroacetic acid extract from each of the four fractions was chromatographed on a 'Dowex 1' column as already described, and the tubes corresponding to the maximal values of the ATP fraction were selected for measurements from the recorded curves for ultra-violet and phosphate content. The incorporation of $^{32}P_i$ into the total ATP fractions was also measured, as well as the phosphate content. The figures in Table 3 demonstrate a *de novo* synthesis of ATP.

Table 3

	4 min	8 min	12 min	16 min
μmoles ATP/ml.	0·0032	0·0043	0·0057	0·0067
Total phosphorus, μmoles/ml.	0·010	0·016	0·018	0·022
Total phosphate incorporation into ATP fraction in μmoles $^{32}P_i$	0·023	0·033	0·0482	0 0612
Total phosphate content of ATP fraction in μmoles P_i	0 076	0·122	0 162	0·199

80 ml. of the membrane fraction was incubated with 8 μmoles of $^{32}P_i$, 100 μmoles of magnesium chloride, 40 μmoles of nicotinamide, 40 μmoles of cysteine, 23·6 μmoles of 3-GAP, 14 μmoles of NAD, 158 μmoles of ADP and 1,000 μmoles of *tris*-acetic acid, *p*H 7 5. Every 4 min, 20 ml. was removed and immediately precipitated in trichloroacetic acid. At the same intervals, unlabelled orthophosphate was added to the rest in an amount of 2·0, 1·3 and 0·6 μmoles, respectively.

The ultra-violet absorbancy and phosphate content were determined in the tube of the ATP fraction with maximum values.

These results agree with our view that human erythrocyte ghosts synthesize ATP. The mechanism by which ATP is formed in human erythrocyte ghosts is likely to be through oxidative phosphorylation on a substrate-level by way of the enzymes glyceraldehydephosphate dehydrogenase and phosphoglycerate kinase.

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PURIFICATION AND LABILITY OF HUMAN FOLLICLE-STIMULATING HORMONE

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THE starting materials for the present investigations were obtained from acetone-dried human pituitary powder by the method of Butt, Crooke and Cunningham[1]. Most of the luteinizing hormone (LH) was removed from the follicle-stimulating hormone (FSH) by absorption on a column of *CM*-cellulose. The first fraction (*CM* 1) eluted from this column with 0·01 M ammonium acetate at *p*H 6·1 contained the FSH. Two fractions, *CP* 1 and *CP* 2, were next obtained from *CM* 1 by elution from calcium phosphate with 1 mM disodium hydrogen phosphate and 0·02 M disodium hydrogen phosphate, respectively, without the previous recommended intermediate step of chromatography on DEAE-cellulose since it was found that this is better effected later using the DEAE-'Sephadex' equivalent. The calcium phosphate fractionation removed much inactive protein and some of the remaining LH.

The amounts of FSH in *CP* 1 and *CP* 2 were in the ratio of 100 : 13 and only 1 per cent of the total activity was lost in another fraction, *CP* 3, obtained by elution with 0·02 M trisodium phosphate. Much of the activity of *CP* 2 was recovered by passage down a column of 'Sephadex *G*-100' (36 × 3·5 cm) eluted with water. The fractions (5 ml.) were scanned for extinction at 280 mμ and showed two peaks with maxima at fractions 10 and 28. The FSH was mainly located in the pooled fractions 8–12 and 13–18, which were of equal potency. The total $E_{280m\mu}$ of these two fractions, 1·43 and 0·62, respectively, did not reflect the biological activities. Fractions 19–25 contained 37 per cent and fractions 26–32 only 17 per cent of the FSH activity of each of the first two fractions.

Passage of *CM* 1 down the same column confirmed that FSH was eluted just after the exclusion volume (fractions 11–17) and that the second ultra-violet peak contained little FSH.

Further purification of FSH was effected on a column of DEAE-'Sephadex' (34 × 3·5 cm) eluted in 5-ml. fractions with 0·005 M phosphate buffer, *p*H 7·0, with a salt gradient to 0·8 M sodium chloride. There was one major ultra-violet peak at 0·41 M sodium chloride (fraction 85) and several smaller ones. The LH activity was mainly confined to fractions 77–79 and FSH activity mainly to fractions 83–87. The FSH activity was not uniformly distributed about the ultra-violet peak but was eluted more in the earlier fractions.

Up to this point the fractions had been dialysed and the protein recovered by freeze-drying; however, the enrichment of FSH activity per mg was not progressing at the rate predicted from the yields of activity and the weights of the fractions, and furthermore the overall yields from a given fractionation were low. This was particularly so when the FSH fraction from DEAE-'Sephadex' was passed down a second column of DEAE-'Sephadex' and only 40 per cent of the FSH activity was recovered. Investigations showed that this could be directly attributed to a major loss of activity occurring during freeze-drying.

Fraction *CP* 1 passed down a column of DEAE-'Sephadex' retained complete activity when stored as eluted at −40° C for 20 h. Subsequent freeze-drying caused an overall loss of 42 per cent of the activity. The lability to freeze-drying became more pronounced on further purification. Thus, after passage of the FSH fraction from DEAE-'Sephadex' through 'Sephadex *G*-100' the FSH fraction remained completely active after dialysis at 4° C for 20 h, but subsequent freeze-drying caused an overall loss of 68 per cent. Freeze-drying without dialysis caused a 45 per cent loss of activity while after storage in solution at 4° C for 14 days about 78 per cent of the original activity was retained. Quantitative recovery of FSH activity in *CP* 1 and other fractions could be obtained by concentration at 4° C of the solutions in dialysis tubing under reduced pressure.

This lability of FSH has previously been noted[2–4]. The extreme lability of purified FSH suggested to us that protective proteins were being removed during fractionation and prompted us to look for the presence of albumin which is known to protect many enzymes from loss of activity during freeze-drying. Investigation showed that on a DEAE-'Sephadex' column, albumin, detected immunologically by the haemagglutination method, was

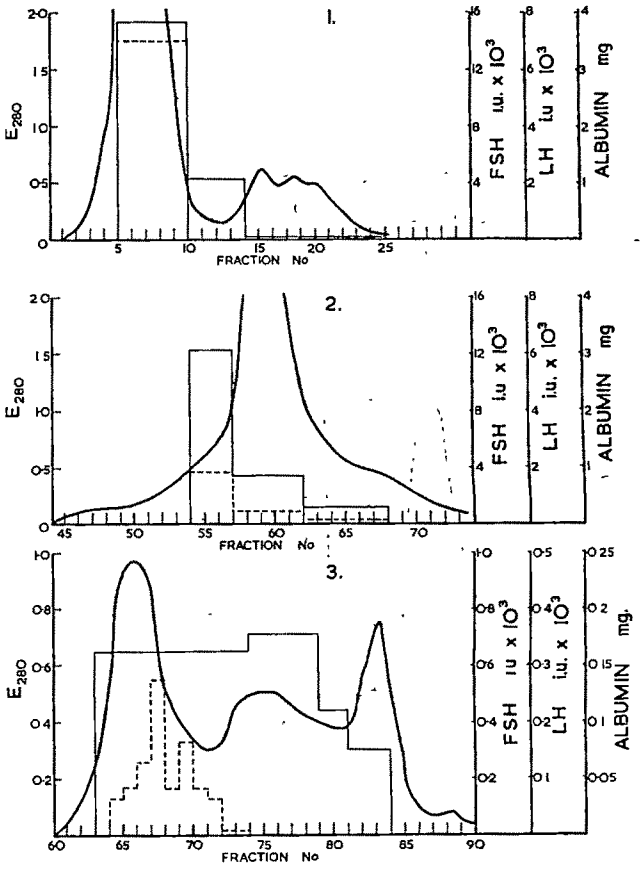


Fig. 1. Scheme for the purification of human FSH. Fraction *CP* 1 is passed down a column of 'Biogel *P*-150' (1) and FSH, LH and albumin are eluted mainly in fraction 5–9. This fraction is now applied to DEAE-'Sephadex' (2) and the eluate collected from fractions 57–61 is finally refractionated on a second column of DEAE-'Sephadex' (3). The FSH activity is eluted slowly over about twenty fractions, fractions 74–78 being virtually free of LH and albumin. ———, FSH; – – – –, LH; · · · · ·, albumin

indeed being removed since it was eluted after the major FSH fraction.

Our recommended procedure for preparing FSH free from LH and albumin is, therefore, an initial fractionation of *CP* 1 on 'Sephadex *G*-100' to remove inactive protein and subsequent fractionation and refractionation on DEAE-'Sephadex'. Recent work suggests that 'Biogel-150' may replace 'Sephadex *G*-100'. Two passages down DEAE-'Sephadex' are generally required to obtain the desired product. The latter columns particularly require to be closely scanned since the precise location of LH, FSH and albumin and the major ultra-violet absorption peak are somewhat variable, presumably due to formation of complexes between the various components. Recovery of activity on the columns as described here was 70–83 per cent.

A typical fractionation is shown in Fig. 1. Specific biological assays for FSH and LH demonstrated that the final product contained the equivalent of 1,000 (with 95 per cent fiducial limits of 730–1,430) mg first I.R.P.-human menopausal gonadotrophin (HMG) per ml. as FSH and less than 20 mg first I.R.P.-HMG per ml. as LH. (These results in I.U. are 143 (104–204) I.U. as FSH and <10 I.U. as LH.)

Purified FSH showed no loss of activity when compared with a control after incubation at 4° C for 2 weeks in 0·05 M cysteine, 0·05 M mercaptoethanol and 0·5 per cent serum albumin. Treatment with 0·05 M sodium nitrite in acetate buffer *p*H 5·0 for 43 h at 4° C caused 85 per cent loss of FSH activity, 64 per cent of which was recoverable after dialysis against 0·1 M mercaptoethanol (35 vol.) for 20 h, indicative of oxidation of –SH to –S–S– by sodium nitrite and reduction back to the original –SH groups by mercaptoethanol. Equal portions of FSH which had been dialysed for 24 h at 4° C against 8 M urea in acetate buffer *p*H 5·0 retained 50 per cent of the original activity, but this was completely lost by alkylation of the free SH groups with iodoacetamide (3·3 per cent; *p*H 7·0) for 4 h at 4° C. Treatment of FSH in formic acid (90 per cent; 1 ml.) at $-10°$ C for 2·5 h with performic acid (0·5 ml.) prepared from a mixture of formic acid (90 per cent; 9·5 ml.) and hydrogen peroxide (30 per cent w/v; 0·5 ml.) caused complete loss of activity, but some 50 per cent of the activity was lost on treatment with formic acid (90 per cent) alone at $-10°$ C for 5 h. FSH activity was found to be labile to acid and treatment with 0·2 M citrate buffer (*p*H 2·2) at 37° C for 25 h or with 0·05 M ascorbic acid (*p*H 2·0) at 4° C for 2 weeks caused complete loss of activity. This is probably associated with the cleavage of *N*-acetylneuraminic acid residues since treatment of FSH with *Vibrio cholerae* neuraminidase at *p*H 5·6 at 37° C for 2 h also caused complete loss of activity. No loss of activity was encountered on treatment of FSH with chymotrypsin at 37° C for 13 or 24 h in 0·2 M phosphate buffer (*p*H 7·0) and attack by trypsin was relatively slow. No loss of activity was encountered at 3–4 h incubation but after 16 h only 15 per cent activity remained. Using five times the concentration of trypsin some 50–60 per cent activity still remained after 8 h, but there was complete loss after 25 h. Pronase caused complete loss of activity after only 2 h incubation at *p*H 7·0 and 34° C and was by far the most destructive of the proteolytic enzymes tested.

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REGIONAL LOCALIZATION OF LYSERGIC ACID DIETHYLAMIDE IN MONKEY BRAIN

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THE hallucinogenic agent, *d*-lysergic acid diethylamide (LSD), profoundly alters integrative aspects of perception. Emotional functioning is also markedly affected and there is considerable evidence of central sympathetic discharge. On the other hand, the discrimination of primary modalities of sensation including touch, posture and kinaesthesia is not disturbed nor is voluntary movement impaired[1].

Neurophysiological investigations have revealed an impaired function in local regions of the brain after LSD administration. Thus, there is a highly specific depression of synaptic transmission in the lateral geniculate body of the cat[2]. In human subjects with subcortically implanted electrodes, LSD causes increased activation in the limbic system which is not present in simultaneously recorded neocortical leads[3].

The characteristic effects of LSD in man are produced after minute doses (less than 1 μg/kg) and persist for 8–12 h. Recently, it has been shown that after the administration of 2 μg/kg LSD intravenously to human subjects, the drug disappears slowly from plasma with a half-life of 3 h, and measurable quantities persist for 8 h (ref. 4). Moreover, there is an excellent correlation between the rate of disappearance of LSD from the plasma and the recovery of a normal performance on a simple arithmetic test. It would thus appear that impairment of mental functioning may be related to a continued presence of the unchanged drug.

In the present investigation we have examined the hypothesis that the effects of LSD on specific areas of mental functioning might be related to a capacity of the drug to selectively concentrate in specific regions of the brain. The regional localization of LSD in the brain of the squirrel monkey has been studied using a sensitive and specific fluorometric method for the measurement of unchanged LSD. The subcellular localization of LSD in several areas of the brain has also been examined.

Four adult squirrel monkeys (*Saimiri sciureus*) were used in these investigations. In one animal, which received 2 mg/kg LSD, the sub-cellular distribution of LSD in various regions of the brain was determined, while in the other three, which respectively received 2, 1 and 0·5 mg/kg of LSD, the regional distribution of LSD in the brain was investigated. LSD (Sandoz) was administered to the conscious animal by a slow intravenous injection over a 10-min period. The dose for each monkey was dissolved in 0·6 ml. saline. Twenty minutes after the end of the infusion the animal was killed by an intracardiac injection of air. The brain was perfused *in situ* with 250 ml. saline by way of the ascending aorta in order to remove as much blood as possible from the brain tissue. The brain was then removed and frozen

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at −25° C in an acetone–dry ice mixture after which it was cut coronally into 3-mm sections using a brain macrotome. Using the atlas of Gergen and MacLean[5], each section was dissected into discrete regions which were kept in ice until analysed.

The LSD content of the dissected areas of brain was determined by the spectrophotofluorometric technique of Axelrod *et al.*[6], which is specific for LSD. As little as 0·001 μg per ml. of LSD can be measured by this method. The mean difference of duplicate determinations was 7·8 ± 2·4 per cent of the values.

The subcellular localization of LSD in various areas of the brain was examined by a method which will be described in detail elsewhere[7]. Tissues were gently homogenized in 10 ml. 0·25 M sucrose with a 'Teflon' pestle. The homogenate was centrifuged at 53,000*g* for 1 h in a discontinuous sucrose gradient prepared by successively placing into a *SW* 25 lusteroid tube 10 ml. 1·17 M sucrose, 10 ml. 0·73 M sucrose, and 10 ml. of the brain homogenate. In this technique the pinched-off nerve endings (synaptosomes) and mitochondria are located at the interface of the 1·17 M and 0·73 M sucrose layers, and the microsomal–myelin layer appears at the interface of the 0·25 M and 0·73 M sucrose layers. These layers, as well as the supernatant fluid, were removed with a Pasteur pipette and assayed for LSD content.

LSD was unequally distributed in the different areas of the brain (Table 1). The highest concentrations of the drug were found in the pituitary and pineal glands. These concentrations were 7–8 times the amount found in the cortex. The anterior pituitary had approximately twice the concentration found in the posterior pituitary gland. Structures of the limbic system, that is, the hippocampus, amygdala, fornix, and septal region, contained 2–3 times the cortical concentration of LSD. Among the deep cerebral structures LSD was 2–5 times more concentrated in the visual and auditory areas and hypothalamus than in the cortex. The extrapyramidal system and thalamus contained approximately 1·5 times the cortical concentration. Among the superficial cerebral structures LSD was about equally distributed between white and grey matter. LSD was not notably concentrated in the visual cortex. The brain stem contained concentrations of LSD similar to that found in the cortex. In one experiment the blood concentration of LSD was determined just prior to killing the animal and was found to be the same as the cortical concentration. The cerebellum contained significantly less LSD than the cerebral cortex. In one animal the concentration of LSD in the iris was measured and found to be 18 times as high as that of the cortex.

Table 1. Localization of LSD in Different Areas of Monkey Brain

	Exp. 1	Exp. 2	Exp. 3	Mean ± *S.E.*
Superficial cerebral structures				
Frontal grey	1·00	1·00	1·00	1·00 ± 0
Frontal white	1·60	1·78	1·24	1·54 ± 0·16
Frontal grey and white	1·30	1·39	—	1·35 ± 0·05
Temporal grey	1·33	—	0·84	1·09 ± 0·25
Parietal grey	0·80	1·22	1·12	1·05 ± 0·13
Parietal white	0·65	0·83	1·18	0·89 ± 0·16
Corpus callosum	1·20	0·56	1·18	0·98 ± 0·21
Visual cortex	0·63	1·28	1·42	1·11 ± 0·24
Extrapyramidal system				
Caudate	1·22	1·39	1·98	1·53 ± 0·23
Putamen	1·47	1·39	1·40	1·42 ± 0·03
Globus pallidus	2·27	1·61	1·84	1·91 ± 0·19
Substantia nigra	1·73	1·44	—	1·59 ± 0·15
Visual and auditory reflex areas				
Optic chiasma	4·72	6·66	5·00	5·46 ± 0·61
Lateral geniculate	3·16	2·39	3·80	3·12 ± 0·41
Superior colliculus	3·17	2·66	3·42	3·08 ± 0·22
Periaqueductal grey	2·13	2·66	—	2·39 ± 0·26
Medial geniculate	—	2·61	4·30	3·45 ± 0·84
Inferior colliculus	1·86	2·39	2·80	2·35 ± 0·26
Hypothalamus				
Anterior	2·19	3·06	2·44	2·56 ± 0·26
Posterior	3·51	2·83	3·70	3·34 ± 0·26
Limbic system				
Amygdala	1·79	2·28	3·40	2·49 ± 0·48
Hippocampus	2·22	1·89	2·80	2·30 ± 0·26
Fornix	3·00	2·66	3·56	3·07 ± 0·26
Anterior commissure	3·95	3·44	—	3·70 ± 0·26
Septum	2·11	—	2·44	2·27 ± 0·16
Cerebellum				
Cortex	0·66	0·83	0·94	0·81 ± 0·09
Dentate nucleus	0·77	—	—	0·77
Pituitary gland				
Anterior	6·00	11·10	14·45	10·51 ± 2·45
Posterior	3·40	6·66	6·70	5·58 ± 1·09
Pineal gland	7·00	5·55	8·00	6·85 ± 0·71
Brain stem				
Midbrain	0·59	1·44	1·50	1·17 ± 0·29
Pons	0·73	1·44	1·00	1·05 ± 0·21
Medulla	0·91	1·28	0·94	1·04 ± 0·12
Thalamus				
Anterior	1·59	2·78	2·20	2·19 ± 0·34
Posterior	1·30	0·50	1·38	1·06 ± 0·28
Blood	—	—	1·06	
Iris	—	—	18·3	

The amounts of LSD administered were 2 mg/kg in exp. No. 1, 1 mg/kg in exp No. 2, and 0·5 mg/kg in exp. No 3. LSD concentrations are expressed as ratio to frontal grey concentration which was 0·94 μg/g in exp. No. 1, 0·80 μg/g in exp. No. 2 and 0·14 μg/g in exp No. 3.

Table 2. Comparison of LSD Content of Various Regions of the Brain

	Ratio to frontal grey* concentration	*P* value
Superficial cerebral structures	1·12 ± 0·03	—
Extrapyramidal system	1·69 ± 0·09†	< 0·001
Visual and auditory reflex areas	3·37 ± 0·26†	< 0·001
Hypothalamus	2·96 ± 0·06†	< 0·001
Thalamus	1·46 ± 0·10†	< 0·05
Limbic system	2·74 ± 0·15†	< 0·001
Pituitary gland	8·05 ± 1·75†	< 0·02
Pineal gland	6·85 ± 0·71†	< 0·005
Cerebellum	0·81 ± 0·09†	< 0·02
Brain stem	1·09 ± 0·19	> 0·8

* Mean ± *S.E.M.*
† Significantly different from superficial cerebral structures (Student's *t* test).

Table 3. Subcellular Distribution of LSD in Monkey Brain
Percentage of total LSD in subcellular fractions

	Supernatant	Microsomes-myelin	Synaptosomes-mitochondria
Hippocampus	75	20	5
Frontal grey	73	19	8
Frontal white	77	20	3
LSD added to homogenate	79	15	6

Sub-cellular fractions of the hippocampus, frontal grey matter, and frontal white matter were prepared as already described. In a separate experiment, LSD was added at 4° C to an intact brain, which was then homogenized and carried through the previously described isolation procedure. In the areas of the brain examined, LSD was largely confined to the soluble supernatant fluid (Table 3). The sub-cellular localization was the same when LSD was added to the homogenate. These results would indicate that LSD does not localize in a specific subcellular brain particle.

After intravenous administration of LSD, the drug is unequally distributed in the different areas of the brain of the monkey, with about a ten-fold variation between structures with the highest and lowest concentrations. These differences in concentration are probably not due to differences in regional blood flow to these areas since these investigations were performed 30 min after the start of the infusion. Although the initial distribution of LSD in the brain would be determined by the regional cerebral blood flow, after 30 min it might be expected that the various tissues would be approaching equilibrium with the blood levels of LSD and regional blood flow would no longer be the determining factor. This is supported by the fact that a comparison of regional blood flow values[8] and LSD concentration shows no significant correlation ($r = -0·03$; $P > 0·9$).

It is also unlikely that the unequal distribution of LSD is simply the result of its lipid solubility and consequent predilection for white matter. There was no apparent difference in the LSD content of cerebral cortex and white matter. Furthermore, LSD was highly concentrated in structures which are not especially rich in white matter such as the pituitary and hypothalamus.

Another factor which should be considered is the vascularity of different regions of the brain. However, since the maximum vascularity in the brain[9] is of the order of 3 per cent and since the blood concentration of LSD was the same as that in the cortex, the different blood content of various regions of the brain could not account for the

magnitude of the differences in regional LSD concentration found. Furthermore, attempts were made to wash the blood from the brain before the determinations were made.

In an investigation of the regional distribution of mescaline-^{14}C in the cat brain, Neff *et al.*[10] found that mescaline, like LSD, was present in high concentration in the pituitary and lateral and medial geniculate bodies. In contrast to the present results with LSD, high concentrations of mescaline were found after 30 min in the cerebrocortical grey matter while white matter contained only 25 per cent as much mescaline.

Arnold *et al.*[11] investigated the distribution of ^{14}C-LSD in the mouse brain by an autoradiographic technique. They also divided the brain into cerebrum, brain stem, medulla, and cerebellum, and determined the total radioactivity in these regions. It was found that cellular areas had higher activity than white matter and that these areas could be arranged in order of decreasing activity as follows: limbic lobe, hippocampus, basal ganglia, grey matter around the third ventricle, cerebral cortex and cerebellum. As the authors point out, these studies do not distinguish between LSD and its metabolites. However, in the regions they analysed, the order of decreasing activity is essentially the same as found in the present investigation.

LSD produces marked perceptual distortions. These effects appear to be the result of affectively determined perceptual alterations and hallucinations. There is, however, no marked impairment of discrimination of the primary modalities of sensation[1]. In the present investigation, there was no particular localization of LSD in the primary sensory areas of the cerebral cortex. The drug, however, was highly concentrated in subcortical visual centres, which have been implicated in the production of visual hallucinations[12]. LSD was also found in high concentrations in the limbic system and in the hypothalamus, areas which are thought to be involved in the mediation of affective behaviour[13]. Monroe *et al.*[3] found that the most significant response to LSD was the appearance of subcortical paroxysmal activity, which was most often limited to the septal, anterior hypothalamic, or amygdaloid-hippocampal regions. Dramatic behavioural changes were usually associated with the electroencephalographic changes in these areas. Certain mental effects of LSD have previously been shown to be related to persistent plasma levels of unchanged LSD[4]. It is possible that some of the effects of LSD on 'emotionality' and affectively determined perceptual alterations might be related to the selective concentration of this drug in areas thought to be involved in these functions.

LSD produces central sympathetic stimulation with effects which include hyperglycemia, mydriasis, pilo-erection, tachycardia, leucocytosis, and a rise in body temperature. These effects are related to the stimulation of mesencephalic and diencephalic structures[14], areas which selectively concentrate LSD.

The above evidence is merely suggestive and obviously no conclusions can be drawn from this investigation as to the mode of action of LSD in producing its characteristic effects. Furthermore, in order to obtain adequate tissue levels for fluorometric assay relatively high doses of LSD were administered in the present study. It is possible that with smaller doses the relative concentration of LSD in those areas which we found had high concentrations might be more pronounced.

In the investigations of subcellular distribution, the localization of LSD in the cell sap would suggest that LSD is not bound to subcellular particles. Arnold *et al.*[11], on the basis of their autoradiographic investigations, concluded that the activity was present in the cytoplasm and not in the nucleus.

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INCREASED RED CELL BREAKDOWN IN ADOPTIVELY IMMUNIZED YOUNG *NZB* MICE

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MICE of the *NZB* strain spontaneously develop haemolytic anaemia with autoantibodies against red cells[1]. Holmes, Gorrie and Burnet have described a positive direct Coombs's test (D.C.T.) in such mice, and have been able to detect this antibody in young mice adoptively immunized with spleen cells from older D.C.T. positive animals[2]. We recently described an antihuman red cell cross-reacting antibody (A.H.R.) in these mice[3], and now, using the spleen cell adoptive immunization technique, have been able to induce this antibody in young animals. Using red cells labelled with chromium-51, we have tried to relate the presence of these two antibodies to increased red cell breakdown which, in man, is an essential feature of autoimmune haemolytic anaemia.

Animals. The *NZB* mice were part of the colony kept in conventional animal housing at Taplow. Donors were selected on the basis of sex and their antibody status. Recipients, 4–6 weeks of age, were separated into three groups prior to the injection of spleen cells (Table 1): those receiving spleen cells from donors which were (*A*) A.H.R. + D.C.T. +, (*B*) A.H.R. − D.C.T. +, (*C*) A.H.R. − D.C.T. −. Each group contained both males and females, individual mice receiving cells from a donor of the same sex.

Spleen cell suspensions. In each group different numbers of donor animals were killed and their spleens taken after perfusion with saline. Spleen cell suspensions were prepared by gentle homogenization of individual spleens in a

Table 1. Groups of Young *NZB* Mice Receiving Spleen Cells from *NZB* Donors

Group	Donor antibody status	No. of recipients Male	Female
A	D.C.T. + A.H.R. +	14	8
B	D.C.T. + A.H.R. −	6	15
C	D.C.T. − A.H.R. −	12	11

D.C.T., direct Coombs's test (+ or −).
A.H.R., antibody against human red cell antigen present in serum (+ or −).

Potter mill and the number of viable cells in each spleen cell suspension was counted in trypan blue. The suspensions were adjusted to contain 33–37 × 10^6 viable cells per 0·1 ml. and inoculations of this amount were given intraperitoneally to the recipients. Female recipients of Group *B* all received cells from one considerably enlarged donor spleen. Group *C* recipients received cells pooled from more than one donor spleen.

Radiochromium red cell investigation. Red cells from normal young *NZB* were labelled with chromium-51 according to the method of Dacie and Lewis[4] using 5 μc. of $Na_2{}^{51}CrO_4$ per ml. of whole blood.

0·2 ml. of a labelled 50 per cent red cell suspension was injected intravenously into each recipient 8 days after the introduction of spleen cells. This interval was allowed since preliminary tests suggested that the likelihood of detecting autoantibodies in recipients was greater after 7 days. Pairs of recipients were killed at successive intervals (usually 2–3 days) between 10 and 36 days after injection of spleen cells.

Whole-body total radiation counts were performed after injection of the labelled cell suspension using a large well counter similar to that described by Warner and Oliver[5], but using a plastic scintillator giving a capacity of 13 × 14 cm with a 7-in. photomultiplier. This well was used in conjunction with an 'Echo' pulse height discriminator and scaler. 0·2 ml. of the original labelled cell suspension haemolysed with distilled water was counted and considered as a standard dose.

Blood radiochromium counts in recipient mice were estimated on 0·2 ml. blood which was haemolysed after withdrawal by cardiac puncture on the day of killing. Counts were performed in a standard 'Panax *LC*4' small well in conjunction with an 'Echo' scaler.

It was assumed that the whole-body radiation count immediately after the injection of labelled red cells was representative of the dose given. In order to compare the blood counts of individual animals, we thought it necessary to correct the counts to a unit dose, by using the formula:

$$\text{Corrected blood count} = \frac{\text{standard dose count}}{\text{whole body count}} \times \text{blood count}$$

Counts were also corrected to unit packed cell volume.

Haematological and antibody evaluation. Haematological values were determined according to the methods of Dacie and Lewis[4] and were limited to the estimation of haemoglobin, packed cell volume and reticulocyte count.

Antibody tests were performed on all the donors before and after they were killed, and on all the recipients on the day they were killed. Direct Coombs's tests were performed on the red cells, and the sera were examined for A.H.R. activity by the immunofluorescent method previously described[3], in which red cells present in the capillaries of human neonatal thyroid provide the substrate.

Occurrence of antibodies. The antibody status of the donors was confirmed in each case before killing. The presence of antibodies in the sera of the individual recipients at the time of killing them is recorded in Table 2. The uniformly negative results obtained in group *C* show that autoantibodies did not develop spontaneously in the recipients during the period of the experiment.

In group *B* the occurrence of a positive D.C.T. was observed in both sexes and in a total of fifteen out of twenty-one recipients. One out of six males and fourteen out of fifteen females were positive. In no animal of either sex in this group was A.H.R. antibody detected.

In group *A* the occurrence of A.H.R. antibody and of a positive D.C.T. test was observed in both males and females. A.H.R. antibody was found in three of eleven males tested, and in three of eight females. The D.C.T. test was positive in most of the mice in this group, but one animal (a male killed on day 22) had a negative D.C.T. although A.H.R. antibody was detected in its serum.

It must be pointed out that these antibody results represent findings on the day on which the individual mice were killed and do not necessarily represent the antibody status for the preceding period.

Blood radioactivity counts. Individual blood radioactivity results obtained are presented in Fig. 1 after correction to unit dose and packed cell volume.

Group *C* males and females gave results suggesting a steady decrease in blood radioactivity over the period of the experiment. It might be anticipated from the individual results in this group that at least some of the injected chromium-labelled red cells normally survive in the blood for as long as 40–50 days.

In group *B* the females show lower levels of blood radioactivity than the controls in group *C*. In one female, red cell survival was less than 20 days. The males show little or no difference from group *C* animals; it must be remembered that in only one male was a positive D.C.T. recorded.

In group *A* animals, both male and female blood radioactivity counts were markedly less than group *C*. With few exceptions, lower levels were found in mice of this group than in either of the other groups. In two mice in group *A* red cell survival appeared to be less than 15 days.

Haematological values. The reticulocyte counts, packed cell volumes and haemoglobin values are shown in Fig. 2.

Within each sex, and for haemoglobin and reticulocyte counts separately, a comparison of groups *A*, *B* and *C* was made, ignoring time, in a one-way analysis of variance design. Since comparison of pairs of groups was also of interest, for example group *A* with group *B*, these comparisons were made in such a way as to ensure that the chance of accepting a difference between two identical means for the three comparisons was not greater than the nominal significance level (5 per cent or 1 per cent)[7]. The results are set out in Table 3, which shows that, ignoring time, there are statistically significant group differences in haemoglobin and reticulocyte values between groups *A* and *C*, and *A* and *B*.

Table 2. Results of Direct Coombs's Tests, and Tests for Anti-human Red Blood Cell Antibody in Young *NZB* Mice receiving Spleen Cells from *NZB* Donors of Differing Auto-immune Status

Donor's antibody status	Results	Recipients' antibody status (days following injection of spleen cells)																							
		8		10		13		15		18		20		22		24		27		29		31		36	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Group *A* D.C.T.+	D.C.T.	+			+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−		+		+	
Group *A* A.H.R.+	A.H.R.	●			−	●	+	●	+	+	+	−	−	+	−	+	−	−	−	−		+		−	
																				−		−		−	
Group *B* D.C.T.+	D.C.T.	−			+	−	+	−	−	−	+	−	+	+	+		+		+		+		+		+
Group *B* A.H.R.−	A.H.R.	●			−	−	−	−	−	−	−	−	−	−	−		+		+		−		+		−
																	−		−		−		−		●
Group *C* D.C.T.−	D.C.T.	−			−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group *C* A.H.R.−	A.H.R.	●			−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

●, Not tested.

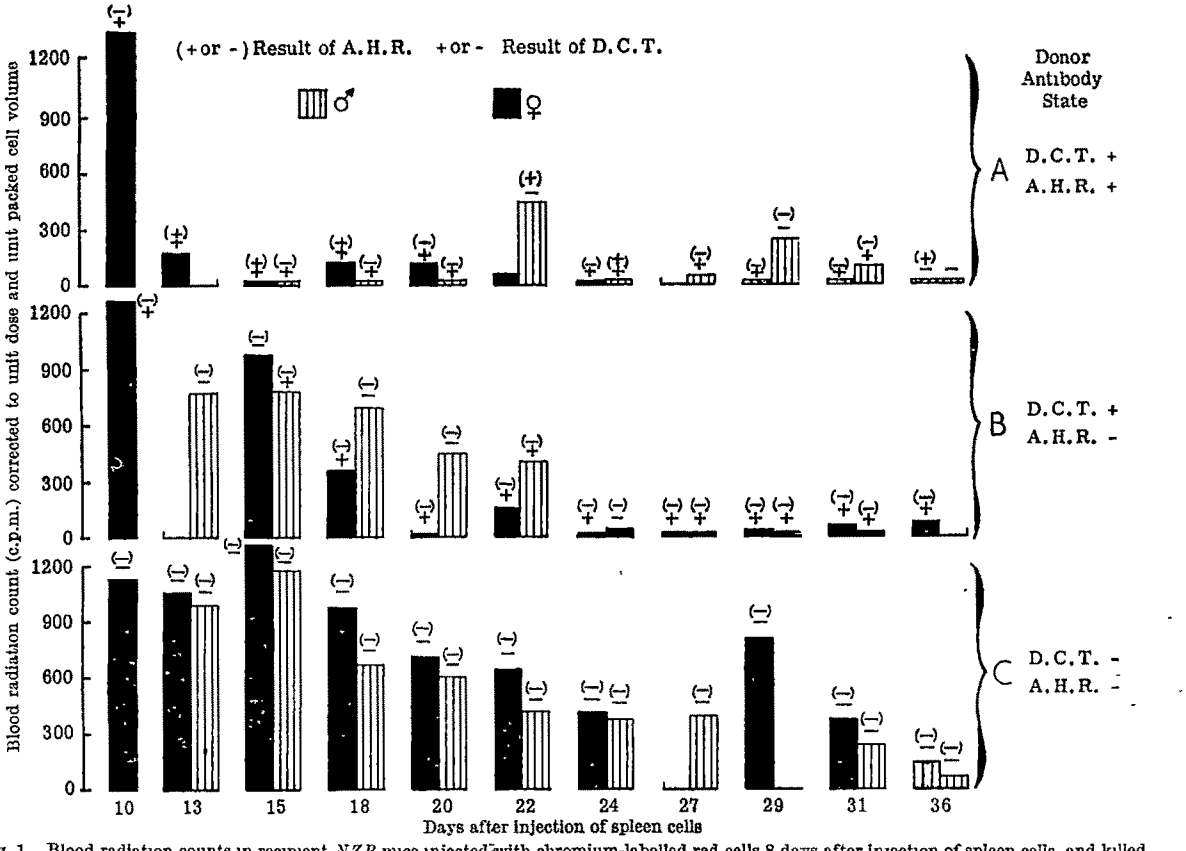


Fig. 1. Blood radiation counts in recipient *NZB* mice injected with chromium-labelled red cells 8 days after injection of spleen cells, and killed 10–36 days thereafter

In man, autoimmune haemolytic anaemia is characterized by an increased *in vivo* red cell destruction associated with the presence of an autoantibody against the red cell. In the *NZB* mice the spontaneous occurrence of anaemia and various red cell autoantibodies have been described[1,2]. We present evidence here to suggest that the anaemia in these mice, like the disease in man, is associated with increased red cell destruction. We have also related the red cell destruction pattern to induction of the two autoantibodies already defined.

Adoptive immunization of *NZB* mice was first performed by Holmes *et al.*[2]. Injecting spleen cell suspensions from old D.C.T. positive *NZB* donors, they were able to transmit the ability to produce a positive D.C.T. to younger animals of the same strain. We have also succeeded in transferring the ability to develop a positive D.C.T. by means of spleen cell suspensions. In addition, we have been able to detect A.H.R. antibody in a group of recipients injected with spleen cells from A.H.R. +, D.C.T. + donors. In this type of transfer experiment it is necessary to establish that any antibody production in recipients is not due to activation of their own immune systems. In control group *C*, neither a positive D.C.T. nor A.H.R. antibody was detected. This shows the absence, in young animals of the age of recipients, of either spontaneous autoantibody production or induction of antibody by injecting spleen-cells from negative donors.

Table 3

		Comparison of groups *A* and *B*	*B* and *C*	*C* and *A*	Overall comparison of *A* and *B* and *C*
Reticulocyte counts	♂	S.* $P<0.01$	N.S.†	S. $P<0.01$	S. $P<0.01$
	♀	N.S.	N.S.	S. $P<0.01$	S. $P<0.01$
Haemoglobin values	♂	S. $P<0.01$	N.S.	S. $P<0.01$	S. $P<0.01$
	♀	S. $P=0.04$	N.S.	S. $P<0.01$	S. $P<0.01$

* S, significant.
† N.S., not significant.

Donor Antibody Status: *A* {D.C.T. +, A.H.R. +} *B* {D.C.T. +, A.H.R. −} *C* {D.C.T. −, A.H.R. −}

In group *B*, it is apparent that the majority of female recipients, receiving spleen cells derived from one female donor, had succeeded in acquiring a positive D.C.T. This contrasted with the males in this group, in which a positive D.C.T. was recorded in only one instance. It is possible that this was due to technical failure in transmission of spleen cells, but as three separate spleen cell suspensions from three different donors were used this seems unlikely.

In group *A*, unlike groups *B* and *C*, we were successful in inducing formation of A.H.R. antibody in both males and females. As can be seen from Table 2, the positive A.H.R. results were obtained only between day 13 and day 24 after cell transfer, whereas the positive D.C.T. results ranged over the whole experimental period. We were particularly interested to note the relatively short duration of these antibody responses; in experiments not described here we have noted also the eventual disappearance of the adoptively transferred positive D.C.T. response, as described by Holmes *et al.*[2]. The transient character of the adoptive immunity investigated here might be due to lack of appropriate antigen, or other stimulus, in the young *NZB* hosts, or might indicate rejection of the spleen cell graft occurring despite the inbred character of the *NZB* strain. Experiments are at present being performed to investigate these adoptive immune responses in irradiated recipients.

The blood chromium levels were determined on only a few mice at each time interval after injection of labelled red cells. However, the results showed definite differences between the groups. In the control group it could be seen that the labelled red cell survival was likely to exceed 40–50 days. The group *B* females showed levels indicating a moderate decrease in labelled red cell survival, although the males in this group showed no difference from the controls, as might be expected from their mainly negative D.C.T. results. The group *A* animals gave individual blood radioactivity counts markedly lower than those

recorded in the control group. In several instances, results showed that mice in group *A* had greatly decreased red cell survival, even when compared with group *B*.

The haematological evaluation is also consistent with this view that the process induced in most of the group *A* mice is an acute haemolytic episode, and confirms the acuteness and severity indicated by the radiochromium results. The spectacular fall in blood chromium levels and rise in the numbers of reticulocytes in the peripheral blood would suggest a considerable rate of red cell breakdown in group *A*. Surprisingly, the fall in haemoglobin is not so marked and is presumably influenced by the extent of red cell compensation. Looking at results in individual animals, it is apparent that certain females in group *B*

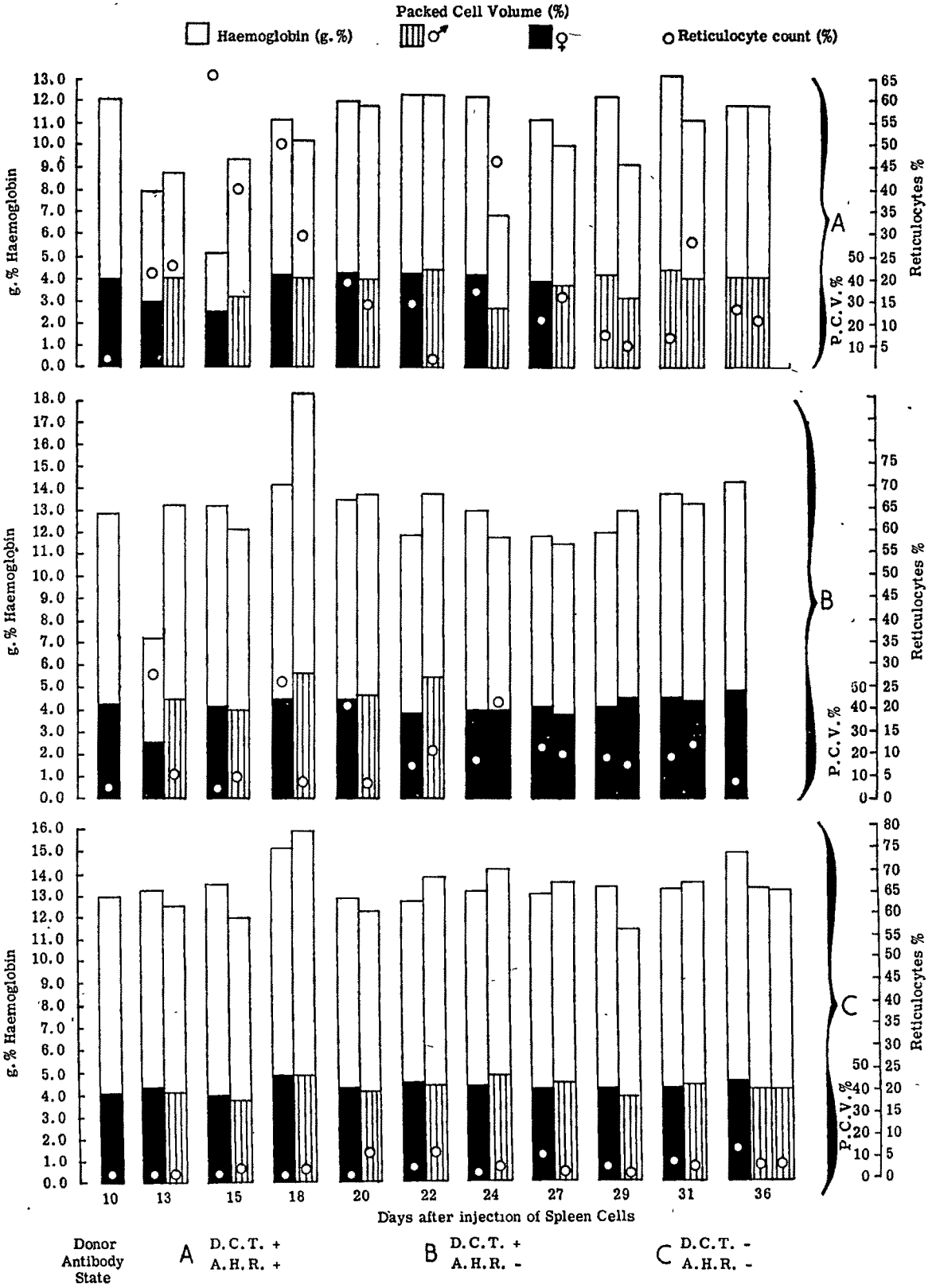


Fig. 2. Haematological values in adoptively immunized *NZB* mice

are anaemic and have a reticulocytosis when compared with group *C*. However, in the group analysis independent of time, group *A* was the only group to show statistically significant degrees of anaemia and reticulocytosis. These results suggest that if the *NZB* donors of spleen cells used to immunize young *NZB* mice adoptively are both D.C.T.-positive and already producing A.H.R. antibody, red cell destruction in the recipients is significantly greater than when donors are positive only by D.C.T. It seems likely, therefore, that the A.H.R. antibody itself enhances red cell destruction. It is also possible, however, the A.H.R. antibody is produced as a result of red cell destruction—which might, for example, reveal hidden antigenic determinants on the red cell surface—but this is perhaps less likely in view of the absence of A.H.R. antibody in group *B* recipients.

Helyer and Howie[6], in describing the spontaneous anaemia associated with the *NZB* mice, noticed the tendency of the males to develop anaemia earlier than the females. The spontaneously occurring A.H.R. antibody appears earlier and also more frequently in males, and we suggest that the earlier and more severe anaemia seen in the spontaneous disease in males[8] is related to this.

Further investigation of red cells, labelled with chromium-51, to estimate the actual half-life of the red cells in the spontaneous disease process are being performed.

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HAEMOGLOBINS OF SHEEP

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TWO allelic adult haemoglobins A and B are found in sheep as well as foetal haemoglobin F[1,2]. When sheep with haemoglobin A are made anaemic by bleeding, the proportion of haemoglobin A falls and another haemoglobin appears (Fig. 1)[3]. Blunt and Evans[4] suggested that this might be a new haemoglobin and van Vliet and Huisman called it haemoglobin C (ref. 5). When bleeding is stopped haemoglobin C gradually disappears and haemoglobin A returns to its former proportion. When sheep with both haemoglobin A and B are bled, only the proportion of haemoglobin A falls on the appearance of haemoglobin C. Sheep with haemoglobin B only, produce no haemoglobin C on bleeding[5,6]. Replacement of one haemoglobin by another under environmental stress is a unique phenomenon, and we have been investigating the relationship between haemoglobin C and the other sheep (or ovine) haemoglobins.

As a first step each of the four haemoglobins was isolated by repeated paper electrophoresis and dissociated into its sub-units at *p*H 4·6 in the presence of human and canine haemoglobin respectively[7,8]. After reassociation, the sub-units formed no hybrid haemoglobins with the sub-units of human haemoglobin. Hybridization with canine haemoglobin resulted in two hybrids, $\alpha_2^{can}\,\beta_2^{ov}$ and $\alpha_2^{ov}\,\beta_2^{can}$, of which the second moved more slowly than the first towards the anode on electrophoresis at *p*H 8·6 (Fig. 2). The $\alpha_2^{ov}\,\beta_2^{can}$ hybrid had the same electrophoretic mobility whether ovine A, B, C or F had been used for hybridization; but the hybrids of the canine α-chain with the non-α-chain of each of the four ovine haemoglobins differed in their mobilities. This suggested that the α-chains of the four ovine haemoglobins were similar and that the differences between these haemoglobins rested in their non-α-chains. This result has been reported independently by van Vliet and Huisman[5]. We will denote the non-α-chains of the ovine haemoglobins A, B, C and F as β^{ovA}, β^{ovB} β^{ovC} and γ^{vF}.

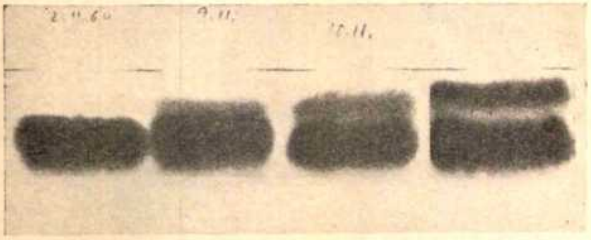


Fig. 1. Appearance of haemoglobin C in increasing proportion after bleeding a sheep with haemoglobin A. The course of bleeding consisted of removing about 2 l. of blood over 4 days. The electrophoretograms represent from left to right the haemoglobin composition on day 1, 7, 8 and 10

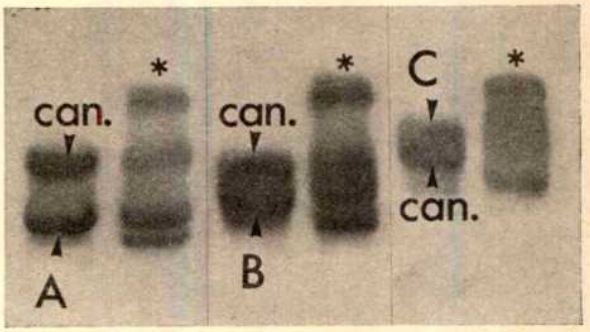


Fig. 2. Hybridization of sheep haemoglobins A, B, and C with canine haemoglobin. From left to right: mixtures of Hb A, B, C, respectively, both before and after dissociation and recombination. The '*' indicates the $\alpha_2^{ov}\,\beta_2^{can}$ hybrid which has the same mobility whether the α_2^{ov} is derived from the ovine Haemoglobin A, B, or C. This suggests that the different mobilities of these three sheep haemoglobins are based on differences in their non-α-chains. (See also van Vliet and Huisman[5].)

Information about the primary structure of the ovine haemoglobins was then obtained by studying their tryptic peptides. Globin was prepared using acid acetone, and

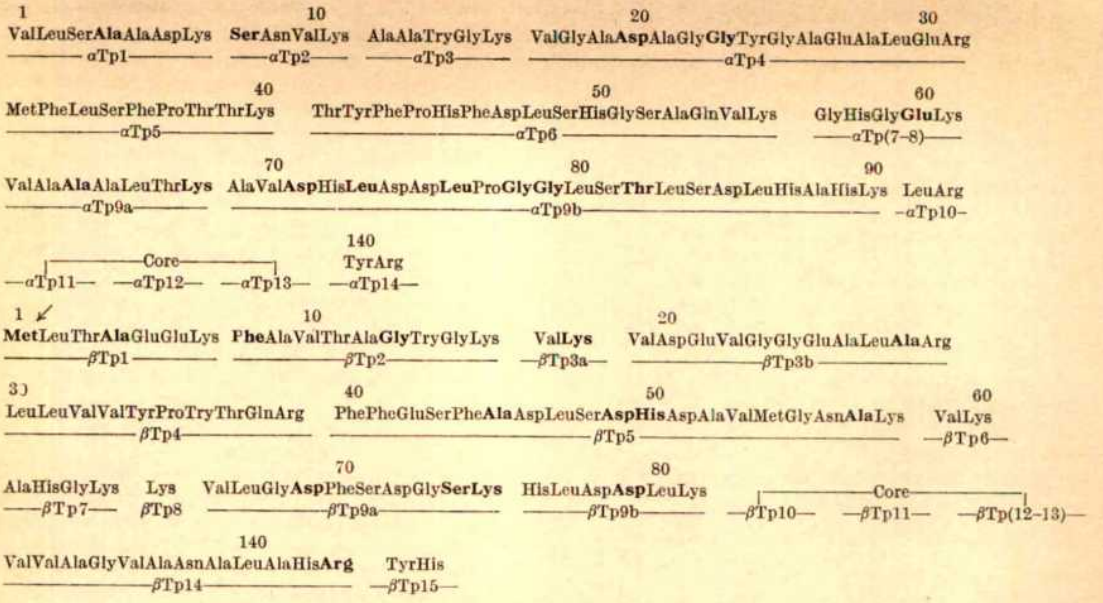


Fig. 3. Most probable partial sequences of the α- and β-chains of ovine haemoglobin A
Bold type indicates the most probable positions of the amino-acid residues which differ in ovine haemoglobin A and in human haemoglobin A. Arrow shows position where a deletion has probably occurred in the evolution of the β^{OVA} chain

digested with trypsin. The peptides that were soluble at *p*H 6·4 were separated from those in the insoluble 'core', and then submitted to paper electrophoresis[9] and chromatography[10]. Those from ovine haemoglobin A were analysed on an automatic analyser[11]. Details of the methods used have recently been summarized[12]. By comparing the amino-acid compositions of the ovine A peptides with those from human haemoglobin A[13,14], it was possible to put the ovine peptides into sequential order and also to infer the most likely sequence of amino-acids within each peptide. The tentative partial sequence is shown in Fig. 3. In a few cases the amino-acid composition of the peptide was not exactly reproducible and further investigation may necessitate minor alterations in the proposed sequences for αTp9b and βTp5.

Peptide maps of ovine haemoglobins A, B, C and F (Figs. 4–7) revealed no differences in the α-chain peptides but several non α-chain differences were apparent. The B peptide map (Fig. 5) differs from that of A (Fig. 4) as follows: An arginine peptide is missing (1). There are two new methionine peptides (2) which are probably structurally related. βTp5 has a lower R_F (3). There is an additional acidic peptide (4). Except for the first difference these results can be correlated with those of Muller[15].

The F peptide map (Fig. 6) differs from that of A (Fig. 4) as follows: There is an additional arginine spot (5), which is probably free arginine. The tryptophan peptide β2 found in haemoglobins A and B is missing but there is an additional peptide nearby which does not contain tryptophan (6). There is a faint additional basic peptide (7)

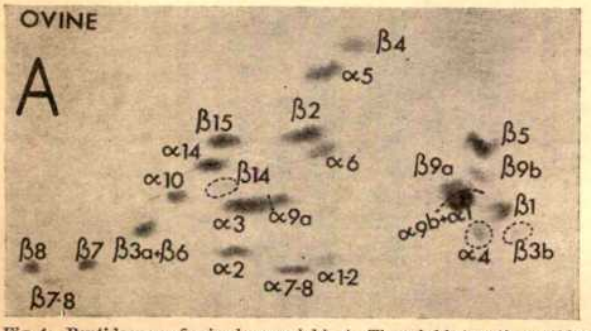


Fig. 4. Peptide map of ovine haemoglobin A. The soluble tryptic peptides of the α- and β-chain respectively are indicated

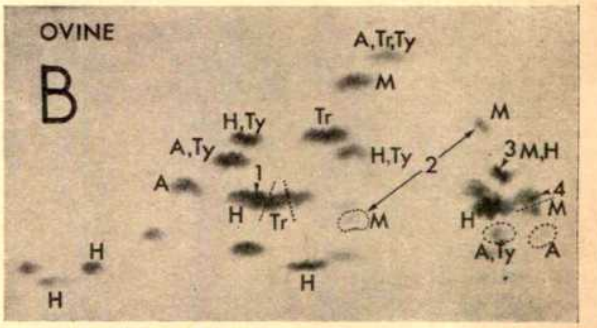


Fig. 5. Peptide map of ovine haemoglobin B. The capitals indicate positive colour tests for: A, arginine; H, histidine; M, methionine; Tr, tryptophan; Ty, tyrosine. The figures refer to remarks made in the text

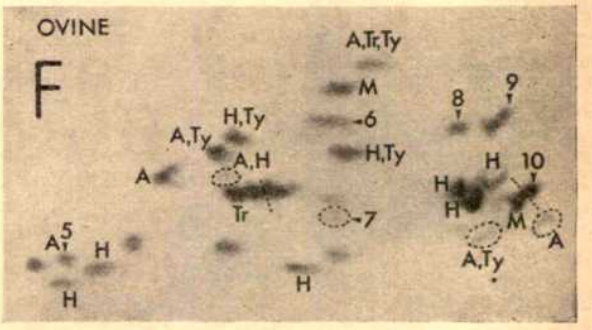


Fig. 6. Peptide map of ovine haemoglobin F. Details as in Fig. 5

and an additional neutral peptide (8). The peptide in the position of γTp5 does not contain methionine (9). There is an additional acidic peptide (10). Peptide maps of foetal haemoglobin from new-born sheep with haemoglobin A and with haemoglobin B were identical.

The C peptide map (Fig. 7) differs from that of A (Fig. 4) as follows: There is an additional arginine positive spot (11) which is probably free arginine. There are two additional basic peptides (12, 13) and an additional neutral peptide (15). The tryptophan peptide β2 is missing, but there is an additional peptide nearby which does not contain trytophan (14). The peptide in the position of βTp5 does not contain methionine (16). The peptide in the

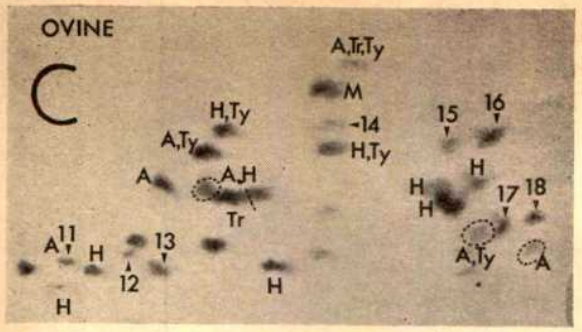


Fig. 7. Peptide map of ovine haemoglobin C. Details as in Fig. 5

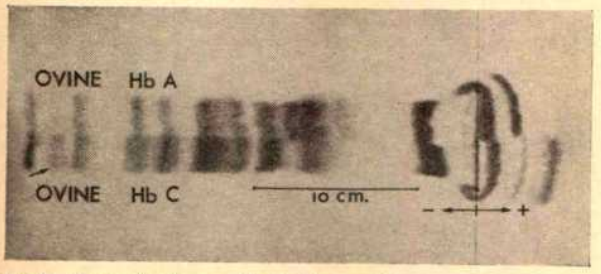


Fig. 8. Electrophoretogram of ovine haemoglobins A and C. The arrow indicates the area staining for arginine, which is absent in A but present in C

position of βTp1 does not contain methionine (17) and there is an additional acidic peptide (18).

The additional arginine positive spot (11) was used to detect small amounts of haemoglobin C in sheep with haemoglobin A in the absence of haemoglobin F. An extended electrophoretogram shows this more clearly (Fig. 8).

Of 94 residues accounted for in the ovine α-chain at least thirteen differ from those of the human α-chain. Assuming that the two chains have the same length (141 residues) there are likely to be at least twenty differences in amino-acid sequence. Ninety-five residues have been accounted for in the β^{ovA} chain of which at least fifteen differ from those of the human β-chain. If this ovine chain is assumed to have one residue less than the human β-chain (145 instead of 146) due to a deletion at position two, one might expect a total of at least twenty-three differences. These differences are what one would expect from the assumption that man and sheep diverged about 100 million years ago and that a mutation giving rise to a change in amino-acid sequence occurs every 8–10 million years[16].

Only seven amino-acid residues could be found in the first tryptic peptide of the β^{ovA} chain whereas there are eight in the equivalent peptide from man. Of the mammalian haemoglobins so far investigated this difference has only been found before in cattle haemoglobin[17]. The N-terminal residue of the ovine peptide was found to be methionine by a modified dinitrophenyl method[18], and this residue is the same as that reported for the N-terminal of the β^{ovA} chain[19]. Digestion of the peptide with chymotrypsin resulted in two products which were found to have the amino-acid compositions Met, Leu and Thr, Ala, Glu_2, Lys respectively. The latter had an N-terminal threonyl so that the partial sequence Met–Leu–Thr–(Ala,Glu,Glu)–Lys can be assumed for β^{ovA}Tp1.

It is believed that the human β-chain evolved from a primitive α-chain by way of the γ-chain by means of gene duplication[20]. During this process an extra residue was added at position two enhancing the molecular divergence of the chains.

N-terminal sequences of human α- and β-chains are:

α Val– Leu–Ser–Pro – –

β Val–His–Leu–Thr–Pro – –

It would appear, therefore, that a deletion has arisen in the ovine β-chain giving it an unexpected resemblance to the ovine α-chain (see Fig. 3). Such a deletion may be an example of evolutionary convergence operating at the molecular level.

The peptide maps of the ovine haemoglobins A and F suggest that there is a greater similarity between the β^{ovA} and γ^{ovF} chains than one might have expected. This was further emphasized by finding that the rather rare methionine residue was N-terminal in both. N-terminal methionyl has also been reported for the non-α-chains of adult and foetal cattle haemoglobin[17]. Zuckerkandl and Pauling[21] have suggested that the gene duplication which gave rise to these peptide-chains of cattle may have occurred considerably later in evolution than that which gave rise to the human β- and γ-chains. This may well apply also to the ovine chains.

Hybridization, amino-acid analysis[22] and peptide mapping have failed to reveal any differences between the α-chains of the four ovine haemoglobins. Therefore it can be assumed with reasonable confidence that these chains have identical amino-acid sequences. The β^{ovA} and β^{ovB} chains are closely related as might be expected for two alleles. Whereas the human allelic haemoglobins differ in one residue, in sheep there are several differences. This may be because the sheep is a much older mammal than man, or possibly sheep with haemoglobin A and haemoglobin B developed separately in different environments. The peptide maps of the two haemoglobins show differenees that might be due to changes in the peptides βTp5, βTp14 and one likely to arise from a core peptide. The β^{ovC} chain appears to differ more widely from both the β^{ovA} and β^{ovB} chains than these two differ from each other. The peptide maps show numerous differences which suggest changes in βTp1, βTp2, βTp5, βTp9, and in the core peptides. Although there is a greater resemblance between the β^{ovC} and γ^{ovF} chains, differences occur at least between β^{ovC}Tp1 and γ^{ovF}Tp1 as well as possibly in the core peptides. One important difference is that unlike the β^{ovA}-, β^{ovB}- and γ^{ovF}-chains the β^{ovC}-chain has no N-terminal methionyl.

Braend and Efremov[23] have found traces of a second haemoglobin in some apparently normal sheep with haemoglobin A. They suggest that this haemoglobin may be a normal rather than an abnormal component, and that a gene linkage may operate between the β^{ovA}- and β^{ovC}-chains similar to that which operates between the human β- and δ-chains. We have consistently found traces of haemoglobin C in all the thirty-one sheep of the Soay and Clan breeds with haemoglobin A or AB we have investigated, but we have never found haemoglobin C in a sheep with only haemoglobin B, even after severe bleeding. When on occasion haemoglobin C did not separate from haemoglobin A on first paper electrophoresis, the rear part of the A band was cut out, eluted, and the eluate after concentration was again submitted to electrophoresis. By this means sufficient haemoglobin C became available to separate from A on paper electrophoresis, and for high-voltage electrophoresis of the peptides to demonstrate the free arginine spot (11) (see Fig. 8).

It would appear, therefore, that the process by which haemoglobin C forms the major proportion of the haemoglobin in anaemic sheep with haemoglobin A is due to an increase in means of synthesis or rate of synthesis rather than to the switching on of a new gene. We have also observed that the rise in the level of haemoglobin C on bleeding coincides with the reticulocytosis. Possibly haemoglobin C is synthesized primarily by the reticulocytes whereas haemoglobin A is primarily synthesized by the normoblasts. This would represent a fundamental difference in the regulation of protein synthesis by the reticulocytes and the late normoblasts of the A sheep but not of the B sheep. It has been proposed that reticulocytes, in view of their difference from normoblasts in size[24] and in metabolism[25], are not part of the normal cell line, but appear under stress as a by-pass of the regular cell production by

way of the normoblasts. The observations in A sheep seem to lend support to this concept.

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COMPARATIVE IMMUNOCHEMICAL PROPERTIES OF NUCLEOPROTEINS: ANTIGENIC CHARACTERISTICS OF DEOXYRIBONUCLEIC ACID PROTEIN FROM A CANINE HAEMANGIOPERICYTOMA

By Prof. R. H. WILSON*, V. JUREVICS, R. DELANEY, T. RITTER, K. RENCZ and A. SCHRAM

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THE question of the comparative immunochemical properties of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), deoxyribonucleic acid protein (DNAP) and the simple alkaline proteins of nuclei, such as histones and protamines, has by no means been completely answered[1–4].

Plescia *et al.*[5] have reported the production of antibodies to *t*RNA. In this work methylated bovine serum albumin (MBSA) was used as an effective carrier for *t*RNA from the yeast cell. Complexes of RNA–MBSA were prepared by adding MBSA (10 mg/ml. water) to RNA (500 μg/ml. in 0·15 M sodium chloride) and injected, as an emulsion with complete Freund's adjuvant (CFA), into the foot pads and intramuscular routes in New Zealand white rabbits. The immune sera were reported to contain antibodies against yeast *t*RNA that cross-reacted with *Escherichia coli t*RNA and synthetic poly-uridine (poly-U). These antisera also reacted with partially purified alanyl *t*RNA and Worthington RNA. These antigens contained no detectable traces of amino-acids and sugars other than D-ribose. Thus, the antigenic determinants involved ribonucleotides presumably in sequence.

If an immunochemical model could be designed for the purpose of determining the characteristics of the sequence of the nucleotides in DNA and RNA, problems concerning the structure of the nucleic acids in different organs and in different species would be simplified.

Wilson *et al.*[1] reported that calf thymus DNA with a molecular weight of $9{\cdot}3 \times 10^6$ was neither antigenic nor haptenic in rabbits. Further studies by the same investigators using deoxyribonucleic acid-histone as an antigen in large quantities over a long period of time revealed that DNAP, free of proteins other than histones, was not antigenic in New Zealand white rabbits. Cross-reaction studies with the histones, DNA and DNAP, from the calf thymus showed that these materials are neither antigenic nor haptenic, in the reported experimental model. However, this does not mean that the DNAP from other sources, such as bacteria and viruses, lack antigenic and/or haptenic properties. It is also recognized that degraded DNA and/or DNAP may be weakly antigenic.

It was therefore thought to be important to the investigation of the comparative immunochemical properties of the nucleoproteins to include in the experimental model DNA, DNAP and histones from a canine cancer haemangiopericytoma.

A 12-year-old male English setter was admitted to the experimental animal quarters with a 37-cm² tumour on the lateral aspect of the left thorax and abdomen. This tumour was removed surgically three times within a period of 6 months. It was growing rapidly and an attempt to remove the entire tumour proved ineffective. The weight of the dog was 52 lb. at the time of its admission to the experimental animal quarters. The gross examination of the tumour revealed that it was subcutaneous, nodular and firm.

A section of the tumour was sent to the Texas A and M College, Department of Animal Pathology, where a diagnosis of haemangiopericytoma was made (Fig. 1). This tumour was described by Stout *et al.*[6] in man and by Mulligan[7] in 68 dogs. Histological examination revealed that it consisted in general of spindle-shaped cells with ovoid or elongated nuclei with considerable cytoplasm which was fibrillar in character. Within the tumour numerous small capillaries lined the endothelium which were closely encircled by the anaplastic and pleomorphic spindle cells; these were considered to be pericytes.

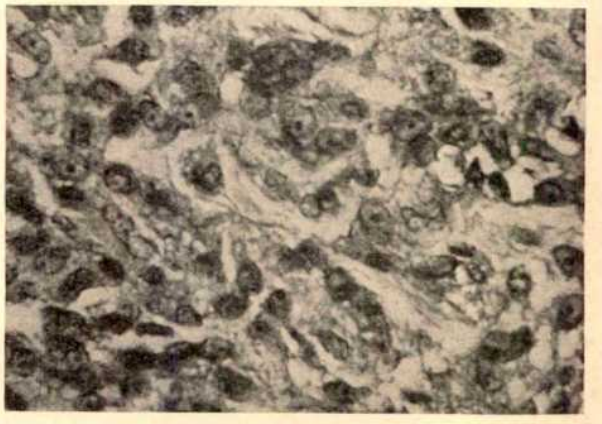


Fig. 1. Photomicrograph of a section of the haemangiopericytoma from which the DNAP was isolated for the immunochemical study

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The circulating eosinophil count was 250 at the beginning of the period of observation. The total leucocyte count was 7,500 and the differential count was 65 per cent polymorphonuclear leucocytes, 30 per cent lymphocytes and 5 per cent other types of leucocytes; the BUN was 1·5 mg per cent and the urine analysis was within normal limits.

The dog was anaesthetized by the injection of 20 ml. of sodium pentothal. The tumour was removed, immediately frozen and kept in the deep freeze at −40° C. The DNA was isolated from fresh frozen tumour using the method of Kay, Simmons and Dounce[8]. This method was carried out essentially as follows: the tumour was placed in a dry-ice container immediately after removal from the dog. It was then stored in a deep freeze at −40° C for 2 days. The tissue was thawed, and all gross fat portions removed. It was then cut into small pieces which were washed five times with 100-ml. portions of 0·1 M aqueous sodium citrate. The mixture was homogenized four times using 250-ml. 0·1 M citrate each time. In the first homogenate 1 ml. of octanol was added to prevent foaming. The homogenate was centrifuged at 1,800*g* for 30 min each time. The final residue was extracted with 1,000 ml. 2 M aqueous sodium chloride. The sodium chloride solution was added in increments until the viscosity of the preparation was that of a thick paste.

The mixture was placed in a refrigerator at 4° C overnight and then centrifuged for 1 h at 1,900*g*. The nucleoprotein was precipitated from the supernatant by addition of two volumes of absolute ethanol extended over a period of 3 h while agitating the contents of the flask.

The precipitated fibres of nucleoprotein were removed from the flask with a glass rod and washed three times with a solution of 75 per cent ethanol and then redissolved in 1,000 ml. of buffer containing 8·1 g sodium chloride and 5·25 g sodium citrate per l. (hereafter called 'standard buffer'). 90 ml. of a 5 per cent solution of sodium lauryl sulphate in 45 per cent ethanol was added to the solution of nucleoprotein. The mixture was stirred for 1 h and then enough sodium chloride was added to bring the final concentration of this salt up to 5 per cent.

This preparation was then placed in the refrigerator at 4° C and left overnight. The mixture containing the nucleoprotein was then centrifuged using a 'No. 40 Spinco' rotor at 19,000 r.p.m. The DNA in the supernatant was precipitated with ethanol as before. The precipitate was washed, redissolved and treated with the detergent as outlined. The last precipitate was dissolved in 120 ml. of the standard buffer solution and frozen. The residual protein in the DNA preparation was determined with the method of Lowry[9].

The DNA content of the final solution was estimated by the diphenylamine method of Dische[10]. The total nucleic acid content which would include the RNA component was determined according to the method of Spirin[11]. The molecular weight of the DNA was determined by the ultracentrifugal method of Burgi and Hershey[12].

Isolation of histones. Histones were obtained from the tumour nucleoprotein described. For this purpose the nucleoprotein was precipitated by addition to 6 volumes of water. The resulting fibres of nucleoprotein were lifted with a rod and placed into a flask containing N/4 hydrochloric acid. Next day the contents of the flask were centrifuged and the residue discarded. The supernatant was then treated with concentrated trichloroacetic acid (TCA) solution to precipitate the histones. Centrifugation of the TCA-treated solution yielded a yellowish sediment which turned white after washing with ether and acetone. The white powder was then dissolved in distilled water, dialysed against distilled water for 2 days, and finally lyophilized. The molecular weight of this histone preparation was determined by the ultracentrifugal method of Archibald[13].

Nucleoprotein isolation and characterization. Nucleoprotein from the tumour was isolated by the method of Mirsky and Pollister[14]. The fresh tumour was obtained from the dog, placed in dry ice, and afterwards stored in a deep freeze at −40° C. It was cut up in 1-cm squares for extraction. Repeated washing with saline citrate buffer, *p*H 7·2 (8·1 g sodium chloride and 5·25 g sodium citrate per l.), removed much of the cytoplasmic material. Residual saline-soluble material was removed by homogenization of the minced tissue in a Virtis '45' blender and centrifugation. This procedure was repeated three times. The residue was then homogenized in 1 M sodium chloride. Centrifugation of the resulting highly viscous solution gave a supernatant which was removed and stored overnight and a residue which was rehomogenized in a new portion of 1 M sodium chloride and also left to stand overnight. Next day the rehomogenized residue was centrifuged and the supernatants combined. The residue was discarded. When this nucleoprotein solution was poured into 6 volumes of distilled water, while swirling with a glass rod, fibres of nucleoprotein precipitated and wrapped around the rod. The rod was lifted, the nucleoprotein redissolved in 1 M sodium chloride, centrifuged and precipitated as before. This procedure was repeated four times. The final precipitate was dissolved in 1 M sodium chloride, rather than dried, and the DNA and protein content was determined using Dische's[10] and Lowry's[9] methods respectively.

The molecular weight of the canine haemangiopericytoma DNAP was $2{\cdot}35 \times 10^6$. The protein content of the DNAP was 66·6 per cent. The DNA content was 33·4 per cent. The molecular weight of the histones was 14×13^3.

Immunological testing procedures. The complement fixation and Ouchterlony procedure were carried out essentially as outlined in Kabat and Mayer[15]. The Arthus test was performed by injecting 0·05 mg of the various antigens in 0·1 ml. intracutaneously and reading the results after 10, 20, 30 min and at 24- and 48-h periods. Control tests were done on non-immune rabbits of the same age. A positive test was recorded when the control was negative and the area of redness, swelling and oedema was greater than 1 cm. The immunization schedule is outlined in Table 1. The antisera for the cross-reactions were obtained from rabbits challenged with the antigens listed in Table 3 (refs. 1 and 2).

The immunochemical data are tabulated in Table 2. The Arthus reaction was remarkably positive 17 days after the initial injection of the DNAP tumour. A qualitative precipitin test was positive 20 days after the initial injection of the experimental DNAP antigen. At this time there were no precipitin lines in the Ouchterlony test after 3 days of observation. The Arthus reaction was again positive 29 days after the beginning of the experiment. The Arthus tests were used not only to detect the presence of circulating antibody, but as a booster injection. The Ouchterlony test was repeated using normal control canine whole serum against the rabbit antisera, and the serum from the dog from which the tumour had been obtained. The Ouchterlony tests at this time were positive both with the normal dog serum and the serum from the experimental animal. The antisera were positive when tested against the DNAP fraction of the tumour with which the animal had been immunized initially. In view of the previous work[1,2] the rabbit antisera were tested against the canine serum protein fractions, namely, gamma-globulin, alpha-globulin, beta-globulin, albumin, fibrinogen and canine tumour DNAP and histone. The canine tumour DNAP fraction was negative. The Ouchterlony tests with the alpha-, beta- and gamma-globulin fractions showed precipitin lines with the beta- and gamma-globulins. The positive precipitin lines obtained with fibrinogen were against gamma- and beta-globulins. There was no precipitin line with albumin.

Immunoelectrophoresis was carried out in order to obtain a more precise definition of the precipitin lines involved. Three precipitin lines in Figs. 2 and

3 were obtained with immunoelectrophoresis. Two of these lines were identified as beta and gamma globulins. The third lines in Figs. 2 and 3 were not identified. No precipitin lines were obtained with the canine haemangiopericytoma histones and DNAP. The complement-fixation test was positive with dog tumour DNAP (Table 4).

The complement-fixation test was positive when gamma globulin and beta globulin were used as antigens against

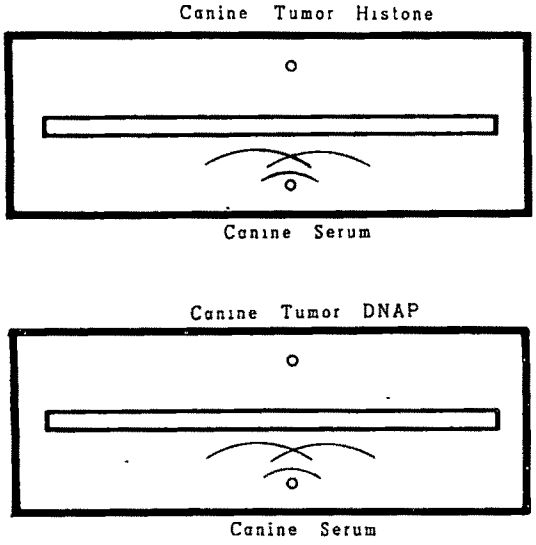


Fig. 2. Rabbit DNAP antisera were placed in the horizontal troughs. Canine haemangiopericytoma histones were placed in the upper well. There were no precipitin lines; there were three lines with non-cancerous canine serum. Canine haemangiopericytoma DNAP was placed in the upper well of the lower slide. There were no lines, whereas with non-cancerous serum there were three lines

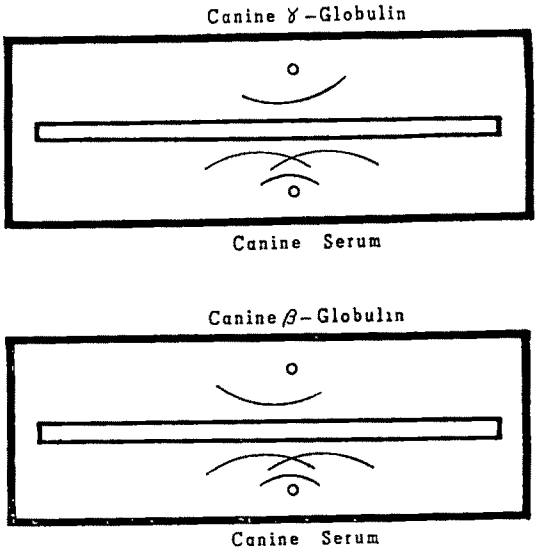


Fig. 3. Rabbit DNAP antisera were placed in the horizontal troughs. Canine beta- and gamma-globulin were placed in the upper well; one precipitin line was obtained with each. Serum from a non-cancerous dog was placed in the lower wells; three precipitin lines were obtained which correspond to the beta- and gamma-globulin lines. The third line was unidentified

Table 1. IMMUNIZATION SCHEDULE USING CANINE HAEMANGIOPERICYTOMA DNAP AS THE ANTIGEN

Rabbit No.	DNAP mg								
	11/2/64	28/2/64	9/3/64	24/3/64	8/5/64	13/10/64	15/4/65	18/5/65	11/6/65
94–*E*	0·5 mg*	0·2 mg†	0·2 mg†	0·2 mg†	0·2 mg†	0·5 mg*	1·5 mg‡	1·0 mg‡	10 mg‡
95–*E*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	1·5 mg	Died 14/5/65	
96–*E*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	Died 22/10/64		
97–*E*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	1·5 mg	1·0 mg Died 31/5/65	
98–*E*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	Died 25/11/64		
99–*E*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	1·5 mg	1·0 mg	10 mg
00–*F*	0·5 mg	0·2 mg	0·2 mg	0·2 mg	0·2 mg	0·5 mg	Died 5/12/64		

* 1 : 1 with complete Freund's adjuvant divided between four toe pads.
† Intracutaneously for Arthus reaction.
‡ Intramuscularly divided between two hips.

Table 2. RESULTS OF THE SEROLOGICAL TESTS WITH ANTISERA FROM RABBITS CHALLENGED WITH CANINE HAEMANGIOPERICYTOMA DNAP

Rabbit No.	20 Days 3/3/64 Precipitin test	20 Days 3/3/64 Ouchterlony test	38 Days 18/3/64 Ouchterlony test		27/3/64 Ouchterlony tests					51 Days 1/4/64 immunoelectrophoresis			
			n-Dog serum	*t*-Dog† serum	α-g	β-g	γ-g	Alb	Fib.	Dog serum	γ-g	β-g	Fib.
94–*E*	Pos	Neg.	1*	1	1	2	1	Neg.	1	3	2	2	2
95–*E*	Pos.	Neg.	2	2	1	2	1	Neg.	1	3	2	2	2
96–*E*	Pos.	Neg	2	2	1	2	1	Neg.	1	3	2	2	2
97–*E*	Pos.	Neg.	2	2	1	2	1	Neg.	1	3	2	2	2
98–*E*	Pos.	Neg.	1	1	1	2	1	Neg.	1	3	2	2	2
99–*E*	Pos.	Neg.	1	1	1	2	1	Neg	1	3	2	2	2
00–*F*	Pos.	Neg.	1	1	1	2	1	Neg.	1	3	2	2	2

* No. of lines on the Ouchterlony plate
† Serum from dog with haemangiopericytoma.

Table 3. RESULTS OF SEROLOGICAL CROSS-REACTIONS WITH THE CANINE HAEMANGIOPERICYTOMA AND CALF THYMUS DNA, DNAP HISTONE AND SALMINE RABBIT ANTISERA
The Ouchterlony test was used to obtain these data

Rabbit antisera against	Antigens tested									
	Bovine serum	Canine serum	Canine tumour DNAP	Canine tumour histone	Canine serum γ-globulin	Bovine thymus DNAP	Bovine thymus DNA	Bovine thymus histone	Bovine serum γ-globulin	Salmine
Canine tumour DNAP	Neg	Pos.	Pos	Neg	Pos.	Neg	Neg.	Neg.	Neg.	Neg.
Bovine thymus DNAP	Pos	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Pos	Neg.
Bovine thymus DNA	Neg.	Neg	Neg.	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.
Bovine thymus histone	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.	Neg.	Neg	Neg
Salmine	Neg.	Neg	Neg.	Neg.	Neg	Neg.	Neg	Neg.	Neg	Neg

Table 4. RESULTS OF SOME SEROLOGICAL TESTS

Rabbit No.	Complement fixation tests with the following antigens				Immunoelectrophoresis on 15/6/65				
	Canine tumour histone	Canine serum β-globulin	Canine serum	Canine tumour DNAP	Canine tumour histone	Canine serum β-globulin	Canine serum γ-globulin	Canine tumour DNAP	Canine serum
94–*E*	Neg.	Pos	Pos.	Pos.	Neg.	Pos.	Pos.	Neg	Pos.
95–*E*	Neg	Pos.	Pos.	Pos.	Died 14/5/65				
96–*E*	Neg.	Pos	Pos	Pos	Died 22/10/64				
97–*E*	Neg.	Pos	Pos.	Pos.	Died 31/5/65				
98–*E*	Neg	Pos.	Pos.	Pos	Died 25/11/64				
99–*E*	Neg.	Pos	Pos.	Pos.	Neg	Pos.	Pos.	Neg	Pos
00–*F*	Neg.	Pos.	Pos	Pos.	Died 5/12/64				

the rabbit DNAP antisera. The rabbit antiserum was tested against the Hyland Laboratories' DNAP latex reagent and was negative in all sera except one. This was tested again and found to be negative.

Histological examination of the rabbit blood did not reveal lupus erythematosus cells. The serological cross-reaction tests with canine tumour DNAP and rabbit antisera against bovine DNAP, DNA, histones and salmine were negative as shown in Table 3.

The present work clearly indicates that there are trace amounts of antigenic constituents in DNA-protein (histone) complexes prepared by the method of Mirsky and Pollister. These antigenic materials may be present in association with the DNA in intact cells, while another possibility is that they are introduced into the DNA-protein complex only after breakdown of the nucleus during isolation. We have identified two constituents of our canine haemangiopericytoma DNAP preparation as beta globulins and gamma globulins using canine serum and serum fractions in immunoelectrophoretic studies with the rabbit DNAP antisera. A third immunoelectrophoretic precipitin line, the component of which is apparently isoelectric at *p*H 8·6, could not readily be identified.

One important observation in this experiment is that the antigenic components of the present dog tumour DNAP account for a very small percentage of the protein portion. The dog tumour DNAP itself did not give rise to Ouchterlony or immunoelectrophoresis precipitin lines in any case; dog tumour histone likewise failed to give positive serological reactions. This fact can be interpreted as follows: there are sufficient quantities of antigens in this dog tumour DNAP to elicit an antibody response in rabbits, while at the same time the antigen quantities are so minute that the sensitivities of known conventional immunological methods fail to give positive reactions. The results obtained in this investigation are consistent with the data previously reported concerning calf thymus DNA, DNAP and histones[1,2].

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ALGAL INFLUENCE ON RADIONUCLIDES IN SETTLING PONDS

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MANY of the larger processing plants handling radioactive material have an adjacent small pond through which low-level aqueous waste solutions are allowed to seep slowly into the ground. Such ponds exist for this purpose on the National Reactor Testing Station. They are a few acres in area and about 10 ft. deep, in gravelly alluvium, and transmit 10–100 gal. aqueous waste per ft.^{2} of basin per day. The chemical character of these solutions varies widely, the *p*H varies from 3–10, and the radiation field at the water surface varies from background to about 0·5 mr./h. Radionuclides contained in solutions moving through the basin sub-soil usually exchange on to the soil particles within a few tens of feet.

Particularly during the warmer months, dense algal blooms turn the pond waters into an organic broth. Although the β–γ radiation-level at the water surface may be only a fraction of 1 mr./h, dried debris (algae, detritus, etc.) which collects on the pond bank can give rise to general radiation-levels of 25 mr./h at 1 cm. The pond algae can itself produce fields up to 100 mr./h/g. Since it is well known that algae concentrate some radionuclides into their structure[1–3] the possibility exists that this mass of organic material could modify the chemical state of the radionuclide in solution, perhaps by chelation, and thereby alter its ion-exchange behaviour with the basin alluvium. Whether this would enhance or detract exchange was not known.

The purpose of our experiments was two-fold: (*a*) to determine if such a modification could be detected, and if so what effect it would have; and (*b*) to determine and evaluate the radiological hazards associated with algal growth in settling ponds.

Examination of the algal bloom showed that it was composed mostly of single-celled, floating or suspended members of the Desmidacae such as *Scenedesmus*, *Closterium*, *Cosmerium*, *Chlamydomonas*, and the like, about 80 per cent by occular estimate. The remainder consisted of *Pamella* and *Protococcus*-like organisms with other divisions represented by *Polycystis* and *Navicula*. Filamentous algae grew along the banks and sometimes in small rafts but did not contribute substantially to the mass of plankton.

Cultures of pond algae were grown in the laboratory plant-growth room in 5-gal. carboys. They were fed a standard nutrient solution and were sparged for 1 min in every 4 h for mixing. Radioactivity in the culture tank was essentially nil. Samples for experimentation were concentrated by centrifuge. The algae were then washed in distilled water, reconcentrated, and placed in 1-l. beakers. Approximately 5 g of wet algae were added to 1 l. of distilled water. 500 p.p.m. calcium was added as calcium nitrate, along with the test radionuclide, strontium-85 or caesium-137, and 5 p.p.m. stable strontium or caesium carrier. The *p*H of the contents of each beaker was then adjusted and maintained at 3·5, 7·0, or 9·0 using sodium hydroxide or nitric acid. At the time of the test each beaker had either 45 μc. strontium-85 or 20 μc. caesium-137. This rather austere regime was used in order to augment the effect of algal modification. The algae remained in these solutions for 3 weeks. The radioactive algae were then removed by centrifugation, washed to remove any activity from the growth solution, dried slowly and weighed. Each beaker contained about 1 g dry algae.

Coincident with the test-growth phase, twenty-four 1-l. beakers of algae were prepared and grown in exactly the same manner as the radioactive group but without the radionuclide. The conditioned water thus produced was used as a comparable leach solution in the experiment to follow. The non-radioactive algae were removed from the cold solutions and discarded. The washed, dried, radioactive algae from the growth phase were then placed in the comparable cold leach solutions and allowed to decompose in the dark for 2 weeks. The radionuclide incorporated either into or on to the algal cells was liberated by dissolution and isotopic exchange to the supernatant leach solution. At this time a third solution was prepared exactly like the first including the radionuclide but excluding the algae. This was used as a blank solution having no algal modification of any type. There were then three sets of solutions, at three different *p*H levels, to be tested and compared. These were (*a*) the original growth solution representing a pond with algae with the radionuclide in the water but not in the algae (growth phase); (*b*) the leach solution representing a pond with algae but with the radionuclide derived solely from nuclides cycled through the algal plant (leach phase); and (*c*) the blank solution representing a pond with algae, with the radionuclides in their original chemical form (blank phase).

Exchange studies were undertaken on synthetic alluvium which was prepared in the following manner. Distribution of particle sizes was determined by sieve analysis of typical alluvial deposits near the pond location. Representative size fractions of this material were then weighed out and thoroughly mixed together. This soil had a cation exchange capacity of 7·93 m.equiv./100 g for strontium and 5·87 m.equiv./100 g for caesium. 1 g of this soil was placed in a screw top vial along with 20 ml. of the test solution and rotated end over end for 24 h. The solution was then centrifuged, a 15-ml. sample removed by pipette, transferred to a counting vial, and counted in a well-type γ counter. The γ activity of the test solution was also determined in this same instrument. The distribution coefficient (K_d) of the material being tested in its test solution was defined to be the ratio of the activity on 1 g soil to the activity remaining in 1 ml test solution at equilibrium.

Table 1. Distribution Coefficients (K_d) of the Radionuclides Strontium-85 and Caesium-137 on Soil

Radionuclide	*p*H	$\bar{x}$ K_d	2*s*	Comparison
Strontium-85				Sr (growth phase)/ Sr (blank phase)
Growth phase	3·5	2·60 ±	0 56	1·011
	7 0	3 35	0 70	0 935
	9·0	3·08	1 00	1·203
Caesium-137				Cs (growth phase)/ Cs (blank phase)
Growth phase	3 5	49·89	16·0	0 685
	7 0	73·38	10 0	0·957
	9·0	73 17	10·0	1·013
Strontium-85				Sr (leach phase)/ Sr (blank phase)
Leach phase	3·5	2·62	1 08	1·073
	7·0	2 91	0·80	1 094
	9 0	3·01	0 60	—
Caesium-137				Cs (leach phase)/ Cs (blank phase)
	3·5	64·67	21 0	0·870
	7·0	72·16	13 0	1 003
	9·0	82·33	23 0	—
Strontium-85				
Blank phase	3·5	2·57	4·80	
Growth phase	7·0	3·58	0·36	
	9·0	2 56	2·20	
Caesium-137				
Blank phase	3·5	72·85	17·2	
	7·0	72·88	14·6	
	9·0	72·22	26·8	
Strontium-85				
Blank phase	3·5	2·44	3·20	
Leach phase	7·0	2·66	1·80	
	9 0	—	—	
Caesium-137				
Blank phase	3·5	74·31	6·20	
Leach phase	7·0	71 20	3·80	
	9·0	—	—	

Finally, two experiments were carried out to determine the ratios of calcium/strontium and calcium/caesium selected by the algae from this system. Cold, washed algae were added to the same basic solution, 500 p.p.m. calcium as calcium nitrate, and 5 p.p.m. stable strontium carrier in 1 l. of distilled water. To this was then added approximately 50 μc. calcium-45. In the case of caesium, 5 p.p.m. stable caesium carrier was used in place of strontium (Table 2). Tests were run in triplicate.

The results of these experiments are listed in Tables 1, 2 and 3. As can be seen from the tabulated data, the distribution coefficients (K_d) for a given radionuclide in the growth phase or leach phase compared with the blank phase, in exchange on to the soil, are very similar. This indicates strongly that there is no detectable modification of the extent of exchange by cycling the radionuclide through the algal cells. There was a considerable difference in the K_d values for the exchange of strontium and caesium on the soil, that for strontium-85 being 3 and that for caesium-137 being 75 (Table 1). The low K_d for strontium is due to competition from calcium for exchange sites on the soil. In this system, the exchange of caesium is not appreciably affected by the calcium concentration. The relatively high K_d's for caesium are due to the marked difference for this element shown by the 10 Å clay minerals such as illite, the predominant clay mineral in this soil.

Table 2. Uptake of Radionuclides by Algae

Radionuclide	*p*H	μc. added	μc. on algae	Uptake (per cent)	m.equiv. inert carrier added	m.equiv. inert carrier on algae	Sr/Ca liquid	Sr/Ca algae	Enrichment factor
Strontium-85	3·5	45	3·51	7·8	$11·4 \times 10^{-2}$	$8·90 \times 10^{-3}$			
	7·0	45	3·46	7 7	$11·4 \times 10^{-2}$	$8 78 \times 10^{-3}$			
	9 0	45	3 78	8 4	$11 4 \times 10^{-2}$	$9·58 \times 10^{-3}$			
Calcium-45	7·0	42	0·13	0 31	25·0	$3·91 \times 10^{-2}$	$4·57 \times 10^{-3}$	$2 33 \times 10^{-1}$	50·8
Caesium-137	3 5	20	1·0	5 1	$3 76 \times 10^{-2}$	$1 92 \times 10^{-3}$			
	7 0	20	0 98	4·9	$3 76 \times 10^{-2}$	$1 84 \times 10^{-3}$			
	9·0	20	0·99	5 0	$3·76 \times 10^{-2}$	$1·88 \times 10^{-3}$			
Calcium-45	7·0	47	0 20	0·43	25·0	$5·3 \times 10^{-2}$	$1 51 \times 10^{-3}$	$3 51 \times 10^{-2}$	23 2

The uptake of caesium, strontium, and calcium by the algal mixture is similar to that shown by a strongly cross-linked ion-exchange resin in a system where specific interactions are absent.

The strontium/calcium ratio on the algae was 51 times greater than the strontium/calcium ratio in solution (Table 2). The enhancement of strontium over calcium is in keeping with the fact that for ions of the same valence, the ion exchanger becomes enriched in the ion with the smaller hydrated radius, in this case strontium.

As a rule, ion-exchangers prefer divalent to monovalent ions, and thus calcium should be preferred to caesium. However, the caesium/calcium ratio on the algae was 23 times that in the original solution. The enrichment of caesium is due to the smaller hydrated radius of the caesium ion as compared with the calcium ion. The caesium enrichment factor was half that of strontium because of the difference in valence between these two ions. Thus, in the caesium–calcium system, the size-charge effects are opposed, whereas in the strontium–calcium system the size-charge effects are acting in the same direction.

Table 3

Radionuclide	*p*H	Per cent	μc.
Strontium-85	3·5	6·5	0·23
	7·0	44·6	1·58
	9·0	43 4	1·54
Caesium-137	3 5	20·5	0·20
	7·0	98·0	0·98
	9·0	68 0	0·68

The similarity of the behaviour of the algal system to an ion-exchange resin is further illustrated by the following calculation: the cation exchange capacity for the algal mixture is about $4{\cdot}8 \times 10^{-2}$ m.equiv./g algae based on the total uptake of strontium and calcium. Knowing that $1{\cdot}88 \times 10^{-3}$ m.equiv. caesium/g algae was sorbed in the caesium–calcium system, there should, by difference, be $4{\cdot}6 \times 10^{-2}$ m.equiv. calcium/g algae. The calculated caesium enrichment factor for this system should be 27. These calculated values agree quite well with the measured quantity of $5{\cdot}4 \times 10^{-2}$ m.equiv. calcium/g algae and the measured caesium enrichment factor of 23.

The results for the uptake of strontium and calcium as reported here are not in agreement with those reported by Kevern for the green unicellular alga *Oocystis eromosphaeria*[4]. Kevern found that this alga took up strontium and calcium in direct proportion to the concentration of these elements in solution. The difference in behaviour of the alga in Kevern's system and that for the algal mixture in our system emphasizes Kevern's warning that the behaviour of one algal system cannot necessarily be extrapolated to another.

Regarding the release of strontium and caesium in our system (Table 3), the release of a higher percentage of sorbed caesium than strontium is probably due to the greater mobility of the caesium ions. Thus, strontium ions with their solvated shell should diffuse less rapidly through the algal membrane than the relatively unsolvated caesium ions. Stated another way, the reaction rate for the isotopic exchange of strontium should be slower than that for caesium, thus less strontium should be released.

Although the exchange behaviour of the radionuclides on the soil is not affected by cycling through the algal cell, the algae do concentrate radioactivity from very low levels in the water to rather higher levels in the algal aggregation. One gram of wet algae from the pond tested produced β–γ levels of 100 mrad/h at 1 cm. It is not difficult to imagine a migrant duck eating several grams of this algae. While this might not be hazardous for a single feeding, chronic feeding of resident ducks on a muscle seeker such as caesium might present an actual hazard. Wave and wind action throw algae on to the pond banks where it dries. Dried algae, flaking off the rocks, can become windborne and transported a considerable distance from the pond.

Finally, it appears that the algae not only concentrate radionuclides in their structure, but the release of these nuclides may be retarded for considerable lengths of time, especially in water of low *p*H. This might form a zone of high activity of undesirable magnitude at the soil–water interface, particularly if the pond were drawn down or drained for any reason. None of these hazardous conditions would develop in settling ponds free from algae. For these reasons it is recommended that an algicide be used to prevent the growth of algae in radioactive-liquid disposal ponds. The algicide used should not affect detrimentally the exchange behaviour of the radionuclides on the soil. It is suggested that algicides such as copper sulphate or sodium hypochloride should be used[5,6].

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NATURE OF IMPULSE ORIGIN AND CONDUCTION IN THE MAMMALIAN HEART

RECENTLY, Dr. C. J. G. Wensing[1] has asserted his firm belief that both the origin and conduction of the wave of excitation in the mammalian heart are neurogenic. His article presents no evidence derived from experiments on the living heart and relies entirely on deductions from anatomical investigations. The article contains many misrepresentations of known facts about the nature of excitation and conduction in heart muscle and certain *ad hoc* assumptions which are required to make the mechanism proposed by his work.

Dr. Wensing states that the retardation of the impulse in the *A V* node is generally explained by pointing to the smaller dimensions of the *A V* nodal fibre. This is not true. Our own investigations[2], and those of others[3], have shown that conduction in fibres of the *A V* node is extremely slow and probably decremental in nature. Among the factors which might contribute to the slowness of this conduction is the small diameter of the fibres, but this is only one of many causes (such as different membrane properties, lower resting potential). Similar slowing and/or block of the impulse can occur in fibres of any diameter if there are appropriate changes in transmembrane potential[4].

Dr. Wensing states that it is impossible to show a direct connexion of specialized fibres between the *SA* node and the *A V* node and that "the explanation of the wave-like propagation of the impulse through ordinary muscle fibres is illogical and inadequate". This we believe to be false. Many recent investigations have indicated that there are tracts of muscle fibres which are both anatomically and physiologically specialized and which act as direct pathways from the *SA* node to the *A V* node[5]. But even if this were not so, there has never been any reason to doubt that conduction from one node to the other could occur through ordinary cardiac muscle fibres. Dr. Wensing states that the number of Purkinje

fibres in the myocardium is very small compared with the number of myocardial fibres so that a single Purkinje fibre would have to activate a considerable number of myocardial fibres. This is true. He adds that the occurrence of this is questionable in view of the non-syncytial nature of cardiac muscle. There is no reason to regard cardiac muscle as being non-syncytial. The recent observation that the intercalated disk has many of the anatomical properties of a membrane is paralleled by evidence that the intercalated disk has a low resistance to the flow of ions and current and does not represent a functional barrier to impulse transmission.

Dr. Wensing would seem to believe that fibres of the Purkinje system are the only fibres in the heart which are able to conduct excitation. (We can see no other explanation for the weight he places on his observation that Purkinje fibres are rare in the atrium, cannot be shown to connect the *SA* node and the *AV* node, and must excite many myocardial fibres in the ventricle.)

Dr. Wensing states that the histological structure of the *SA* node and the *AV* node provides no sound basis for explaining their function. In fact many investigators have found the *SA* node and the *AV* node to be histologically distinct from other parts of the heart. We are unable to imagine how one could predict from histological examination that a particular structure had the ability to generate impulses.

Dr. Wensing states that previous investigators have been misled in their identification of the location of the *SA* node by the presence of a nearby structure in the sulcus terminalis which possesses thin fibres and a considerable amount of connective tissue in the interfibrillar meshes. Dr. Wensing, however, identifies the *SA* node as being the area which stains most intensively with his acetylcholinesterase stain. In this conclusion he may or may not be correct, but in no event has he identified the *SA* node of the living heart by any of its functional properties and shown that the site so identified corresponds to the area located by his stains.

Dr. Wensing asserts that the generation of the impulse results from the action of the dense network of autonomic interstitial cells. He further asserts that these cells cause the muscle cells to contract by secreting a "neurosecretory product". We believe that this conjecture lacks any experimental support.

Dr. Wensing supposes that the delay which occurs at the *AV* node results from the fact that the nerve network becomes intensively plexiform and meshlike at the *AV* node. He offers no evidence that conduction in such a mesh would be slow. He also suggests that the network of autonomic interstitial cells between the meshes of this plexus generates the beats which occasionally arise at the *AV* node.

Dr. Wensing remarks that although acetylcholine does not penetrate ordinary nerve fibres it may well penetrate the nerve fibres of which he speaks and that such penetration explains why acetylcholine causes *AV* block. This would seem to be little more than conjectural.

We find, therefore, that the only demonstrative evidence in the article by Dr. Wensing consists of his anatomical descriptions of a network of nerve fibres intimately associated with the *SA* node, the *AV* node and the bundle of His. The rest we believe to be surmise.

Against this we may place the results of some 80 years of investigation which we have reviewed elsewhere[2] and which shows among other things that: (1) Cardiac muscle fibres conduct excitation as if they were syncytially interconnected. (2) The cells of the *SA* node, when investigated with intracellular microelectrodes, behave exactly as they would be expected to if they were the source of the action potential. They show spontaneous depolarization during diastole, and the slope of this spontaneous depolarization changes in the expected way under the influence of all physical and chemical agents which alter the heart rate. (3) Cells which show spontaneous depolarization during diastole are found in those parts of the heart which have long been known to be possible sites of origin of extrasystoles. (4) The spread of excitation from the *SA* node to the *AV* node has been mapped by the use of intracellular and extracellular recording. A full and complete parallelism exists between the spread of excitation and the spread of the action potentials in the appropriate cardiac cells. (5) Exactly analogous arguments apply to the spread of excitation in the *AV* node, the His-Purkinje system and the ventricular myocardium. In each case the spread of excitation corresponds exactly to the spread of the action potentials of the appropriate cells. (6) The electrical activity of single cells of the *AV* node, like that of single cells of the *SA* node, changes in exactly the way one would expect if transmission through those cells determined both the *AV* nodal delay and the variations in that delay which result from the action of physical and chemical agents.

In brief, we believe that Dr. Wensing has ignored an extensive, complex and extremely well-documented set of facts which have provided an almost complete picture of how the cardiac impulse arises, how it spreads and how it is influenced by physiological and pharmacological changes. In place of this, he offers a set of facts about the anatomy of certain nerves, a number of apparently incorrect assertions about the traditional view, a number of *ad hoc* assumptions about possible mechanisms, and no experimental information whatsoever.

It should be noted that the nerve network postulated by Dr. Wensing has the following properties: (1) it is heavily concentrated at the *SA* node and generates impulses in the same way as does the *SA* node; (2) it connects the *SA* node and the *AV* node in the same way as do the fibres of the atrium; (3) it is heavily concentrated at the *AV* node and is there subject to slow conduction just as *AV* nodal fibres are; (4) its peripheral distribution is exactly that of the His–Purkinje system.

Dr. Wensing thus replaces the traditional configuration of cardiac cells responsible for impulse generation and conduction in the heart by a nerve network of identical configuration and functional properties. He further requires that a conjectural system of autonomic interstitial cells transmit excitation from this nerve network to cardiac cells immediately adjacent to it throughout its entire course.

There would seem to be no reason for regarding impulse origin or conduction in the mammalian heart as neurogenic.

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Electrophysiological investigations of the specific conductive tissue in the heart are based on the derivation of action potentials. These are physical phenomena which can be initiated by chemical changes. If one rejects this assumption, a further discussion has no sense. The chemical processes which cause these changes in potential may themselves be initiated by intrinsic (that is, inherent to the fibres) or by extrinsic (that is, beyond the territory of the proper fibre) factors.

The crucial question is, what must be considered the initiator of the heart beat? Is it the chemical processes,

which secondarily cause changes in potential, or is it the changes in potential themselves? Prof. Hoffman and Dr. Cranefield's arguments will be discussed in sequence.

They state that the small diameter of the nodal fibres is not a valid argument for the *A V* delay in itself, although they admit that the specific diameter may play a part in the entire process. In Paes de Carvalho's paper[1], as well as in my own[2], the greater diameter of the nodal fibres was emphasized. When the data of Paes de Carvalho and Truex[3] are compared, it appears that scarcely any difference exists between the diameter of the nodal fibres in the stratum which reveals the greatest delay, on one hand, and the atrial fibres, on the other. I fully agree with Prof. Hoffman and Dr. Cranefield that there are more causes for this great delay, among others, the various membrane-characteristics. From papers cited by them[2,4] it would seem that these characteristics vary, depending on the nature and period of the excitation and, moreover, on pharmacological influences. The concentration of acetylcholinesterase is highest exactly in the middle part of the node. The question should then arise of whether these membrane-characteristics are specifically inherent to the cells under consideration, or initiated by specific reactions of these cells which are induced by their environment.

Prof. Hoffman and Dr. Cranefield start axiomatically from the assumption that conduction is solely inherent to specific fibres. In this respect, one of Prof. Hoffman's experiments[5] deserves closer attention. He states that in preparations of Purkinje fibres, the transmission of impulses may be delayed or even blocked if adequate changes occur in the transmembranous potential. Moreover, in his paper he points out that during an intracellular excitation of a Purkinje fibre, the response at the second electrode in the fibre, situated at a greater distance from the source of the impulse, is registered in advance of one closer to this source. When examining the experiment more closely, it is assumed that it is possible to derive potentials from isolated Purkinje fibres with mutual distances of more than 1 mm. The registration of intracellular potentials, with which I am familiar, is a considerable achievement; but the registration of potentials from a single fibre at so great an interval is even greater than that.

The foregoing implies that the action potentials registered by Prof. Hoffman and Dr. Cranefield will be initiated by various Purkinje fibres. Moreover, when applying intracellular stimulation, there must be a subsequent conduction perpendicular or oblique to the longitudinal axis of the fibre. In this case, more structures must be penetrated than a single intercalated disk alone; that is, two cell membranes and the intercalated connective tissue. It seems improbable that this conduction occurs through anastomoses between the fibres, the more so since anastomoses are seldom encountered between Purkinje fibres which show a straight course. Notwithstanding this fact, there would seem to be the possibility of conduction and the only factual substrate appears to be the intercalated nervous network.

There is a considerable number of papers on the occurrence of specialized muscular tissue in the atria, but the number of those describing direct anatomical connexions between both nodes is very limited[6,7]. A closer examination of these papers shows that they are vulnerable to considerable criticism. I have already listed the physiological data supporting direct conduction.

According to recently published data[8], the separation between cardiac muscle cells has no physiological bearing, which suggests that I probably attached too much importance to the function of the intercalated disk. The question arises, however, of whether the existence of an electrical synapse between various bundles of muscular tissue is feasible.

Prof. Hoffman and Dr. Cranefield agree with me that the histological structure of both nodes does not constitute a sound basis for explaining their function, as it would be difficult to derive a function from a structural basis. When describing the nervous network in the *SA* node, however, I made a comparison with the marginal organ of the jellyfish. This organ shows not only a close morphological resemblance to the *SA* node, but also a functional one.

On studying the relevant literature, one arrives at the conclusion that a small number of investigators has been misled in their identification of the *SA* node[9]. I do not hesitate, therefore, to identify the described nervous network with the *SA* node, the more so since it is a simple matter to distinguish between the nervous network and the nodal cells in Champy–Coujard preparations. Moreover, the exact location of the *SA* node in two pig hearts has been determined by direct electrophysiological registration with the aid of the Durrer-team.

I should like to emphasize that the nerve stainings which were applied to trace the nervous network have been based on the visualization of compounds occurring in that network; that is, catecholamines and acetylcholinesterases.

There are no experimental data on the conduction in those structures which I described in the *A V* node, but it is well known that the velocity of conduction depends to some extent on the diameter of the fibre. In the central part of the plexus the diameter is smaller, whereas the distance to be covered in a plexus is considerably longer.

The assumption that the *A V* rhythm is initiated by the nervous network in the meshes between the plexus was, in fact, based on the same considerations as were given when the *SA* node was dealt with. It would be inconsequent to interpret functionally the structure of the *SA* node and to ignore that in the *A V* node.

Prof. Hoffman and Dr. Cranefield are partly correct in blaming me for not taking sufficiently into account the existing ideas on excitation and conduction of impulses. On the other hand, however, I did not, and do not, ignore these concepts. It is beyond any doubt that specialized muscular tissue has a definite function in the conductive process, but I repeat that it is my firm belief that the nervous component is of primary importance. The precise interaction of both structures, however, is not yet known. In this respect it is appropriate to refer to the great histological differences which occur between the conductive tissue in ungulates and carnivores.

I agree with Prof. Hoffman and Dr. Cranefield that it is difficult to derive functional data from a histological basis. It was for this reason, therefore, that my paper was entitled: "Evidence for . . . etc.". I emphasized the fact, however, that it is also intolerable to ignore histological data when interpreting electrophysiological results. I cannot accept Prof. Hoffman and Dr. Cranefield's views that the nervous structures referred to here have no excitative or conductive function.

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HYDRODYNAMIC ANALYSIS OF DOLPHIN FIN PROFILES

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DOLPHIN fins have well-streamlined cross-sections that may be useful as hydrofoils or airfoils designed to operate in the Reynolds number range around 10^6. Possible applications might be torpedo fins and propeller or pumpjet blades, boat propellers, rudders, keels, struts and hydrofoils, helicopter rotor blades, windmill vanes, and sailplane wings and control surfaces.

Photographs of cross-sections of one dolphin tail fluke and two dorsal fins were obtained from animals that had died of natural causes. The cross-sections were obtained about midway between the bases and the tips of the three fins. Fin offsets were measured from the photographs and plotted on a 2-m scale. The best curves possible were drawn through the measured points; the maximum deviation being 0·2 per cent or about 0·02 cm. About eighty points were read from the 'best' curves and run on an IBM 7094 computer at the Naval Ordance Test Station to obtain pressure distribution using the Douglas two-dimensional airfoil programme which assumes potential flow. Pressure distribution is generally the most significant factor in analysing the performance of hydrofoil or airfoil cross-sections.

The profiles and pressure distributions of the three fins are shown in Fig. 1. Fin *A* is a tail fluke section of a common dolphin (*Delphinus bairdi*), fin *B* is a dorsal section of a Pacific striped dolphin (*Lagenorhyncus obliquidens*), and fin *C* is a dorsal section of a Dall porpoise (*Phocoenoides dalli*). The lower portion of Fig. 1 illustrates the upper half of the cross-section of each fin; the semi-thickness to chord ratio, y/c, is plotted against chord length from the leading edge. The upper portion of Fig. 1 shows the pressure distribution where:

$$C_P = \frac{P - P_0}{\rho\, V_0^2/2}$$

and P_0 is the depth pressure, P the local static pressure on the fin, ρ, mass density of the flowing fluid, and V_0, the fluid speed relative to the fin.

The fin chord lengths are between 12 and 18 cm long. The fin offsets are listed in Table 1. The effect of angle of attack on the pressure distribution of fin *A* is shown in Fig. 2. Maximum thickness and its location, leading edge radius, trailing edge thickness, and minimum pressure coefficient, C_p, and its location are listed in Table 2.

The three fins are similar in that their maximum thicknesses are all located between 32 per cent and 36 per cent chord length of the leading edge; all are fairly thick (15·2 per cent to 21·1 per cent chord length) and have a relatively large leading edge radius (2·4 per cent to 4·2 per cent chord

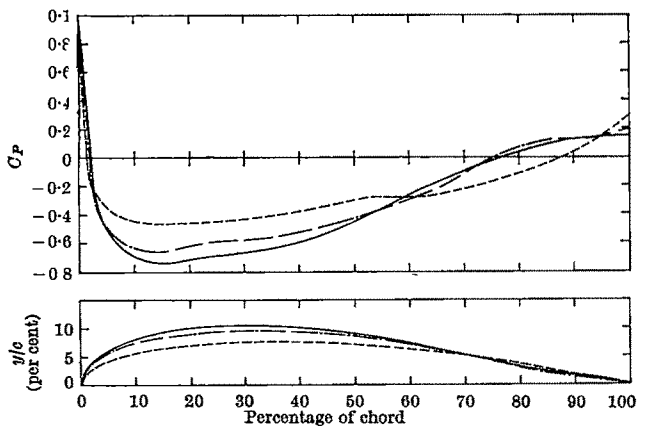


Fig. 1. Profiles and pressure distributions of three dolphin fins. *A*, ———, Tail fluke of common dolphin, *B*, – · – · –, dorsal fin of Pacific striped dolphin, *C*, - - - - - -, dorsal fin of Dall porpoise

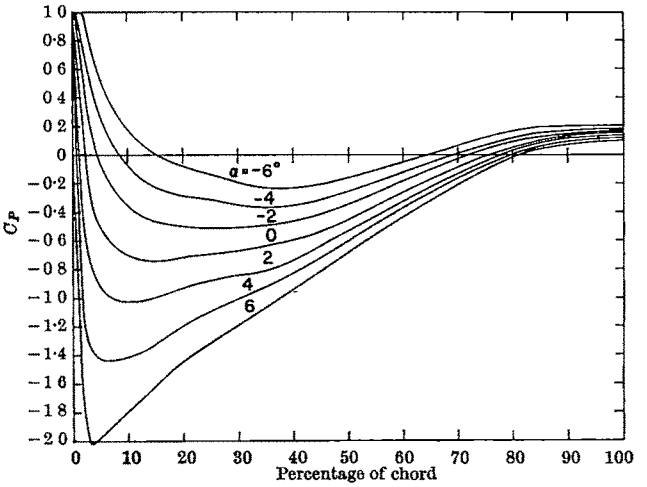


Fig. 2. Effect of angle of attack on the pressure distribution of Fin *A*

length); their minimum pressures are located between 14 per cent and 16 per cent chord length of the leading edge; all have a fairly long region of gentle adverse pressure gradient (increasing C_p) followed by a steep adverse pressure gradient; all trailing edges are relatively thin, and tail fluke *A* and dorsal fin *B* have cusped trailing edges. Fins *A* and *B* are surprisingly similar since they are cross-sections of different kinds of fins from different species. Fin *C* is a thinner section and came from Dall porpoise, reported to be the fastest of the three species[1].

All fins were generally symmetrical although some warpage appears in the photographs near their trailing edges. Fin offsets for the computer study are based on the semi-thickness and, therefore, symmetrical sections. The pressure distribution is strongly affected by small changes in fin profile; consequently, some of the minor waviness in the pressure distributions of Figs. 1 and 2 may not be real.

Analysis of the fin pressure-distributions indicates that the fin sections are ideally designed for operation at Reynolds numbers, R_e, in the vicinity of 10^6 where these fins operate. The low-drag airfoil sections designed for airplane wings operating at R_e around 10^7 and above are relatively poor in the range of $R_e = 10^6$, since the laminar boundary layer extends too far into the adverse pressure gradient region and separates, causing excessive drag[2].

Table 1. Dolphin Fin Offsets

Distance from nose (% chord)	Fin semi-thickness (% chord)		
	Fin *A*	Fin *B*	Fin *C*
0	0·00	0 00	0·00
2	3·97	3·80	2·84
5	6·07	5 71	4·24
10	8 05	7·44	5·61
15	9·28	8·51	6·48
20	9·96	9·12	7·04
30	10·53	9·65	7 62
40	10·17	9 46	7·56
50	8·98	8·60	7·42
60	7·20	7·19	6 43
70	5 22	5·28	5·35
80	3·16	3·22	3·85
90	1·57	1·74	2 08
100	0 23	0 45	0·14

Table 2. Dolphin Fin Characteristics

Fin designation	Maximum thickness (% chord)	Distance from nose to max. thickness (% chord)	Leading edge radius (% chord)	Trailing edge thickness (% chord)	Minimum C_P	Distance from nose to min. C_P (% chord)
A	21·1	32	4·2	0·46	−0·74	15
B	19·3	33	3·8	0 90	−0·66	14
C	15·2	36	2·4	0 28	−0·46	16

The fin pressure-distributions are not similar to the more commonly known airfoils[2,3]. The fin profiles are a compromise between the less-known special airfoils *FX*05-191 by Wortmann[4] and *EA*6(-1-)-018 by Eppler[5] designed for the R_e region of 0·5–3·0 × 10^6. The differences between the designs of Wortmann and Eppler are sufficiently great to indicate that a compromise design is significant and may have unexpected beneficial characteristics. The favourable pressure gradient near the nose and the gentle adverse pressure gradient to trigger turbulence, combined with the cusp-shaped pressure gradient of fins *A* and *B* to provide a near-uniform boundary layer form parameter, appear to optimize the length of laminar flow and eliminate both laminar and turbulent boundary layer separation. The difference in adverse pressure gradient shape of fin *C* from those of fins *A* and *B* may be due to its reduced thickness ratio. Thinner sections of a given airfoil family have reduced adverse pressure gradient; consequently, the adverse pressure region can be shifted backwards and modified without causing turbulent boundary layer separation.

The thicknesses of the trailing edge of the fins lie between 0·28 per cent and 0·90 per cent chord length. Airfoils experience essentially no drag increase for thickness ratios up to about 0·5 per cent chord length at R_e around 10^6 and only a small drag increase at 0·8–1·0 per cent thickness ratios[6]. The photograph of fin *B* showed evidence of slight deterioration, so its trailing edge may not be as thick as reported.

The thick-fin sections are probably needed primarily for strength. Thick sections also permit greater angle-of-attack, α, variations without producing excessive minimum pressure peaks at the leading edge that can result in flow separation and cavitation. Fig. 2 indicates that two-dimensional fin angles of attack up to 4° (7° for a fin aspect ratio of 4·0) should be permissible without producing separation and high drag. This angle is in the reasonable range of maximum α for propulsion by fluke undulation and for dorsal fin angle of attack resulting from manoeuvring. During acceleration at low speed, the fluke angle of attack could be considerably greater since added drag is not so important and the fluke would not stall until α reached about 20°.

From the point of view of cavitation, fins *A*, *B*, and *C* at $\alpha = 0°$ should reach speeds of 16·3 m/sec (31·6 knots), 17·3 m/sec (33·6 knots), and 20·7 m/sec (40·2 knots), respectively, before cavitating near the water surface. However, a realistic angle of attack is about 3° or 4°. Fin *A* would cavitate at 13·9 m/sec (27·0 knots) at $\alpha = 2°$, 11·7 m/sec (22·7 knots) at $\alpha = 4°$, and 9·9 m/sec (19·2 knots) at $\alpha = 6°$. It is likely that cavitation would be avoided by dolphins since cavitation is known to produce considerable surface damage to metals and might be painful to dolphins. If the fin profiles had been thinner or if the maximum thickness had been more rearward, the nose radius would have been smaller, making cavitation begin at much lower speeds at $\alpha = 4°$ but at higher speeds at $\alpha = 0°$.

Maximum speeds of tame dolphins trained for speed runs are 7·7 m/sec (15 knots) for the Pacific striped dolphin[7], 8·3 m/sec (16·1 knots) for the Pacific bottlenose dolphin[8], and 11·0 m/sec (21·4 knots) for the Pacific spotted dolphin[9]. Maximum top speed reported in the technical literature for the common dolphin is 10·3 m/sec (20 knots)[10].

I thank Dr. Samuel Ridgeway for sectioning the dolphin fins and supplying photographs and Mr. Dave Nelson for advice and for helping to operate the computer programme.

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LOCALIZED HYPERCHONDROPLASIA INDUCED BY CRUDE PAPAIN ADMINISTERED TO MICE

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MICE can tolerate a single injection of up to 50 mg/kg body-weight of crude papain when injected intravenously; this is significantly higher than the amount of papain that can be tolerated by rabbits or guinea-pigs[1]. Two injections of papain given to mice on consecutive days usually result in the closure of the proximal tibial epiphyseal plates[1].

This article presents results of investigations of the effects of papain on the epiphyseal plate in tibiae when relatively low doses of papain are administered to mice.

Crude papain was prepared fresh in 0·9 per cent saline, and the solution was filtered before use. Female Charles River white mice weighing approximately 25 g were divided into three groups: group I, ten control mice were injected subcutaneously with 0·10 c.c. 0·9 per cent saline for 5 consecutive days; group II, ten mice were injected with 1·0 mg of papain in 0·10 c.c. 0·9 per cent saline for 5 consecutive days; group III, sixteen mice were injected with 1·0 mg of papain in 0·10 c.c. 0·9 per cent saline on the first and third day of the experiment. All the mice were killed on day 21. The tissues were prepared for histological investigation using Peterfi's double-embedding method and stained with Mallory's stain or periodic acid–Schiff (PAS) as previously described[2].

In the control group, the epiphyseal plates of the tibiae and femora comprised orderly arranged columnar rows of chondrocytes. The proliferating cells were transversely compressed with deeply staining nuclei and cytoplasm and ranged from 8 to 14μ in length. The mature and senescent chondrocytes had vesicular nuclei and vacuolated cytoplasm with some PAS-positive granules. They were hypertrophied and attained sizes ranging from 11–20μ. The metaphysis was comprised of longitudinally arranged trabeculae of bone with calcified cartilage cores (Figs. 1–3).

The group given 1 mg of papain daily for 5 consecutive days was the only one of the three groups which suffered from the toxic effects of papain. In this group, four out of ten mice died before the termination of the experiment. The most striking feature of this group was the almost complete destruction of the epiphyseal plates of both femora and tibiae in all the remaining mice. The few remaining chondrocytes were irregularly arranged (Figs. 4–6). These features are in accord with earlier published descriptions[1]. The metaphysis was comprised of few

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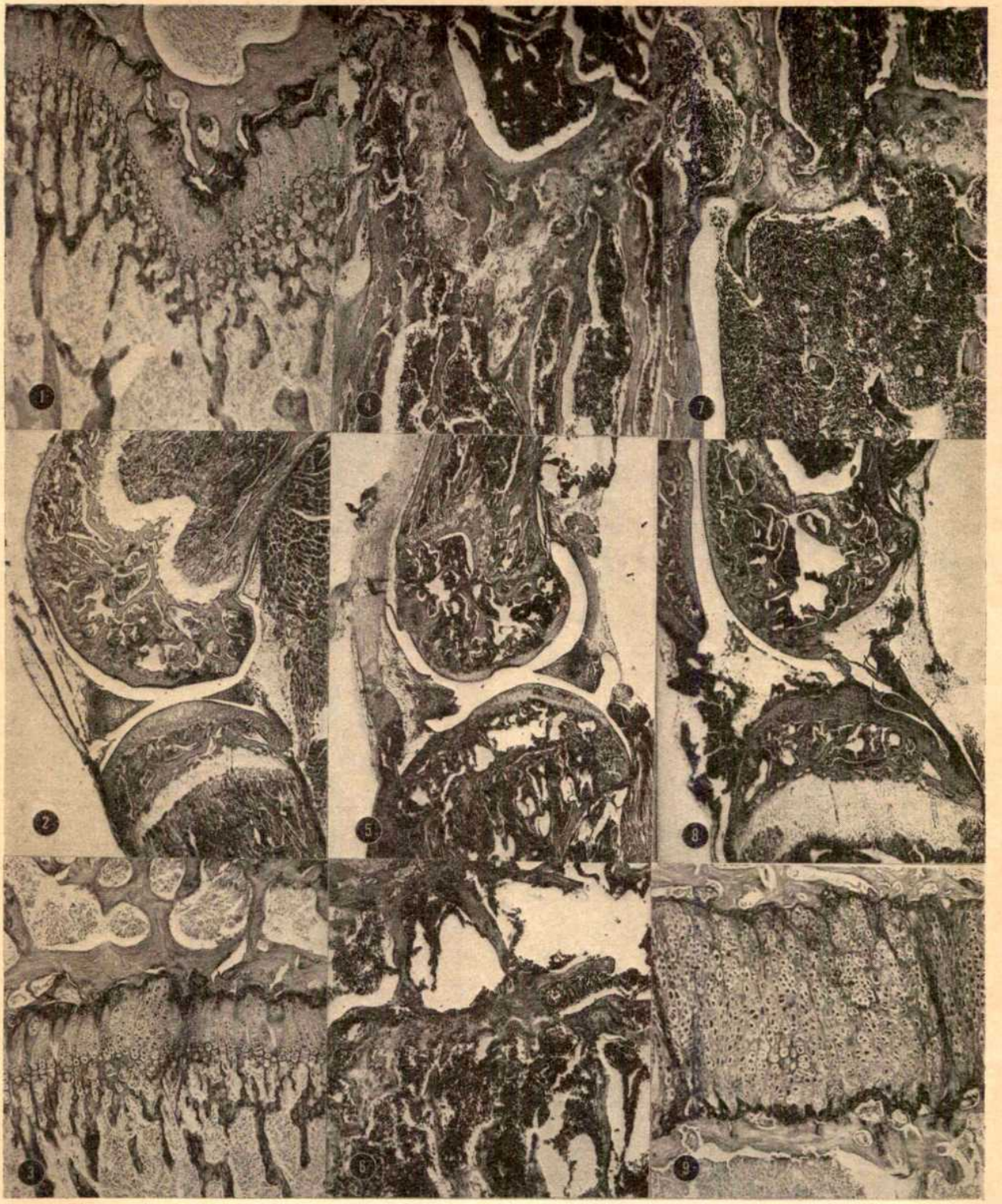


Fig. 1. Distal femoral epiphysis from same normal control mouse as shown in Fig. 2. PAS stain. (×61)

Fig. 2. Knee area showing femur above and tibia below. Mallory's stain. (×17)

Fig. 3. Proximal tibial epiphysis from same normal control mouse as shown in Fig. 2. PAS stain. (×61)

Fig. 4. Distal femoral epiphysis of same mouse as shown in Fig. 5. Note almost complete destruction and disorganization of epiphyseal plate. Mallory's stain. (×61)

Fig. 5. Knee area showing severely damaged epiphyseal plates. Taken from a mouse given a total of 5 mg of papain subcutaneously. Mallory's stain. (×17)

Fig. 6. Proximal tibial epiphysis from same mouse as in Fig. 5. Mallory's stain. (×61)

Fig. 7. Distal femoral epiphysis from same mouse as shown in Fig. 8. Note severely damaged epiphyseal plate and disappearance of metaphyseal trabeculae. Mallory's stain. (×17)

Fig. 8. Knee area showing atrophied femoral epiphyseal plate (above) and hyperchondroplastic tibial epiphyseal plate (below). Taken from a mouse treated with a total of 2 mg of papain given subcutaneously. Note areas of recovery on lateral margins of the plate. Mallory's stain. (×17)

Fig. 9. Proximal tibial epiphyseal plate showing increased number and sizes of proliferating chondrocytes. Note transverse stratum of bone under the plate. PAS stain. (×61)

trabeculae which, when present, were somewhat heavier than the control group.

In the mice given 1 mg of papain per injection on two alternate days, the femoral epiphyseal plates were bilaterally destroyed in all the mice in this group (Fig. 7), and had a similar appearance to those of the 5 mg papain-treated group. However, in the tibiae, only six out of sixteen mice presented this bilateral pathology. In the remaining ten mice, the tibiae had greatly widened epiphyseal plates due to hyperchondroplasia of the proliferating chondrocytes (Figs. 8–9). This manifestation was observed in both right and left tibiae in four of the mice, and only in the left tibiae in the remaining six. The proliferating chondrocytes were not only significantly increased in number but also in size (8·5–29μ), and became irregularly arranged. The cells were deeply staining, with PAS-positive granules in the cytoplasm, and were not compressed as in the normal mice but were variously shaped (Fig. 9). Only a few mature and senescent hypertrophic cells were present, and they were located in areas adjacent to the active invasion of metaphyseal capillaries. These cells had vesicular nuclei and vacuolated cytoplasm containing PAS-positive granules; they were larger than the same cell types in the controls, ranging from 20 to 29μ in size. The metaphysis was comprised of a

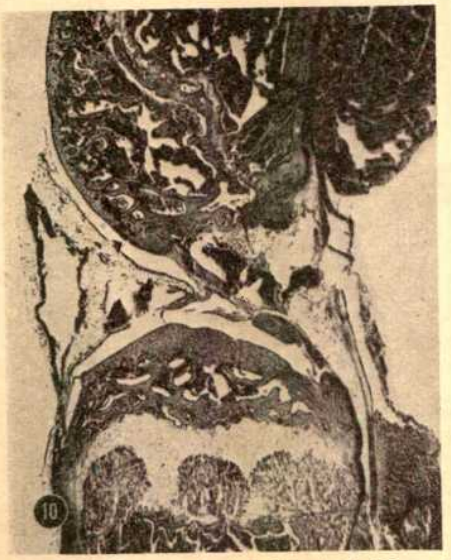


Fig. 10. Knee joint of a mouse treated with a total of 2 mg. of papain subcutaneously. Note destroyed femoral epiphysis above and tibial epiphysis below in recovery phase. Mallory's stain. (×17)

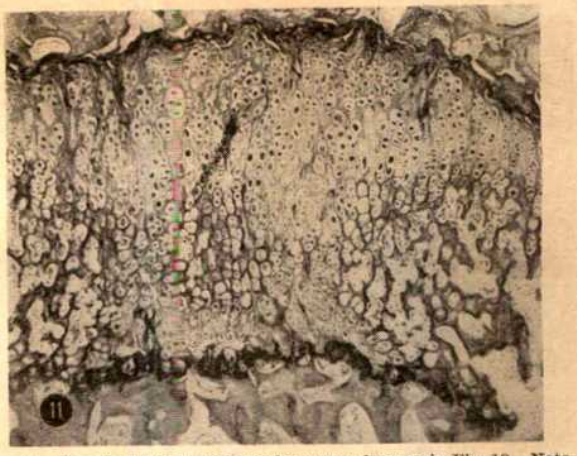


Fig. 11. Proximal tibial epiphysis from same knee as in Fig. 10. Note the presence of a number of senescent cartilage cells with concomitant destruction of those cells. PAS stain. (×61)

transverse stratum of bone which formed adjacent to the undersurface of the cartilaginous plate. The longitudinal trabeculae had all but disappeared, indicating an almost complete inhibition of osteoblastic activity. This was similar in appearance to the growth arrest phenomenon which was due to nutritional deficiencies[3].

In some of the mice tibiae, papain no longer appeared to interfere with the metabolism of the chondrocytes. This was manifested by the reappearance of maturing and senescent epiphyseal chondrocytes with concomitant invasion of capillaries to break down the chondrocytes (Figs. 10 and 11), and is similar to the recovering phase of Park's nutritional deficiency experiments[3]. Thus, the epiphyseal plate began to have a normal appearance again.

Low doses of papain administered to mice appear specifically to alter the normal metabolism of specific chondrocytes, yet to allow them to proliferate. The process of orderly maturation and senescence is interfered with, but the mechanism by which this occurs is not known. I can only concur with Park's conclusion[3] that the "capillary-osteoblast complexes had been powerless to invade the cartilage until the maturation of the cartilage cells had been completed"[3].

This investigation was supported by a U.S. Public Health Service grant *AM*–02745–07, from the National Institutes of Arthritis and Metabolic Diseases.

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SOMATICALLY UNSTABLE MUTANTS OF *Aspergillus nidulans*

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SOMATICALLY unstable mutants have, for obvious reasons, been largely ignored in mutation studies and excluded from breeding experiments. Nevertheless, it is becoming clear that they may constitute an important and frequent class of hereditary changes[1–9], and their occurrence and properties must be accounted for in any comprehensive theory of mutation. In the *Aspergilli* such mutations are relatively common and in a few cases their origin has been traced to extrachromosomal[10–13] and chromosomal[14] causes. To pursue the problem further, unstable mutations have been specifically sought in *Aspergillus nidulans* both spontaneously and following treatment with mutagens. The present article describes the frequencies and some of the properties of unstable morphological mutants that occur spontaneously in this species.

To determine the frequency with which unstable mutants arise, conidia, hülle cells and ascospores of Birmingham wild-type isolates, 1, 26, 27 and 36, and their white and yellow spore colour derivatives (obtained by ultra-violet irradiation) have been examined. These isolates belong to heterokaryon compatibility group *B* (ref. 15), and as such are thought to have basically similar genetic backgrounds[16]. Since their isolation, from the Birmingham area, all have been kept in stock tubes at 5° C, being propagated by mass hyphal transfers every 3 months.

All colonies were grown on a modified Czapek minimal medium (containing KH_2PO_4 instead of K_2HPO_4), solidified by New Zealand Agar (supplied by G. T. Gurr, Ltd., London), the plates being incubated at 25° C. Conidial suspensions were obtained from 10-day-old colonies (propagated from the stock tubes by mass

Table 1. Types and Frequencies of Mutants obtained from Isolates belonging to Heterokaryon Compatibility Group B. (The Data for Each Isolate are pooled over its Different Spore Colours)

Isolate	Tissue	Total No. of colonies	Percentage of mutants	Types and No. of unstables							Total No. of unstables	No. of stables
				Crimson	*Mycelial*	*Fawn*	*Minute* types			Others		
							a	*b*	*c*			
1	Conidia	13,969	0·179	2	6		5	1	6	1	21	4
	Hülle cells	18,946	0·101	7		1		5	4	2	19	1
	Ascospores	14,458	0·263	5	7	1	7		14	3	37	1
26	Conidia	19,128	0·120	2	2	2	3	1	7	4	21	2
	Hülle cells	14,506	0·041		4						4	2
	Ascospores	19,270	0·322	10	13	1	21	5	7	5	62	
27	Conidia	15,692	0·110	3	1		1	3	5	3	16	2
	Hülle cells	11,097	0·090	7		2			1		10	
	Ascospores	19,366	0·340	18	19	1	13	5	5	1	62	4
36	Conidia	16,771	0·143	4	5				5	4	18	6
	Hülle cells	19,035	0·078	2		1		7	4		14	1
	Ascospores	27,158	0·269	14	17	3	18	2	14	5	73	
	Totals	210,702	0·180								357	23

hyphal transfers). Mature perithecia were sampled individually, twenty being selected at random. Attached conidial heads were removed and the hülle cells, obtained by rolling the perithecium over the surface of an agar plate, were spread. The clean perithecia were then burst individually and four samples of ascospores were taken from each. All plates were examined for abnormal colonies after 7 days' incubation and such colonies were tested by growing samples of their conidia and by making mass hyphal transfers. Mutants were classified as unstable if, on propagation by conidia, the colonies produced were of two types, mutant and normal, and if normal sectors were produced by the mutant colonies.

The frequencies and types of mutants obtained are summarized in Table 1, and the main features are as follows: (1) The mean frequency of mutant colonies over all strains is $1{\cdot}8 \times 10^{-3}$. (2) The relative frequency of unstable to stable mutants is 16 : 1. (3) Ascospores consistently give the greatest frequency of unstable mutants. (4) There are only four general classes of unstable mutants, namely, *Crimson*, *Mycelial* (not to be confused with the *Mycelial* described by Roper[17]), *Fawn* and *Minutes*. The minute class can, however, be subdivided into 3 phenotypically distinct classes each having in common a much slower growth rate than the normal. (5) There is no evidence that the frequency of unstable mutants differs between the various spore colour strains of the same wild isolate. The data, given in Table 1, have therefore been pooled over the spore colour variants.

The mutants have the following descriptions:

Crimson, white when young, turning through orange to a bright crimson at about 7 days. Conidia are few and smaller than normal. Growth rate is approximately 0·8 of normal.

Mycelial: white mycelium with golden patches of hülle cells especially in the centre. Conidia few and very small. Growth rate is approximately 0·9 of normal.

(The foregoing two mutants were first described by Croft[13].)

Fawn: fawn colonies with fewer, slightly smaller conidial heads than on a normal colony and also very small abnormal heads. Growth rate is approximately 0·8 of normal.

Minute, type a: this is phenotypically identical to the minute variant described by Faulkner and Arlett[18]. Growth rate is approximately 0·4 of normal. *Type b*: pinkish with a knobbly appearance due to stunted hyphal growth and a cover of hülle cells. Conidia few and very small. Growth rate is approximately 0·3 of normal. *Type c*: this is density dependent, greyish if small but covered with hülle cells and pale yellow if large, but at all densities they have characteristic abnormal heads. Growth rate is approximately 0·4 of normal.

The 'other' category of Table 1 consists of unstable mutants which only occur very rarely.

A comparable investigation has been carried out on Birmingham isolate 29 and strain 29*y*. These belong to heterokaryon compatibility group *A* (ref. 15) and are therefore thought to have a basically different genetical background from group *B* (ref. 16).

The results show that unstable mutants are also present in this group with an equally high frequency (Table 2). They are, however, phenotypically different from those obtained from heterokaryon compatibility group *B*. Here the relative frequency of unstable to stable mutants is 24 : 1, with ascospores again giving the greatest frequency. There is, however, a significant difference (P for $\chi^2 < 0{\cdot}001$) for the total number of mutants between the two spore colours.

The high frequency of unstable mutants recovered in the samples of spores and cells from the colonies of the thirteen strains examined could arise from two alternative effects: (i) a high mutation rate to the unstable state; (ii) a low mutation rate coupled with the accumulation of unstable mutants within the colonies propagated by mass hyphal transfers because they are either not selected against, or are actually favoured by selection during the growth of a colony.

To distinguish between these alternatives two types of colony from the same strain were examined for the frequency and types of unstable mutants they gave rise to when propagated by conidia. These were (*a*) colonies that had been maintained entirely by mass hyphal transfer from the time the isolates were first obtained from the wild, and (*b*) colonies with normal phenotypes that had been initiated from single conidia 7 and 10 days before the frequency of unstable mutants was determined. It is possible that in the first type of colony (*a*), unstable mutants accumulated over a period of many years. In the second type of colony (*b*), however, unstable mutants could only have arisen since the colony was initiated by a wild-type haploid spore, either 7 or 10 days previously. Irrespective of the method of propagation and their age, all the colonies yielded the same range of types of unstable mutants (Table 3). A χ^2 analysis of the total frequency of unstable mutants showed no significant difference between them ($\chi^2_2 = 0{\cdot}053$). Thus there is no evidence that these mutants are accumulating in the mass hyphal cloned material.

Two further sets of observations support this conclusion. First, because all the unstable mutants have slower rates

Table 2. Percentage Mutants obtained from Birmingham Isolate 29 and its Yellow Spore Colour Derivative

Strain	Tissue	Total No. colonies	Per cent unstables	No. stables
29 Wild-type (green)	Conidia	5,979	1·154	4
	Hülle cells	7,044	0·71	
	Ascospores	407	2·457	
29*y*	Conidia	6,681	0·045	
	Hülle cells	3,686	0·054	
	Ascospores	3,826	0·105	
Totals		27,623	0·499	

Table 3

Type of colony sampled	Total No. of colonies	Percentage of mutants	Types and No. of unstables							Total No. of unstables	No. of stables
			Crimson	*Mycelial*	*Fawn*	*Minute* types			Others		
						a	*b*	*c*			
a	6,629	0·196	2	1	1	1	3	5		13	
b_1	4,644	0·237	1	3			2	3	1	10	1
b_2	2,919	0·445	2	5	1		4	1		13	

Types and frequencies of mutants obtained from colonies of the same isolate propagated in different ways: *a*, by mass hyphal transfers of an old clone, b_1, by single conidia 7 days before sampling; b_2, by single conidia 10 days before sampling.

of growth than the normal strains from which they arise (see earlier) they are continually being overgrown by the normal sectors to which they invariably give rise. Secondly, the mutants, once they arise, are almost impossible to retain by any normal method of propagation. Indeed, it is only possible to retain the mutant phenotype for more than a single propagation by continual propagation with conidia from the mutant areas of the mutant colonies.

In view of these findings it seems extremely unlikely that there has been any accumulation of unstable mutants in the long-standing isolates. On the contrary, it seems more likely that the estimate of the frequency of unstable mutants is minimal in that many mutant spores and cells may revert to normal before they produce a scorable macroscopic colony. Thus it can be assumed that the high frequency of unstable mutations is due to a high mutation rate.

These investigations have shown that the spontaneous rate of mutation to an unstable state is very high. Thus the highest rates observed in these experiments, which are probably minimal estimates, are $5{\cdot}8 \times 10^{-4}$ for *Crimson*, $6{\cdot}9 \times 10^{-4}$ for *Mycelial* and $7{\cdot}3 \times 10^{-4}$ for minute *type a*, all from ascospore samples (Table 1). Apart from the exceptionally high spontaneous mutation rate to the vegetative petite condition in certain strains of yeasts, these spontaneous mutation rates for the unstable mutants are among the highest recorded (see table in ref. 19).

A further similarity with vegetative petite is that these same types of unstable mutants can be specifically induced with mutation rates as high as 100 per cent with various acridine dyes[13,20]. Whether or not these mutants are also like vegetative petites in being extrachromosomal mutations is at present being investigated. The evidence so far is equivocal, neither fitting a conventional extrachromosomal explanation nor a conventional chromosomal one. Whatever the outcome of any further analyses, it is already clear that the unstable mutants possess many novel properties in addition to their high mutation rate.

I thank Prof. J. L. Jinks for guidance and criticism during the preparation of this report, and the Science Research Council for a research studentship.

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CHANGES IN MITOTIC ACTIVITY DURING EARLY CALLUS DEVELOPMENT

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WHEN an inoculum is taken from a tuber of Jerusalem artichoke, *Helianthus tuberosus*, and transferred to a suitable medium a callus culture is established, the development of which depends on vigorous cell division. For the analysis of at least the early stages of the process, the state of the cells with respect to the division cycle at the time of excision is a matter of some significance and it is this which is the subject of this article.

The observations that are presented have been made with cultures grown on an agar medium which contains a mineral salts mixture, sucrose, 2 : 4-dichlorophenoxy acetic acid and coconut milk. Essentially similar results have also been obtained using a liquid medium of the same composition[1]. After a lag phase (the length of which depends on the age and state of the tuber from which the inoculum is taken) rapid division begins. These early divisions are in the superficial layers only[2]. The first divisions occur in the outermost layers of cells and with these the long axes of the spindles are predominantly parallel to the surface. With subsequent divisions the spindle is at right angles to the surface, giving rows of cells also at right angles to the surface.

Important evidence regarding the initial state of cells is provided by observations on the course of change in the number of cells with time. The results of one set of such determinations are presented in Fig. 1. These results were obtained with an experimental design which has been described in detail elsewhere[2]. It is evident that even after division begins increase in the number of cells does not occur uniformly with time. In the cultures of this experiment there is no change during the first 36 h, but afterwards an abrupt increase occurs which is completed within a few hours. This is followed by a period of about 12 h during which the number of cells remains constant. After this a second abrupt rise is observed. It may be emphasized that it is only the superficial cells that divide, but the data show that these do so more or less synchronously. The data suggest that a wave of mitosis occurs immediately before the first abrupt increase and that immediately before the second sharp increase another wave of mitosis is completed following the interphase which succeeds the first mitosis. A high mitotic index (12 per cent) has been observed immediately before the first increase but not earlier, and the interpretation proposed is further supported by counts of cell pairs that are seen in certain of the macerates on which the determinations of total cell number are made. Each pair is held together within the wall of the parent cell and the members of the pair are separated by the thin wall formed at the end of mitosis. Each pair is therefore the consequence of an

immediately preceding division. Pairs can be seen only when the first sharp increase in total cell number begins. Thereafter the proportion of these increases sharply as the first increase in total cell number is completed. In due course a phase is reached in which the proportion of pairs remains approximately constant, and this corresponds to the phase of more or less constant total numbers which precedes the second sharp increase in total numbers (see Fig. 2).

Clearly the evidence indicates that at the time of excision all the cells in the explant are at about the same stage with respect to the division cycle, that those that are stimulated to divide all approach the first mitosis, during the initial lag period, at approximately the same rate. Certain observations indicate that the stage from which the progress to mitosis begins in the lag period is early in the pre-DNA synthesis period. Samples from a population have been taken at intervals, the individual explants fixed in ethanol–acetic acid (3 : 1), sectioned at 50μ and Feulgen stained[3]. The density of staining has been measured microspectrophotometrically, and these observations have confirmed those of previous workers[4,5], who showed that immediately after excision, with respect to DNA content, all the cells are in the $2C$ condition and that they attain the $4C$ condition before division occurs. The approximate time of the transition has been analysed in cultures in which tritiated thymidine has been added to the cultures and the proportion of labelled nuclei determined[6] on sections from explants cultured for different periods. The results are shown in Fig. 3, in which the corresponding change in the total number of cells is also shown. It is evident that while in this experimental series the first increase in cell number begins after 24 h, nuclear labelling begins after 12 h but before 24 h. While cell number is increasing from 24–36 h there is little or no increase in the proportion of labelled nuclei, but afterwards this increases again while the total cell number is more or less stationary. The data are not sufficiently critical to provide a basis for the estimation of the duration of the S and G_2 phases preceding the first division wave or of the durations of the three phases during the second division cycle, but they do show that all the cells are in the pre-DNA synthesis phase (early G_1) at the time of excision. This corresponds to the observation that at the beginning of the experimental period the cells are in the $2C$ state with respect to DNA content.

It is significant that nuclear labelling is not observed during the first 12 h. However, an acceleration in respiration is observed immediately transfer to the culture medium is effected[1]. The enhanced respiration no doubt promotes accelerated metabolic activity and it may be supposed that a progress through G_1 begins immediately the cells are transferred to the culture medium. This in turn suggests that at the time of excision the cells are uniformly in early G_1.

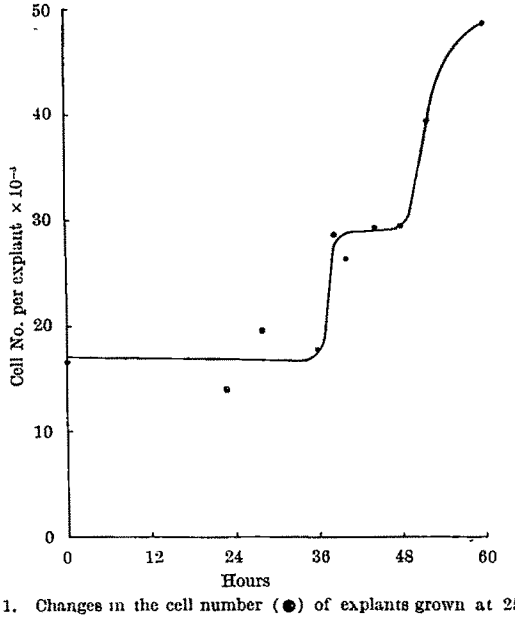


Fig 1. Changes in the cell number (●) of explants grown at 25° C

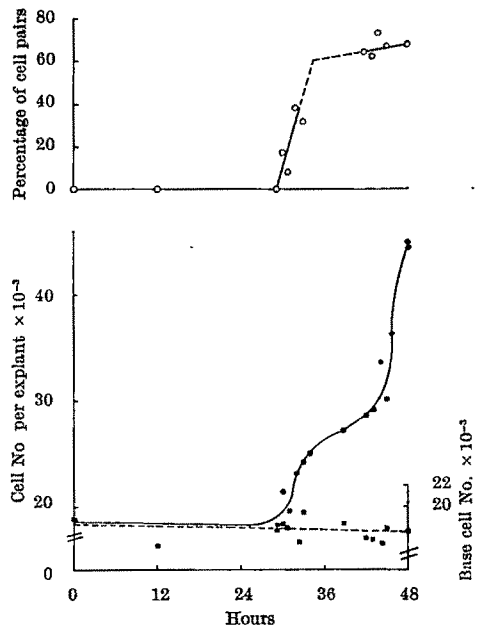


Fig 2. Changes in the cell number (●), base cell number (■) (cell number—number of cell pairs), and the percentage of cell pairs (○), during the growth of explants at 25° C

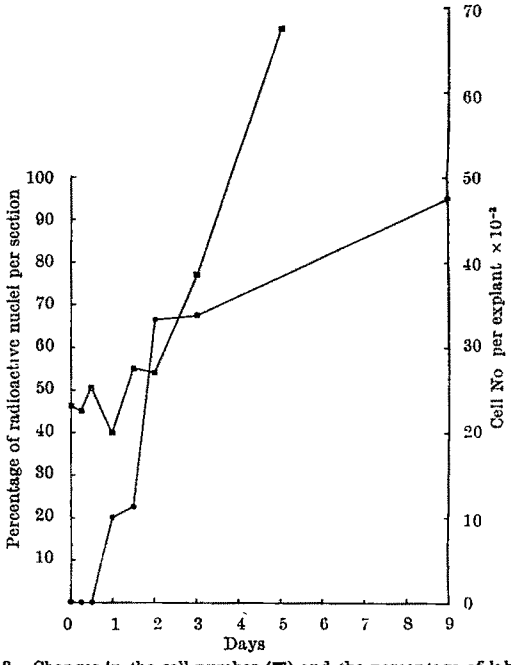


Fig. 3 Changes in the cell number (■) and the percentage of labelled nuclei per section (●) of explants cultured at 25° C on a medium containing ^{3}H thymidine (0·96 μc./ml. specific activity 3·0 c./mM)

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LETTERS TO THE EDITOR

ASTROPHYSICS

Resonant Rotation for Venus?

CAN the rotation period of Venus be commensurate with its synodic period? If the rotation period is 243·16 days retrograde, the axis of Venus which points toward the Earth at one inferior conjunction will point toward the Earth at all subsequent inferior conjunctions. This value for the rotation period of Venus lies within the errors of the measured rotation period[1-3]. If the moment of inertia of Venus about this axis is minimal, it is possible that the planet is locked into this synodic commensurability with its permanent bulge or longest axis pointed toward the Earth at each inferior conjunction. In other words, the presence of the Earth may have stabilized the sidereal rotation period of Venus at the value of 243·16 days retrograde. To investigate the stability of such a commensurate spin angular velocity for Venus, we shall use a method similar to that used to determine the stability of the spin angular velocities of Mercury which are commensurate with its orbital angular velocity[4].

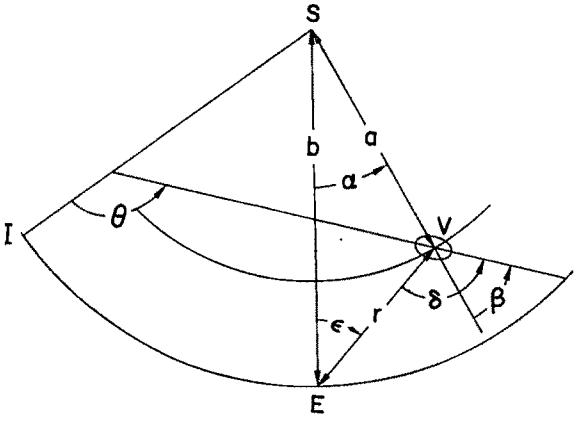


Fig. 1

The orbits of Venus and the Earth are approximated by concentric circles with the spin axis of Venus perpendicular to its orbit plane. It is also assumed that the axis about which the moment of inertia is minimal points approximately toward the Earth at inferior conjunction. Fig. 1 illustrates the angles and co-ordinates involved in the analysis. The points S, E, and V represent, respectively, the Sun, the Earth, and Venus. The semi-major axes of the orbits of Venus and the Earth are denoted by a and b. The distance between the Earth and Venus is r. The long axis of Venus is inclined by an angle θ to the inertial line SI, δ is the angle between the Earth–Venus line and the long axis of Venus, β is the angle between the Sun–Venus line and the long axis, ε is the angle between the Sun and Venus as seen from the Earth, and α is the difference in the heliocentric longitudes of the two planets. In the absence of solar tides, the equation of motion is[5]:

$$C\ddot{\theta}+\frac{3}{2}(B-A)\frac{GM_{\oplus}}{r^3}\sin 2\delta+\frac{3}{2}(B-A)\frac{GM_{\odot}}{a^3}\sin 2\beta=0 \qquad (1)$$

where A, B, and C are the principal moments of inertia in order of increasing magnitude, $M_{\oplus}$ and $M_{\odot}$ are the masses of the Earth and the Sun, and G is the gravitational constant. The condition for a commensurability of the type discussed here is that the average value of $\dot{\beta}$, which we shall denote by $\langle\dot{\beta}\rangle$, be a half-integer multiple of $\dot{\alpha}$. We have the following angular relationships for commensurate motion:

$$\begin{aligned}\dot{\beta}&=-n_v+\dot{\theta}\\ \langle\dot{\beta}\rangle&=p\dot{\alpha}\\ \langle\dot{\theta}\rangle&=n_v+p\dot{\alpha}\end{aligned} \qquad (2)$$

where p is a half integer and n_v is the mean orbital angular velocity of Venus. A retrograde rotation with sidereal period equal to 243·16 days corresponds to $p=-5$. As we wish to investigate the stability of small changes in $\dot{\theta}$ about its mean value $\langle\dot{\theta}\rangle$, we introduce the angular velocity $\dot{\gamma}=\dot{\theta}-\langle\dot{\theta}\rangle$. If conjunction occurs among inertial line SI at the time $t=0$, then:

$$\begin{aligned}\beta&=\gamma+p\alpha\\ \delta&=\gamma+(p+1)\alpha+\varepsilon\end{aligned} \qquad (3)$$

Substituting equations (3) into (1) and averaging over a synodic period, we obtain (see ref. 4):

$$C\ddot{\gamma}+\frac{3}{2}K(p)(B-A)\frac{GM_{\oplus}}{b^3}\sin 2\gamma=0 \qquad (4)$$

where $K(p)$ is determined by numerical integration on an IBM 7094 computer. The values of $K(p)$ for various values of p are listed in Table 1. Perturbations of the orbit of Venus by the Earth also contribute to $K(p)$. However, for the resonance in question ($P=-5$), this contribution is negligible.

The commensurate rotation rates are seen to be stable in the absence of any external torques other than those due to the permanent deformation of Venus from axial symmetry. The resonance will remain stable if the net additional torque ($-T$) satisfies:

$$|T|<\frac{3}{2}K(p)(B-A)\frac{GM_{\oplus}}{b^3} \qquad (5)$$

A solar tidal torque must be acting on Venus. If this were the only significant additional torque, the criterion for stability would be:

$$\frac{B-A}{C}>\frac{4\times10^{-2}}{K(p)Q}=\frac{1{\cdot}6\times10^{-2}}{Q} \qquad (6)$$

for $p=-5$. Here $1/Q$ is the specific dissipation function[6]. Estimates between 10 and 20 for the Earth's tidal effective Q are derived from observation of the secular acceleration of the Moon[6]. These low values may be primarily due to dissipation in shallow seas[7], and therefore may not be applicable to Venus. Estimates of the Q for the Earth's mantle are on the order of several hundred[8]. A stable resonance with a Q of this order for Venus would require a value of $(B-A)/C$ comparable with that of the Moon. It is not at all certain that Venus could maintain such a deformation because the larger surface gravity of Venus would imply greater internal stresses than those supported by the Moon.

Table 1

p	$K(p)$
−0·000	0·908
−0·500	1·479
−1 000	2·857
−1·500	3 831
−2·000	4·311
−2·500	4·394
−3·000	4·204
−3·500	3·851
−4·000	3·417
−4·500	2·958
−5·000	2·513
−5 500	2·001
−6 000	1·735
−6 500	1·417
−7·000	1·147
−7·500	0·921
−8·000	0·734
−8·500	0·582
−9 000	0·460
−9·500	0 360
−10·000	0·282

If Venus had a primordial forward spin, the solar tides could not account for the present retrograde rotation. This has led some authors to propose the existence of other torques on the planet, notably of atmospheric origin (Gold, private communication (1964); MacDonald[6]). As the mass of the Venusian atmosphere is thought to be comparable with that of the Earth's oceans, such proposals seem quite reasonable. If the atmosphere is capable of pushing Venus through the otherwise stable synchronous state of rotation (with respect to the Sun), present control of the rotation of Venus by the Earth is difficult to understand.

In a previous communication[4], we discussed stable resonant spins for a planet in an eccentric orbit. We found that a necessary condition for capture into a resonance was the existence of a significant term in the tidal torque which would damp librations about the commensurate spin value. For Venus a similar condition must be met by the total additional torque T. As yet we have been unable to find any physical basis for the existence of such a term.

A refined measurement of the rotation period of Venus will be awaited with great interest.

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Detection of Hydrogen Emission Line 166α in *M*17

The existence of recombination lines of highly excited states of hydrogen is well established through recent radio observations[1,2] of various galactic H II regions. This communication reports the detection and analysis of a new line $n_{167} \rightarrow n_{166}$ (1,424·736 Mc/s) in *M*17, which extends our previous results[1] to lower frequencies. This particular $n \rightarrow n-1$ transition lies closest to the well-known 21-cm hyperfine line of interstellar hydrogen. We also suggest a convenient notation for the various transitions.

Our data were obtained with the Harvard 60-ft. telescope equipped with a cavity maser 10-channel radiometer having a resolution of 80 kc/s. The antenna beamwidth is 52 min of arc, and its aperture efficiency is 0·38 at the frequency of operation. The total noise temperature of the system is approximately 100° K. The maser used in this investigation is essentially the same as that used by Lilley *et al.*[1].

Our profile for *M*17 is shown in Fig. 1. Using our observational results (Table 1), we obtain, in a manner completely analogous to that described by Lilley *et al.*[1], $T_e = 5{,}900 \pm 2{,}400°$ K, where T_e is the computed electron temperature. This result agrees satisfactorily with the previous[1] determination of $6{,}800 \pm 3{,}000°$ K and $5{,}500 \pm 2{,}400°$ K and with Hoglund and Mezger[2], who obtained $4{,}060^{+430}_{-380}$ K.

In Fig. 2 we have plotted all reported values for *M*17 of the observed linewidth versus wave-length of observation. These values fall nearly on a straight line as expected for purely Doppler broadening as suggested in Fig. 2. Thus, our present result agrees with the conclusion arrived at by Lilley *et al.*[1] that the linewidth can be entirely accounted for by thermal and turbulent broadening to $\nu \sim 1{,}420$ Mc/s.

Table 1 Summary of Observational Results

ν (Mc/s) = 1,424	$\Delta\nu_L$ (kc/s) = 200
T_A (°K) = 0·195 ± 0·030	$\Delta\nu_L$ (corrected) = 183 ± 60 (kc/s)
T_L/T_C = 0·83 ± 0·17 per cent	V (km/s) = 20 ± 7

The above quantities are defined in ref. 1.

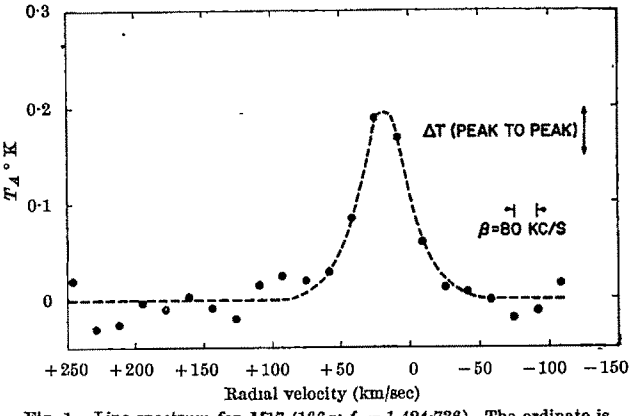


Fig. 1. Line spectrum for *M*17 (166α; f_0 = 1,424·736). The ordinate is antenna temperature. The abscissa is velocity with respect to the local standard of rest

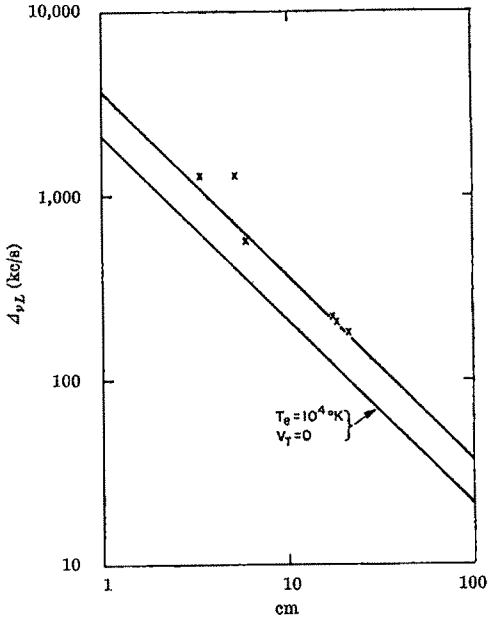


Fig. 2. Plot of linewidth ($\Delta\nu_L$) versus wave-length for all observed $n \rightarrow n-1$ lines in *M*17

We have also observed a region near *W*75 (ref. 3) and have obtained evidence for a line, although the signal-to-noise ratio is not sufficient for quantitative analysis.

To avoid cumbersome notation for future work on lines of excited hydrogen, we suggest a notation widely used in optical spectroscopy. For example, the $n_{167} \rightarrow n_{166}$ transition reported in this communication would be labelled simply 166α. The transition $n_{200} \rightarrow n_{198}$ would be designated 198β. The number represents the principal quantum number of the lower state, and α, β, γ . . . designate respectively transitions of type $n \rightarrow n-1$, $n \rightarrow n-2$, $n \rightarrow n-3$, etc. This notation is consistent with Lyman α (1α), Hβ (2β), etc.

The use of the line 166α (at 1,424 Mc/s) will enable the many hydrogen-line (1,420 Mc/s) systems around the world to examine both ground state and excited hydrogen.

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PHYSICS

Dislocation Velocities

THE purpose of this communication is to discuss the various techniques which have been used to obtain information concerning the velocities of dislocations, and particularly to point out the differences between techniques. In view of these differences, suggestions are offered as to how the various results ought to be interpreted in order to provide the most useful information.

Three techniques have so far been proposed for determining the effect of stress on dislocation velocity. The method of Johnston and Gilman[1] consists of isolating a single dislocation half-loop; locating the positions of its ends by etch-pitting; applying an essentially rectangular stress-pulse of known intensity and duration; re-etching to find the distances which the ends of the half-loop move. The distance moved divided by the pulse duration is then the average velocity associated with the given stress. In a variation of this method first used by Stein and Low[2], fresh dislocations are introduced by scratching the surface of the specimen immediately prior to application of the stress-pulse. After stressing, the specimen is etched and the distance from the scratch to the furthest etch-pit is used in the calculation of average velocity. Finally, as a means of reducing the experimental complexities of these two methods, Guard[3] suggested that in the plastic régime during a simple tension or compression test the cross-head speed of the testing machine be suddenly altered by a substantial amount and the resulting change of flow stress noted; then through an equation relating the plastic strain-rate to the dislocation population and its average velocity, inferences could be made concerning the stress dependence of this velocity.

It is vitally important to recognize that these three methods relate to three totally different physical situations. A dislocation examined by Johnston and Gilman has moved through a crystalline region free from the influences of other dislocations. The factors affecting its motion are only the applied stress and the intrinsic drag of the lattice as modified by impurity content, other point defects, etc. On the other hand, a dislocation observed by Stein and Low is additionally influenced by local variations in the applied stress resulting from the stress fields of the scratch and all of the dislocations adjacent to it. In the experiment of Guard each dislocation contributing to the overall effect is moving 'through' a rather dense array of neighbouring dislocations, and probably experiences many fluctuations of the local stress field as well as entanglement with its neighbours. (If the entire dislocation population were mobile, and everywhere moving with the same velocity, then the preposition 'through' is superseded by 'with' and the following argument is invalid; but it is not likely that these conditions prevail.) Thus the three methods attempt to describe the average response of dislocation velocity to applied stress, for dislocations in three different environments ranging from isolation to conglomeration.

Should the methods then be expected to yield equivalent results? The answer to this question depends on the details of the interactions among the dislocations. For any particular crystal, some indication is given by its macroscopic stress–strain curve. Regions of strain-hardening indicate strong interactions which should produce fundamentally different results in the different situations, whereas regions of easy glide indicate weak interactions and approximately the same results might be expected. However, the answer to this question is really not important.

The real significance of such experiments lies in the fact that they provide part of the basis for quantitative prediction of stress–strain-time behaviour on the macroscopic scale[1,4–6]. This behaviour is obtained by integration under suitable boundary conditions, of a strain-rate relation of the form:

$$\dot{\varepsilon} = \varphi \rho v \qquad (1)$$

Here ε is the plastic strain, and the dot indicates time derivative; φ is an orientation factor which depends on the configuration of dislocations present, and the strain (it may, therefore, be termed a structure factor); ρ is the length of dislocation line per unit volume (also called dislocation density because one of the important measuring techniques related to this quantity is surface etch-pitting to determine the number of line intersections per unit area); and v is the average dislocation velocity.

The integration of equation (1) involves specification of the forms of φ, ρ and v. It has been usual to assume that φ is a constant, or that changes of φ have a negligible effect on the final results. As regards ρ, experiments indicate that this is approximately a linear function of plastic strain:

$$\rho = \rho_0 + \alpha\varepsilon \qquad (2)$$

The earliest velocity–stress relations were either fitted to the empirical formula:

$$v = (\tau/\tau_0)^m \qquad (3)$$

or to the relation derived by Gilman[7]:

$$v = v^* \exp\{-D/\tau\} \qquad (4)$$

These formulae each contain two parameters (τ_0, m and v^*, D) which can be adjusted to provide a best fit for the data. Integration of equation (1) based on the other relations has been quite successful in providing quantitative descriptions of macroscopic phenomena in the small-strain, short-time régime. For long times a mechanism of ageing must be included; for large strains, a description of hardening.

Some investigators have described hardening in terms of an 'effective stress' experienced by the dislocations. The reasoning is that long-range stress fields will develop as the dislocation population increases, and that a 'back stress' will tend to impede the progress of moving dislocations. The average dislocation velocity is then supposedly governed by the effective stress. That is, in equations (3) or (4) τ is replaced by this effective stress. However, such analyses must always conclude that after yielding this effective stress simply equals the yield stress. Thus any hardening introduced in this manner is essentially empirical. Moreover, the concept of effective stress is inimical to the equilibrium requirement for the spatial average stress to equal the applied stress.

Chen, Gilman and Head[8] provide a more satisfying approach to the mechanism and description of hardening. They have investigated the effect of a fluctuating stress field on the average velocity of a moving dislocation, assuming that its instantaneous velocity is given by equation (4). For sinusoidal fluctuation of τ about a mean value equal to the applied stress, they show that the average velocity is smaller than the velocity obtained by simply using the applied stress in equation (4). The reason is that more time is lost by the dislocation in traversing the low-stress regions than is gained in the high-stress regions. On this basis it seems sensible to discuss an 'effective drag parameter', or 'effective stress exponent' if relation (3) is being used, rather than conceptually to violate equilibrium with the notion of an 'effective stress'.

At this point the argument may seem entirely semantic, but there is an important advantage to be gained by thinking of D or m (and indeed possibly even v^* or τ_0) as functional quantities rather than as constants. This relates to the method of Guard[3] which has received considerable attention[9–14], particularly during the past year. Considerable effort has been expended in relating the results obtained using this method to those of the other two methods. The fact is that this technique is particularly well suited for

investigating the dependence of (at least one of) the velocity-stress parameters on strain and strain-rate.

The data available clearly indicate that dislocation velocities are dependent on factors other than stress. Even for relatively strain-free crystals the concentration of certain point defects seems to be very influential. Certainly dislocation interactions affect average velocity at some stage of the deformation process and hence the velocity relation must be related somehow to strain. More generally it can be asserted that dislocation velocity is influenced by stress and structure. The term structure is intended to be descriptive of crystalline imperfection, and is somewhat analogous to entropy. Temperature also influences the velocity relation, but so far there is no experimental evidence to indicate whether or not any influence is expected by such variables as strain-rate, hydrostatic pressure, etc.

In summary, there is no reason to believe that dislocation velocities in a given material are determined solely by the resolved shear stress. Investigation of velocity dependence on other appropriate parameters may serve to delineate the true functional form of the velocity relation. This would provide greater insight into the mechanism governing plastic flow and a better quantitative description of the process.

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A New Method for determining Surface Densities by Evaluation of the Decrease in Concentration of Single Stationary Drops

Surface densities can be determined from the decrease in concentration and the estimated total area of a large amount of bubbles or drops made to pass through a solution[1]: this method is not accurate since the amount of substance adsorbed by the bubbles or drops is dependent on the hydrodynamics of the system.

An alternative method is now suggested, by which surface densities can be determined directly. The method is based on the fact that adsorption on to the surface of a single drop consisting of a solution of a sparingly soluble substance may lower considerably the original solution concentration. In this method, a drop of known volume and surface area with an initial concentration c_1 is formed, and the surface tension is determined after equilibrium is reached. The final bulk concentration c_2 will be lower than the initial concentration c_1, as solute is adsorbed at the surface. The final bulk concentration c_2 can be determined by comparing the equilibrium surface tension value of the drop with surface tension-concentration data determined by any other suitable method in which adsorption on to the surface does not modify appreciably the bulk concentration. Garner and Garfias[2] have shown that reliable surface tension-concentration data can be obtained from the dimensions of a small air bubble immersed in a large volume of solution.

If it is assumed that all the adsorption of the solute is confined to a superficial layer one molecule thick, and that the concentration in the bulk of the drop is constant right up to the superficial layer, then the surface density is the difference per unit area between the initial and final amount of solute present in the bulk of the drop, as given by the equation:

$$n = \frac{V(c_1 - c_2)}{S}$$

where n is the surface density in g moles/cm^2; V, the drop volume in cm^3; c_1 and c_2, initial and final concentration in g moles/cm^3; and S, the drop surface area in cm^2.

It can be shown that surface adsorption can lower considerably the concentration of drops, by considering a drop of undecanoic acid solution, which at equilibrium has a final concentration c_2 of $1{\cdot}5 \times 10^{-8}$ moles/cm^3. For a drop with a volume of 0·020 ml. and a surface area of 0·30 cm^2 the amount of solute contained in the bulk of the drop is 3×10^{-10} g moles. Dervichian[3] has reported that the equilibrium film has a density of 4×10^{-10} g moles/cm^2, so there are also $1{\cdot}2 \times 10^{-10}$ g moles adsorbed at the surface of the drop. The total amount of solute present is therefore the sum of that in the bulk and in the surface, that is, $4{\cdot}2 \times 10^{-10}$ g moles, which would require that initially the drop should have been formed with a solution containing $2{\cdot}1 \times 10^{-8}$ g moles/cm^3 of undecanoic acid; in other words, for this particular example, the concentration is 40 per cent lower after surface adsorption has taken place.

Besides the fact that the suggested method is confined to the investigation of the surface densities of a few sparingly soluble substances, there is an additional restriction imposed by the nature of the method. In effect, surface densities cannot be determined for all ranges of concentration: the maximum concentration for which the surface density may be determined by this method corresponds to that in which the total amount of solute (bulk plus surface) per unit volume gives the saturation concentration.

Experiments at liquid–air and liquid–liquid interfaces are being conducted by one of the authors (F. J. G.) and will soon be reported.

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GEOPHYSICS

Time Variations of Geomagnetic *H* and *D* at Disturbed Stations

The cylindrical co-ordinate system, defined by the components H, D and Z, used to describe the geomagnetic field brings some noteworthy consequences when going from the field to its time gradients. Although of a quite elementary geometrical nature these effects are sometimes overlooked in magnetic cartography and research work.

The use of the direction co-ordinate D, determined by the field itself, is due to practical reasons: it is the direction of a compass needle. H and D completely describe the horizontal part of the field, as do X and Y or any other pair of horizontal magnetic 'elements'.

Fig. 1 shows the prevalent H and D as determined at a certain station at a certain time. We further have the disturbance field P, α due to a crust anomaly. We could of course easily produce this disturbance ourselves by placing a magnet in a fixed position close to the instruments. We further assume that we know the 'normal' field H_n, D_n which would prevail if P did not exist.

Transforming to geographical north and east components, we obtain:

$$X = H \cos D \qquad X_n = H_n \cos D_n$$
$$Y = H \sin D \qquad Y_n = H_n \sin D_n$$

Since P,α are constant, the time variations in the xy-frame are equal:

$$\dot{X}=\dot{X}_n$$
$$\dot{Y}=\dot{Y}_n$$

This leads to the following expressions for the 'normal' time variations in terms of those observed:

$$\dot{D}_n=\frac{H\dot{D}}{H_n}\cos\,(D_n-D)-3438\,\frac{\dot{H}}{H_n}\sin\,(D_n-D)$$

$$\dot{H}_n=\frac{H\dot{D}}{3438}\sin\,(D_n-D)+\dot{H}\cos\,(D_n-D)$$

where $\dot{D}_n$ and $\dot{D}$ are expressed in minutes of arc per time unit. As can be seen the change in one 'normal' component is a function of $\dot{D}$ as well as $\dot{H}$.

Let us apply these formulae to the following practical case, approximately valid for the Kiruna Observatory 1954–55:

$H=11{,}000\gamma$	$D=-7°$ (observed)
$H_n=12{,}100\gamma$	$D_n=+1°$ (estimated)
$\dot{H}=+2\gamma$/year	$\dot{D}=+9'$/year (observed)

H_n and D_n were estimated from a magnetic chart. Inserting these values in the foregoing equations yields:

$$\dot{H}_n=+6\gamma\text{/year}\qquad \dot{D}_n=+8{\cdot}1'\text{/year}$$

These values are considerably different from the observed values.

The observed values H, D indicate a significant magnetic anomaly, that is, deviation from the 'normal' field, in the sense that we will get a similar deviation whichever pair of horizontal components we use.

The pair $\dot{H}$, $\dot{D}$ does not constitute an anomalous annual change of significance. The anomaly is only a geometrical effect. In the north-east system we obtain:

$$\dot{X}=\dot{X}_n=5.5\ \gamma\text{/year}$$
$$\dot{Y}=\dot{Y}_n=28.6\ \gamma\text{/year}$$

Physically, the results given here are only an illustration of the well-known fact that there is little or no correlation between small-scale field anomalies (with sources in the crust) and the anomalies of the secular variation (with sources in the core).

The time variations of the disturbance field P,α which would make $\dot{X}\neq\dot{X}_n$, $\dot{Y}\neq\dot{Y}_n$, are generally much smaller than the secular variation and difficult to detect in short time spans. This stability of the thermoremanent magnetization (and other kinds of rock magnetism) is essential for the archaeomagnetic and palaeomagnetic research.

In magnetic cartography no deeper meaning should be attached to local anomalies in $\dot{H}$ and $\dot{D}$ without a closer look. As a supplement isoporic maps based on $\dot{X}$ and $\dot{Y}$ are recommended. The field stations, including the 'secular stations', providing most of the data for magnetic charts are often disturbed, simply because it is almost impossible to find undisturbed stations in some areas.

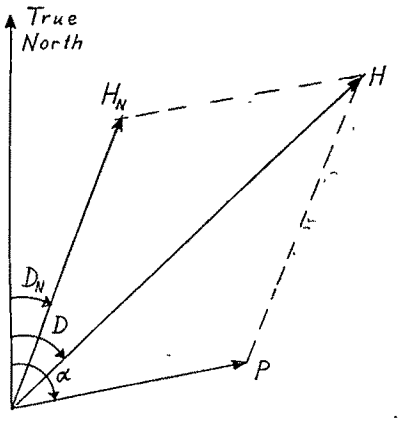


Fig 1

The previous discussion is of course applicable to all time variations, such as storms, diurnal and lunar variations, bays, pulsations, sudden commencements. For example, before attributing physical significance to the amplitudes in H and D of Sq at a magnetic observatory it may be worth while to check (by means of observatory data and magnetic charts) whether or not the field is under influence of a local anomaly. $Sq(H)$ and $Sq(D)$ may differ considerably from $Sq(H_n)$ and $Sq(D_n)$. It is always safer to work in X and Y. Another pair of useful horizontal components are Hgm, Dgm where Hgm is the component in the direction of geomagnetic north.

It is usual practice (with important exceptions) to locate magnetic observatories in areas which are as free as possible from local disturbances. This is often ascertained by means of a local survey. If the field gradients are sufficiently small the site is considered suitable. There may, however, be larger, regional anomalies which are not detected in this way. In this case the recorded 'disturbed' values $\dot{H}$, $\dot{D}$ may be representative for a fairly large area. Still, they do not necessarily indicate a real anomaly in the time variations studied.

A synoptic study of the amount of local disturbance at the magnetic observatories may be helpful to users of magnetic data. The whole discussion is, of course, somewhat obscured by the vagueness of such conceptions as 'normal field' and 'disturbance'.

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GEOLOGY

The Malvern Lower Cambrian Strata

IN a recent communication R. St. John Lambert and D. C. Rex[1] imply that the palaeontological evidence is insufficient for any claim to be made that Lower Cambrian strata are exposed in the Malvern Hills. Whereas there is some doubt concerning the presence there of Middle Cambrian rocks, there is little doubt about the existence of Lower Cambrian. The earliest fossiliferous strata in Shropshire, which occur above the Wrekin Quartzite but at least 450 ft. below the Olenellid *Callavia* Limestone, were described by Cobbold[2] as yielding two of the brachiopod species described by Matley[3] from the Malvern Quartzite of the Malverns, namely, *Micromitra* (*Paterina*) *phillipsi* (Holl) [olim *Kutorgina cingulata* auct.] and *Obolella groomi* Matley[4]. Later, a third brachiopod species known elsewhere only in the Shropshire *O. groomi* fauna, *Micromitra* (*Paterina*) *rhodesi* Cobbold, was collected by H. F. Metcalfe from the Malvern Quartzite in association with the previously recorded forms[5]. It is significant to learn that the "last major isotopic event in the Malvernian occurred 590 ± 20 m.y. ago"[1], for this has an important bearing on the problem of the date of the base of the Cambrian. It would be of even greater interest to learn the isotopic age of the palaeontologically dated Lower Cambrian Comley Sandstone glauconitic rocks from Shropshire which are being investigated by the Oxford group.

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Volcanic Chronology of Lanzarote (Canary Islands)

The relative or absolute chronology of Late Tertiary and Quaternary events in the Eastern Canary Islands is mostly unknown. Pacheco[1] and Hausen[2] both attribute the cinder cones and olivine basalts of the central volcanic region of Lanzarote to the Quaternary on general grounds; the preservation and freshness of form. The northern and southern uplands of Famara (671 m) and Ajaches (561 m), built of weathered lavas and pyroclastics with deep valley dissection, are similarly attributed to the early Tertiary; a formation termed the Tableland Series by Hausen and, in Ajaches, the Ajaches Volcanic Series by Driscoll *et al.*[3].

To elucidate the volcanic chronology a number of systematic themes were followed and linked by field mapping at the scale 1 : 25,000. Foremost among these was the use of an established raised beach succession to provide a broad grouping and relative dating of the volcanics where they outcropped at the coast. Within this grouping, field mapping reveals a more complex picture not discussed here.

Zeuner's raised beach records for the eastern Canaries lack sections, textual discussion and has few sites[4]. It was not used in this investigation. A raised beach succession reported by Driscoll for the Tertiary Ajaches area of south Lanzarote probably relates to Quaternary eustatic shifts of sea level. Platforms and marine deposits at 55, 35, 23–25, 15, 6 and 1 m, and a shore platform are described. Possible marine erosion levels at 200 m and 300 m were suspected. I have made an attempt to extend this sequence to the entire island.

The 55-m level appeared morphologically flanking the Tableland Series outcrop in the low central region of the island and on the eastern side of the Famara Upland. Blocks of crystalline limestone ejected from more recent volcanoes may represent the shoreline and its deposits north-east of Tinajo.

The 15-m level appears as a cliff line without deposits cut into lavas from Mt. Teneza and buried by later lavas. In the Ajaches area the beach overlies lavas from Mt. Roja, the West Coast Basalts of Driscoll and not the Tableland Series as assumed by Hausen and Pacheco. The 25-m beach reported in Ajaches by Driscoll refers to the surface of terrestrial deposits; the marine beach is 8–10 m. lower. Marine and terrestrial deposits are probably above the former intertidal zone, as is usual on this coast, and the old sea level would appear to be near 15–17 m. Relict potholes and rolled boulders appear at this height. A marine conglomerate at 8–9 m covered by lava from Atalaya de Femés at Berrugo probably relates to the 15-m level. It has a similar fauna but lacks corals. Shingle bands at various heights on the cliffs of the east Ajaches coastline are poorly rounded, lack marine fauna and are interpreted as interbedded gravels with the Ajaches Volcanic Series, unrelated to Quaternary sea level.

The 6-m and the 1-m beaches form the best basis for the broad grouping of volcanoes and lava fields (Fig. 1). The 6-m level is extensively found: a hard limestone and conglomerate with a warm water fauna, particularly *Strombus bubonius*, a gastropod now absent from the Canaries. On the calm south-east facing (Sotavento) coast the beach is depositional in embayments, but on lava-flow headlands it often lacks a precise morphology. Limestone and small shells impregnate angular basal scoria of the sub-horizontal laval flows, frequently appearing to be older than the solid lava just above, but there is no sign of contact metamorphism. In the Ajaches area, lavas from Atalaya de Femés reach the sea at Janubio and Berrugo where they are older than the 6-m beach. The 1-m beach deposits are less calcareous, virtually recent in fauna and parallel present beach contours. Erosionally the 1-m level intercliffs 6-m deposits while north of Arrecife (east coast) a deep beach section showed the 6-m and 1-m deposits separated by a rotlehm[5] extending below sea level. A rotlehm is often found below the 1-m conglomerate where it occurs alone.

Above 55 m, no marine deposits are found. The erosional levels of *c.* 200 m and 300 m fail to appear in the Famara. However, Mt. Ubique (308 m), a cone some distance south of the Famara, the only preserved cone of the Tableland Series, is strongly cliffed on the south-east flank in contrast to its other smoothly aggraded slopes. This may indicate marine action to its foot at *c.* 150–160 m although the old abrasion platform is covered by later lavas.

The age and presence of this cone isolated in the central volcanic region indicate that this low region, with small Tableland Series exposures, is due to regional inequality of the Tertiary eruptions, and not to later faulting forming a 'tectonic graben' (Hausen). Similarly there is no field evidence for the faults invoked for the Famara and Ajaches escarpments; indeed, there are no faults in any exposures of the Tableland Series. Pacheco's thesis of long continued and strong erosion from the west must still stand. On the Famara Upland field mapping shows that the gently rising plateaux culminating in rocky outcrops of columnar basalt are the sub-original lava

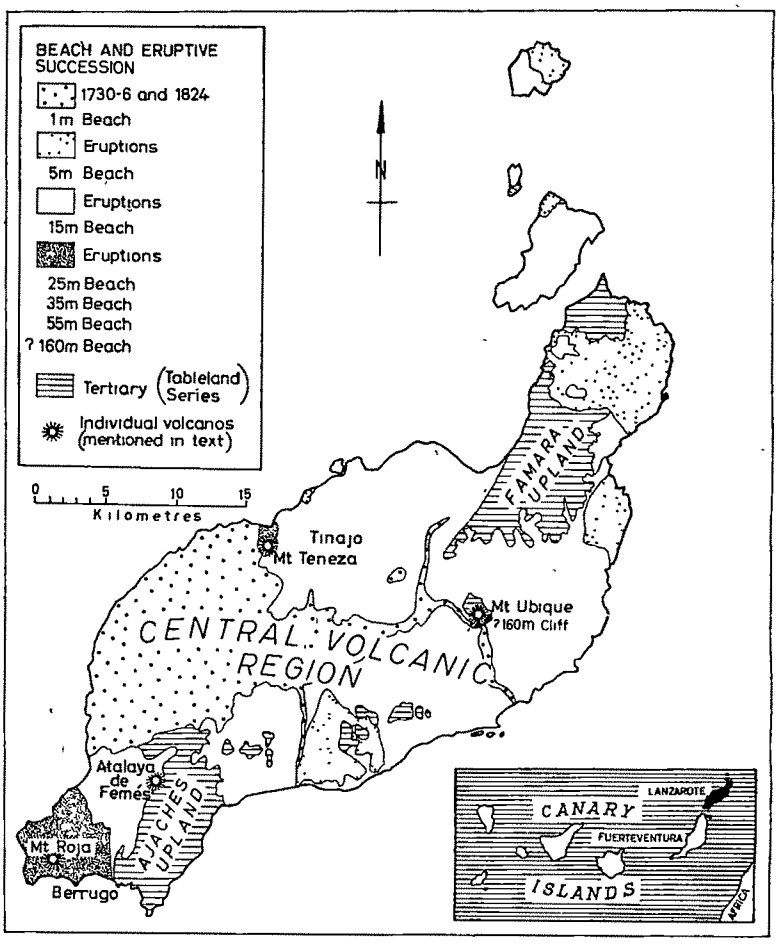


Fig. 1. Map of the volcanic chronology of Lanzarote based on the raised beach succession

fields and throat fills of old volcanoes. Scoria and lapilli are seen dipping off the old necks. The deep radiating barrancos emphasize the original topography and are a true consequent drainage pattern. Many have since been blocked and partly filled by lavas from shield volcanoes, at least one of which is younger than the 15-m stage. In the more closely dissected Ajaches area this is less easy to demonstrate, but throat plugs and lapilli cone structures are found. Benches at high levels here appear to be dependent on structure.

The eruptive nature of the Tertiary volcanics is very similar to that of the Quaternary volcanics of the central region, a fact noted by Hartung[6]. They are not the fissure eruptions envisaged by Pacheco, nor do they merit the name 'Tableland', being in no way similar to the Antrim or Deccan plateaux basalts. The broad grouping revealed by Fig. 1 shows that the lavas between the 15-m and 5-m stages were the most extensive. While Quaternary vulcanism is known in the Tertiary Uplands and particularly the Famara, its reiterated manifestations are in the low central region. The eruptions between the 5-m and 1-m stages are less extensive, but this may merely reflect a shorter time interval. A linear pattern linking eruptions within and between these groups can be seen. The historic eruptions of 1730–36 and 1829 are unaffected by raised beaches.

There is no certain Quaternary vulcanism anterior to the 55-m stage and most of it post-dates the 15-m stage. The 25-m and 35-m stages recorded for Los Ajaches (and Fuerteventura, see below) are entirely missing; they are probably buried. The apparent break in vulcanism from the early Later Tertiary to the Middle Pleistocene may be in part illusionary due to a later burial by lavas, but it seems unlikely in view of the preservation of cones of both ages only partly modified. Since the Middle Pleistocene, successively younger lavas have eliminated the higher beaches, providing clear evidence of the progressive youthfulness with decreasing height of the raised beach successions. This constitutes an important local proof that the lower beaches are younger, a proof independent of the concept of 'intercliffing'.

The 160-m level and the raised beach series below 55 m can be tentatively correlated with similar successions on the west coast of Fuerteventura[7] and Morocco[8]. Cliff-foot heights and faunal associations provide the basis. The sequence may date back to the end of the lower Pleistocene (55 m = Sicilian following Lecointre[8]) while the 150–160 m level recorded on Mt. Ubique may date to the Lower Pleistocene or Late Pliocene (= Villafranchian). In conjunction with preservation of form and sub-original topography it suggests an age closer to Miocene or Pliocene for the upper succession of the Tableland Series rather than the early Tertiary and post-Eocene ages of Hausen and Pacheco. The presence of the cone Mt. Ubique seems to preclude any higher transgressions. No interbedded marine deposits are known within the Tableland Series.

The tectonic stability of the region which these tentative correlations imply is supported in the field. Spanish survey marks are inadequate for precise levelling and variations in exposure prohibit the detection of slight changes of level, but there is no sensible variation on the 6-m or 1-m beaches. The 55-m beach encircling Los Ajaches and morphologically present elsewhere is a major stage (40 m–65 m), but nowhere is there any sign of disturbance. The absence of evidence of faults in either the Tertiary or Quaternary formations seems to preclude large-scale tectonic activity in these periods despite extensive vulcanism although minor seismic activity associated with imminent eruptions is not unknown[9]. Such stability is noteworthy so close to a continental margin and particularly in view of attempts to see North African structural lineaments in the Canaries with the possible tectonic implications of this. While the Casablanca area is a reputedly stable part of the Moroccan Meseta, many parts of the Atlas owe their origins to Quaternary tectonics. The deep seas (4,500 ft.) between the islands and Africa may indicate a separation from the African basement and partial insulation from tectonically active zones.

This succession, established for Lanzarote, may serve as a useful point of reference in studies on the more vulcanologically complex islands of the Central and Western Canaries.

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METALLURGY

Strain Hardening in Fatigue

Fractographic studies[1–3] using optical and electron microscopy have shown that, for many metals, high-strain fatigue endurance is determined by crack propagation of the type designated stage II by Forsyth[4]. This is contrary to the statistical argument put forward by Gittus[5] and the dislocation intersection model of Gilman[6], neither of which is in accord with fractographic evidence.

In a recent theory[7], Grosskreutz has related high-strain fatigue endurance to the ductility at fracture in a tensile test, as proposed by Coffin[8], and the strain-hardening coefficient, n.

We picture crack propagation occurring by plastic flow away from the crack tip during the tensile part of a cycle and this flow is not completely reversed by compression. The deformation causes strain hardening ahead of the advancing crack and inhibits plastic flow in the subsequent cycle. Continued crack propagation can only occur if further strain, and thus strain hardening, occurs at the crack tip. We therefore anticipate a continuous increase in strain hardening from the crack nucleus to the crack position at final fracture.

A technique reported by Williams[9] has been used to measure microhardness on the fracture surface and has shown that a continuous increase in strain hardening exists. Specimens of type 316 steel which failed with lives between 5×10^2 and 7×10^5 cycles were mounted in a transparent medium which was polished away parallel to the fracture surface. When the fracture surface was reached, islands of metal were exposed (Fig. 1), these being taper sections of the particular local feature. It has been verified that the ripples present are of the form reported by Laird and Smith[1]. Microhardness indentations were placed on regions of high taper magnification. The hardness values obtained thus give a measure of the condition very close to the fracture face; this cannot be done directly on fracture faces or on longitudinal sections. Indentations were made using the Reichart microhardness tester loaded at 31 g. A typical result is shown in Fig. 2 with error bars drawn at one standard deviation; note that the annealed material and material in the necked down region of a fractured tensile test piece have diamond indentation ocular readings of 106 and 84, respectively. Measurements in the gauge portion indicate that some progressive hardening occurs during testing, but this is a second-order effect compared with the local hardening at the fracture face.

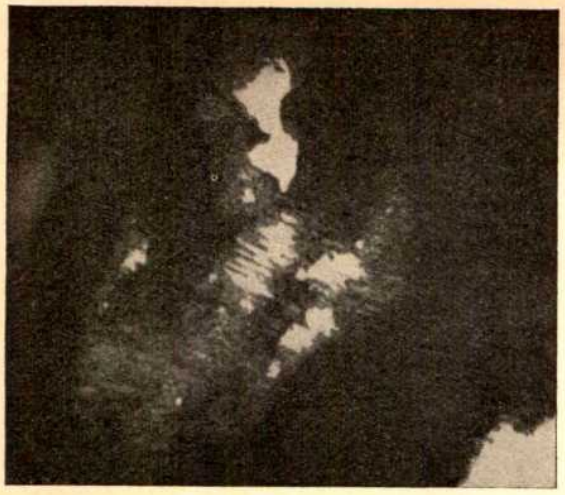


Fig. 1. Islands of metal showing fracture surface ripples revealed by taper sectioning. Specimen subjected to high strain fatigue. Failed in 12,435 cycles. (*c.* × 800)

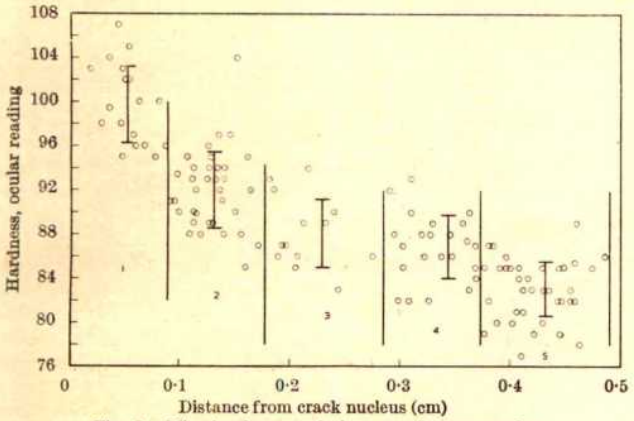


Fig. 2. Microhardness variation over fracture surface

It is believed that a continuous increase of strain hardening during crack initiation and propagation is a common feature during testing of ductile metals, even when 10^7 or more cycles are required for failure. It is also suggested that the foregoing results indicate that strain hardening to some level appropriate to the ultimate tensile strength is not necessary before a crack can advance.

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CHEMISTRY

Partition Isotherms and the Mixed Second Virial Coefficient

In a recent publication[1] Sewell and Stock have presented their results for the effect of an inert gas, nitrogen, on the solubility of carbon tetrachloride in non-volatile liquids used as stationary phases in gas-liquid chromatography. Although not stated, it is to be understood that the method is based on the original proposals and work of Freeguard[2] and Stock, and it is the aim of this publication to direct attention to the consistency of Freeguard's results and to indicate a method whereby the later results may be used to evaluate B_{12}, the second virial coefficient that accounts for the interactions between unlike molecules of the mixed gases.

To avoid confusion we adopt the nomenclature of the previous publication[1]; thus the fugacity, p_2^*, of the pure vapour at a pressure p_2 is given by:

$$\ln p_2^* = \ln p_2 + B_{22} \cdot p_2/RT$$

where B_{22} is the second virial coefficient of the pure vapour. The activity of the vapour is given by the ratio of the fugacity at the pressure p_2 to that of the saturated vapour at the temperature T.

Therefore

$$\ln a_2 = \ln p_2^*/p_2^{*0} = \ln p_2/p_2^0 - B_{22}(p_2^0 - p_2)/RT \quad (1)$$

where a_2 is the activity of the vapour and p_2^0 the saturated vapour pressure. Let x_2 be the mole fraction solubility of the vapour in the non-volatile solvent and γ_2 be the solute activity coefficient at this concentration. Since vapour and solute are in thermodynamic equilibrium, it follows that:

$$\ln a_2 = \ln x_2\gamma_2 = \ln p_2/p_2^0 - B_{22}(p_2^0 - p_2)/RT \quad (2)$$

In the presence of nitrogen, when P is the total pressure and $P-p_2$ its partial pressure, we have:

$$\ln x_2'\gamma_2' = \ln p_2/p_2^0 + P[B_{22} - (1-y)^2(B_{11} - 2B_{12} + B_{22})]/RT - B_{22}p_2^0/RT \quad (3)$$

where x_2' is the new mole fraction solubility of the vapour, γ_2' the corresponding value of the activity coefficient and B_{11} the second virial coefficient of nitrogen. We note that the situation has been simplified by the assumption that the effect of applied pressure on γ_2 is negligibly small when P assumes values the maximum of which are not very much greater than 1 atmosphere. Adopting the assumption[2] (but with some reservation) that for y, the mole fraction of the solute in the gas phase, we may write $y = p_2/P$, and subtracting equation (2) from equation (3):

$$\ln x_2'\gamma_2' - \ln x_2\gamma_2 = P[B_{22} - (1-p_2/P)^2(B_{11} - 2B_{12} + B_{22})]/RT - p_2B_{22}/RT \quad (4)$$

from which

$$RT(\ln x_2'\gamma_2' - \ln x_2\gamma_2) = B_{12}[2P\,(1-p_2/P)^2] - B_{11}[P\,(1-p_2/P)^2] + B_{22}[p_2(1-p_2/P)] \quad (5)$$

From the results of a previous investigation[3] it may be shown that over a wide range of concentration ($x_2 = 0$ to $x_2 = 0{\cdot}45$)

$$\ln x_2\gamma_2 = A \ln x_2 + C \quad (6)$$

where A and C are constants characteristic of the solvent (we use A and C for dinonyl phthalate and A' and C' for squalane).

Therefore

$$A.RT(\ln x_2' - \ln x_2) = \mathrm{f}(B,p_2,P.) \quad (7)$$

where $\mathrm{f}(B,p_2,P.)$ is used as an abbreviation for the right-hand side of (5).

If we consider the equilibrium condition for a second and different non-volatile liquid in the same atmosphere, we have

$$A'.RT(\ln x_{22}' - \ln x_{22}) = \mathrm{f}(B,p_2,P.) \quad (8)$$

where x_{22} and x_{22}' are the initial and final mole fraction vapour solubilities, respectively.

Therefore

$$A \ln(x_2'/x_2) = A' \ln(x_{22}'/x_{22}) \quad (9)$$

Furthermore, if $A = A'$ (which is almost exactly the case for the dinonyl phthalate/carbon tetrachloride and squalane/carbon tetrachloride systems), then

$$x_2'/x_2 = x_{22}'/x_{22} \quad (10)$$

It is this relationship (10) that has been demonstrated[2] to hold over a range of pressure to $P = 22$ cm mercury by the technique as later described by Sewell and Stock but

using an apparatus with less efficient mixing; it was for this reason that the results were considered of insufficient precision for the calculation of B_{12}. However, since in all cases $x_2' < x_2$, a negative value of B_{12} was indicated, in keeping with the predictions of the theory of corresponding states.

To calculate a value of B_{12} from all data[4] equation (7) was used in conjunction with Coggan's[5] multi-fit programme using the *Atlas* I computer at the University of Manchester. In terms of the criterion

$$\Sigma(x_2^{\text{observed}} - x_2^{\text{calculated}})^2$$

the best value for each set of data, namely -997 for dinonyl phthalate and -540 for squalane, was approximately three times better than the corresponding states value of -158. The value -790 which is five times the theoretical value of B_{12} appears to give a reasonable fit to both systems, particularly when $P-p_2$ approaches 1 atm., and is therefore suggested for calculations in gas-liquid chromatography. There is evidence, however, that structural alterations in the mathematical model would enable a very much more accurate fit of data to be made. The theoretical implications of such a model are at present being examined by Dr. Coggan and myself.

I thank Dr. G. C. Coggan for his help in connexion with the data processing and Mr. P. A. Sewell and Dr. R. Stock for making all their results available.

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Joule–Thomson Effect: Non-ideality and Association

It is generally asserted in text-books on thermodynamics and the properties of gases that the Joule–Thomson effect demonstrates conclusively that real gases are non-ideal. This is indeed the case, but not in the sense that usually seems to be understood.

The Joule–Thomson effect is a measure of the extent to which, at constant pressure, the volume of a fixed mass of gas fails to be strictly proportional to the absolute temperature. Such departures from the relation $pV \propto T$ occur in: (i) non-ideal gases in which there is no chemical reaction; (ii) ideal quantum gases at very low temperatures; (iii) associating gases (ideal and non-ideal).

Case (ii) has been considered briefly by Landsberg[1]. The purpose of the present communication is to emphasize the relevance of case (iii) (exemplified by the large[2] Joule–Thomson effect exhibited in experiments[3] conducted for another purpose on the associating gas $2NO_2 \rightleftharpoons N_2O_4$); and to point out that, even when the possibility of association is taken into account, it is still possible to use experimental observations on the Joule–Thomson effect as one of the sources of compelling evidence of non-ideality.

Now whereas for inert non-ideal gases there may be either a decrease or an increase in temperature, in an ideal associating gas the Joule–Thomson expansion always leads to a decrease in temperature. That is, $(\partial T/\partial p)_H = -(\partial H/\partial p)_T/C_p$ is always positive (but, provided that a dimer is one of the associated species present, resembles the value for an inert non-ideal gas in tending to a non-zero limit at low pressures). For increase in pressure at constant temperature leads to increased association, and therefore, since enthalpies of dissociation are positive, to decrease in H. Thus, $(\partial H/\partial p)_T$ for an ideal associating gas is always negative, which implies that $(\partial T/\partial p)_H$ is always positive.

It is found from experiment that in certain cases $(\partial T/\partial p)_H$ is negative, as for hydrogen and helium at room temperature and atmospheric pressure, which compels the conclusion that these systems are non-ideal, and cannot be ideal associating gases. That is: the Joule–Thomson effect does not of itself demonstrate non-ideality (as distinct from association); but inversion of the Joule–Thomson effect does.

This conclusion is akin to one concerning empirical equations of state. If at low pressures an expression $pV = RT + Bp$ is found to apply, a negative value of B might be attributable to association, to non-ideality, or to both together; but a positive value of B cannot occur for an ideal associating gas, and so is conclusive proof of non-ideality.

The point considered here is one more example of similarities in the effects of non-ideality and of chemical reaction (compare, for example, "deviations from Nernst's distribution law"). It should be emphasized that in particular instances, as indicated here, it is possible to show conclusively that the system considered is non-ideal.

I thank Prof. F. Gray for valuable discussion of these matters. This communication was prepared during the tenure of the Edward A. Deeds fellowship of Queen's College, Dundee.

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Effect of Interstitial Atoms on a Lattice-vacancy Diffusional Process

An investigation has been made of the mechanism of diffusion of chromium into iron–carbon systems over the temperature range 950°–1,265° C. At temperatures above 825° C, the diffusional mechanism of chromium into iron is complicated by a change in crystal structure from a body-centred cubic lattice (α-phase) to a face-centred lattice (γ-phase). At low concentrations of carbon, a lattice-vacancy diffusional mechanism has been postulated, and a moving boundary model set up to represent this condition[1]. The results have confirmed that at concentrations of carbon of less than 0·16 per cent only lattice-vacancy diffusion was effective. At concentrations of carbon above this, the interstitial atoms hinder diffusion.

This communication presents results illustrating this effect for concentrations of carbon up to 1 per cent (Table 1).

Fig. 1, a plot of $\dfrac{\text{diffusivity of chromium in iron } (\gamma\text{-phase})}{\text{diffusivity of chromium in iron } (\alpha\text{-phase})}$ versus concentration of carbon, shows that for concentrations of carbon up to 0·16 per cent this ratio is independent

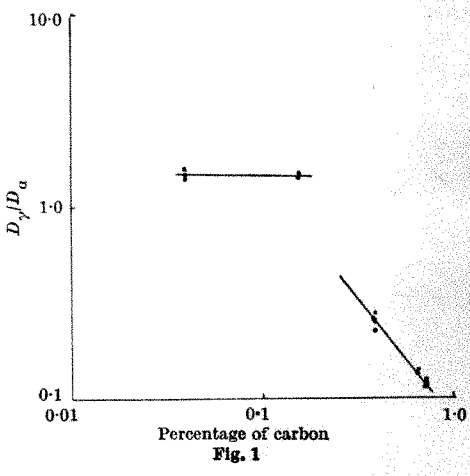


Fig. 1

Table 1

Carbon concentration / Temp. (° C)	0·04% D_γ/D_α	0·16% D_γ/D_α	0·4% D_γ/D_α	0·68% D_γ/D_α	0·75% D_γ/D_α
950	1·7	1·5	0·25	0·15	0·13
1,050	1·45	1·40	0·25	0·14	0·11
1,125	1·50	1·45	0·28	0·14	0·11
1,200	1·45	1·40	0·21	—	0·12
1,265	1·35	—	0·25	—	—

D diffusion coefficient cm²/sec.

of concentration of carbon as predicted by the model. At higher concentrations of carbon, however, the ratio $\frac{D_\gamma}{D_\alpha}$ is reduced tenfold and is inversely proportional to the concentration of carbon. This indicates that the interstitial diffusion mechanism is now controlling, the effect of the carbon atoms being more pronounced on the face-centred cubic lattice as expected.

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Contact Angles of Liquids on Organic Solids

The interfacial tensions between a solid surface (S), a liquid (L) and its vapour (V) can be related as follows[1]:

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2\varphi\sqrt{\gamma_{SV}\gamma_{LV}} \quad (1)$$

where the interaction parameter φ is virtually unity for intermolecular forces of dispersion type but rather less than unity for immiscible phases in general. When combined with Young's equation[2]:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV}\cos\theta \quad (2)$$

the result is:

$$\cos\theta = 2\varphi\sqrt{\frac{\gamma_{SV}}{\gamma_{LV}}} - 1 \quad (3)$$

While this is broadly in accordance with Zisman's well-established empirical relationship[3] for low-energy substrates, it fails to account for its rectilinear form.

It seems likely that the curvature implied in equation (3) arises through failure to recognize that low-energy solid surfaces can deform appreciably at an interfacial boundary[4] (Fig. 1).

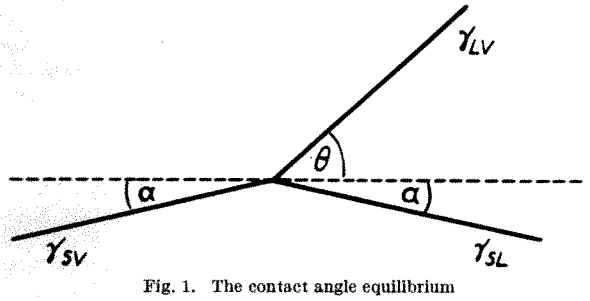


Fig. 1. The contact angle equilibrium

If the angle subtended to the horizontal plane by the surface of the deformed solid is α, then obviously:

$$\frac{\gamma_{LV}}{\sin 2\alpha} = \frac{\gamma_{SL}}{\sin(\theta - \alpha)} = \frac{\gamma_{SV}}{\sin(\theta + \alpha)} \quad (4)$$

and it follows that:

$$\frac{\gamma_{LV}}{\gamma_{SV} + \gamma_{SL}} = \frac{1}{\sqrt{1 + \beta\cos^2\theta}} \quad (5)$$

where:

$$\beta = \frac{4\gamma_{SV}\gamma_{SL}}{(\gamma_{SV} - \gamma_{SL})^2} \quad (6)$$

Writing $y = \frac{\gamma_{LV}}{\gamma_{SV}}$ and using equation (1), it follows that:

$$\cos\theta = (2\varphi - \sqrt{y})\sqrt{\frac{(1 - \varphi\sqrt{y})(1 + y - \varphi\sqrt{y})}{y(1 + y - 2\varphi\sqrt{y})}} \quad (7)$$

This may be compared with:

$$\cos\theta = \frac{2\varphi}{\sqrt{y}} - 1$$

for the case when deformability of the substrate is ignored.

The symmetrical form of the deformation, on which this result depends, is indeterminate from equilibrium of interfacial tensions alone, since any rigid-body rotation imposed on the system of forces cannot affect equations (4) in any way. But, in the absence of absorption, the properties of the solid phase below the interface are invariable and the condition of energy minimization would be thought, therefore, to lead to the symmetrical configuration shown in Fig. 1. This, in any case, is the natural modification of Young's equation, wherein both angles α are zero. Again if β is allowed to become sufficiently large, the limiting form of equation (5) is Young's equation, but in cases of greatest interest with organic substrates β is a number of order unity and equation (2) is then a very inadequate approximation to (5).

It is easily shown that equation (7) deviates hardly perceptibly from a rectilinear form provided φ is not too far from unity.

For the particular value $\varphi = 7/8$ it may be shown that the function given by the expression (7) intersects the straight line to which it approximates at its midpoint. It will be seen that the curve is virtually indistinguishable from this line (Fig. 2). On the other hand, if the deformation of the surface is neglected the corresponding dependence shows considerable curvature. It is generally recognized[5] that Zisman's rectilinear plots refer to solids where $0{\cdot}8 < \varphi < 1{\cdot}0$ so that the rectilinear form follows naturally as a result of surface deformability for such materials.

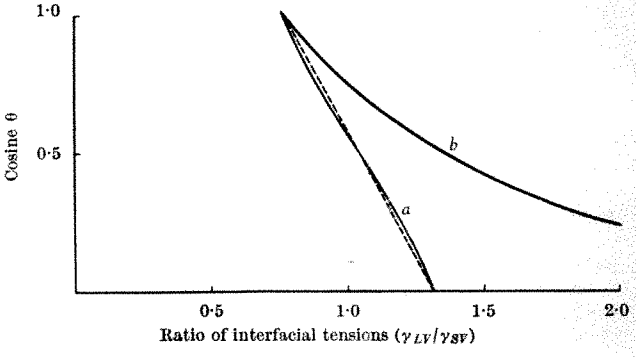


Fig. 2. The relation between cosine θ and the interfacial tension ratio. *a*, Calculated for a deformable substrate; *b*, calculated on the basis of Young's equation

The slope of the Zisman plots is readily related to the interaction parameter. Referring to equation (7), it is seen that $y = \varphi^2$ corresponds to $\cos\theta = 1$ and $y = 1/\varphi^2$ corresponds to $\cos\theta = 0$, so that the slope in question is given by $\varphi^2/1 - \varphi^4$. For forces that are of dispersion type, and φ exactly equal to unity, the Zisman line would be parallel to the vertical axis and contact dependent on the stringent requirement $\gamma_{LV} = \gamma_{SV}$, that is, $y = 1$. In this limiting case the rectilinear property is, of course, attained exactly.

For solid materials, the vector diagram usually known as Neumann's triangle[6] can only properly be applied to interfacial tensions derived from the Helmholtz free energy for the system as a whole. Defined in this way there is included a contribution from the deformational energy of the solid phase which, unfortunately, bears no simple relation to the surface free energy[4]. Since the relation (1) is more naturally applied to specific surface

free energies, it is perhaps inevitable that φ cannot be regarded as strictly constant for interfacial tensions as well.

In some organic solids, notably polytetrafluoroethylene, stress relaxation may be relatively rapid on account of the known facility for molecular chain rotation and translation[7], a tendency which should be enhanced in the surface layers on account of diminished packing density. In such cases the distinction between interfacial tension and intrinsic surface energy will tend to disappear, although some dynamic effects concerning contact angles might well be attributed to the actual rate of stress relaxation in the interfacial zone.

The rectilinear relationship established empirically by Zisman *et al.* would, therefore, seem to be consistent with the view that wetting phenomena depend significantly on surface deformability as well as on intrinsic surface energy effects. Consequently, Young's equation does not describe the boundary conditions as well as Neumann's triangle, in spite of the prevailing view that the latter applies only to liquids. As a corollary, it may be conjectured that the surface layers of organic solids are partially disoriented and exhibit a degree of mobility more characteristic of a liquid or gel. Dynamic effects would, on the same view, depend on the actual magnitude of the relaxation time for molecular re-orientation at the interface[8].

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$Ca_8Si_5O_{18}$ and the Nature of "γ-Dicalcium Silicate Hydrate"

Hydrothermal treatment of mixtures of γ-Ca_2SiO_4 and quartz has yielded a new compound of probable composition $Ca_8Si_5O_{18}$, or $8CaO.5SiO_2$. This is formed, together with other products, over a wide range of conditions (bulk $CaO : SiO_2$ molar ratio 1·5–2·0, temperature 180°–600° C, pressure 10–200 bars, time 3–14 days). It has not yet been obtained in a pure form, but a preparation containing it with only small proportions of γ-Ca_2SiO_4 and wollastonite was obtained by treating a mixture of bulk $CaO : SiO_2$ ratio 1·75 for 14 days at 600° C and 200 bars.

The crystals were of micron size and anhedral. Selected-area electron diffraction showed them to be orthorhombic, with *a*, 11·54 Å; *b*, 5·09 Å; *c*, 28·86 Å. Systematic absences were found of *h0l* reflexions with *h* odd, and of *0kl* reflexions with *l* odd; the space group could therefore be *Pcam* or $Pca2_1$. The principal X-ray powder spacings (in Å) were at 3·60 (*ms*), 3·05 (*vs*), 2·84 (*s*), 2·69 (*s*), 1·95 (*ms*), 1·895 (*ms*), and 1·806 (*ms*). The X-ray powder pattern was indexed and indicated cell parameters differing slightly from those obtained by electron diffraction: *a*, 11·45 Å; *b*, 5·09 Å; *c*, 28·70 Å. The *ab*-face of the unit cell is virtually identical in parameters with those of γ-Ca_2SiO_4 (ref. 1) and kilchoanite (a polymorph of $Ca_3Si_2O_7$) (refs. 2 and 3). Comparison of indexed X-ray powder patterns showed that the Fourier transforms of $Ca_8Si_5O_{18}$ and kilchoanite are closely similar. The infra-red absorption spectra of the two compounds are also very much alike.

These facts strongly suggest that the new compound has an interstratified structure formed by the regular alternation in the *c*-direction of layers of γ-Ca_2SiO_4 and kilchoanite. The unit cell of γ-Ca_2SiO_4 has $c = 6·78$ Å and atomic contents $4[Ca_2SiO_4]$ (ref. 1); that of kilchoanite has $c = 21·95$ Å and atomic contents $8[Ca_3Si_2O_7]$ (refs. 2 and 3). The unit cell of the interstratified compound would thus be expected to have $c = 28·73$ Å and atomic contents $4[Ca_8Si_5O_{18}]$. The predicted and observed cell parameters agree closely with each other, and the postulated chemical composition is compatible with the experimental evidence.

An apparent compound of composition $2CaO.SiO_2.0·26–1·00\ H_2O$ has been reported by various investigators as the product of hydrothermal treatment of β- or γ-Ca_2SiO_4 at 160°–300° C under pressures of saturated steam, and is generally known as "γ-dicalcium silicate hydrate" (ref. 4) or "$C_2SH(C)$" (ref. 5). The X-ray powder pattern, infra-red absorption spectrum, and thermal weight-loss curve of this material have been redetermined and are consistent with the view that it is a mixture consisting predominantly of calciochondrodite ($Ca_5(SiO_4)_2(OH)_2$) (ref. 6) and $Ca_8Si_5O_{18}$ or a phase closely resembling it. Electron microscopy and selected-area electron diffraction (ref. 7) show that it also contains kilchoanite.

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Absorption Spectra of Methylene Blue in Benzene

Dyes are generally investigated in aqueous media because of their insolubility in benzene and similar solvents. We have found[1] that many dyes, if extracted with benzene at a suitable *p*H, pass into the benzene medium, very often with a change of colour. This communication reports our preliminary results with methylene blue.

In Fig. 1, curves *A* and *B* are the absorption spectra of methylene blue in water and in a buffer of *p*H 11·2, respectively; it is evident that the shoulder of curve *A* at 610 mμ becomes quite prominent in the buffer. The shoulder has been ascribed by Rabinowitch and Epstein[2] to the dimer. The benzene extract of the dye solution in the aforementioned buffer is red in colour; curve *C* shows the absorption spectra in benzene. This red form of methylene blue was reported by Lewis[3] to be formed by the addition of hexane to an alcoholic solution of methylene blue. Lewis considered this to be a new form of dye. We recovered this red form of the dye as a solid (partly soluble) from the benzene extract by freeze-drying technique; the same could be dissolved in water and the absorption spectra are shown by curve *D* in Fig. 1. It would be seen that this red form has the peak exactly at the shoulder of the curves *A* and *B*. We conclude that the red form of methylene blue is not a new form of the dye as supposed by Lewis but is the dimer—its red colour in benzene being due to a solvent effect. The alternative idea that the red form is the carbinol base is discounted by the fact that the aqueous solution of the red form does not become blue on addition of acid. The shift in the position of the peak (curves *C* and *D*) is remarkably large. If the red form is dried strongly and dissolved in dry benzene, the position of the peak shifts somewhat towards longer wave-length, as is shown by the curve *E*. Evidently the effect of moisture on the absorption spectra of the red form of the dye is quite strong.

The absorption spectra of the red form in benzene solution are highly sensitive to acids or polymers containing sulphate and similar end-groups and to comparatively high concentrations of hydroxylic compounds. The peak at 515 mμ in benzene is not only shifted under such conditions to longer wave-lengths but shows a number of subsidiary peaks. Fig. 2 illustrates two such shifted absorption spectra by the addition of about 0·2 mM polymer sulphate (curve *B*) and by the addition of a large quantity of ethyl alcohol (curves *C*–*E*). Curve *A* shows the absorption maxima of the dye in benzene under the same condition. This is similar to the solvent effect observed by Sheppard[4] on merocyanine dye (polymethine dyes). All the optical measurements were recorded from a Hilger 'Uvispek' photoelectric spectrophotometer using 1-cm stoppered silica cells at room temperature (≈ 27° C). Studies of other cationic dyes in benzene and similar solvents using our extraction technique[1] are being made.

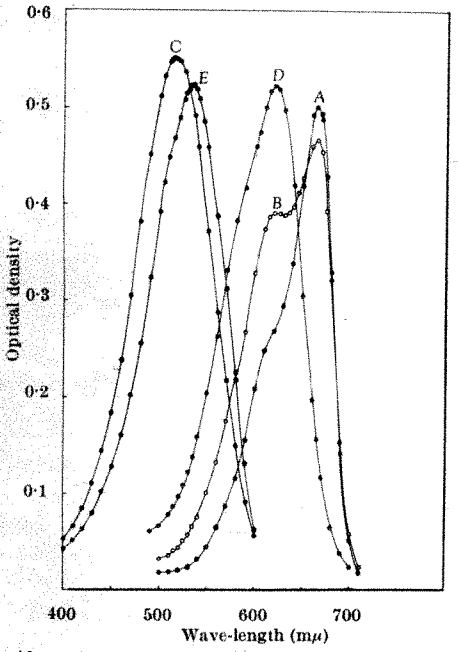


Fig. 1. Absorption spectra of methylene blue in *A*, water; *B*, buffer *p*H 11·2; *C*, benzene (red form); *D*, water (red form); *E*, dry benzene (dried red form)

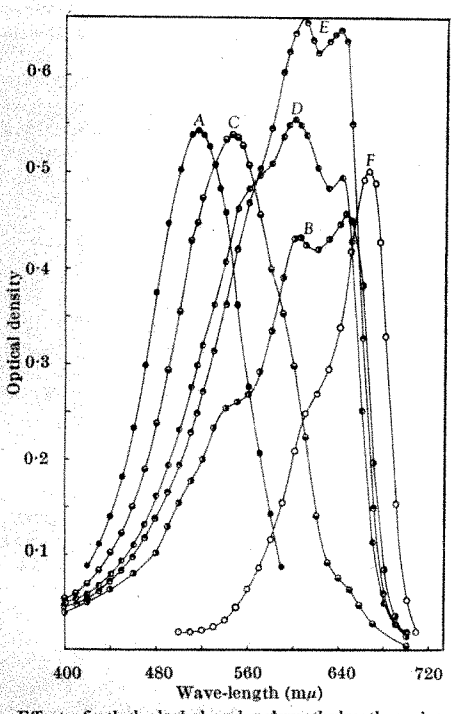


Fig. 2. Effect of ethyl alcohol and polymethylmethacrylate (PMMA) having sulphate end-group on red form of methylene blue in benzene. *A*, in benzene; *B*, on addition of PMMA; *C*, *D*, *E*, on addition of ethyl alcohol 20 per cent, 70 per cent and 90 per cent by volume, respectively; *F*, methylene blue in water

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BIOCHEMISTRY

Effect of Bailey's Tropomyosin Purification on EGTA-sensitizing Activity

Ebashi discovered that EGTA or Ca^{++} insensitive, non-relaxing actomyosin could be made EGTA sensitive by a third protein which exhibited physico-chemical properties characteristic of Bailey's tropomyosin B (ref. 1). However, he found no EGTA-sensitizing activity in Bailey's tropomyosin, and his new protein lost its activity when subjected to Bailey's procedures required for purification. Ebashi concluded that his EGTA-sensitizing protein was a new 'tropomyosin-like' protein or a native form of tropomyosin *B*. He offered no explanation concerning the difference between his 'tropomyosin-like' EGTA-sensitizing protein and Bailey's tropomyosin *B*.

In repeating Ebashi's preparation I noticed a striking discrepancy in the effect of the ammonium sulphate precipitation (60 per cent saturation) on the activity of the protein. In his experience[1] this precipitation led to inactivation and therefore had to be omitted from the purification procedure. In my experience this led to an increase in specific activity. Ammonium sulphate essentially free of trace contamination with heavy metal was used in our procedure; the usual commercial preparations are known to contain such trace contamination.

This situation suggested a parallel to that encountered by Kominz *et al.*[2,3] in determining the SH content of tropomyosin *B*. The 'salting-out' steps in the Bailey preparation led to a loss of SH groups due to the presence of heavy metals in commercially available ammonium sulphate. According to Drabikowski[4], even in the absence of heavy metals, the Bailey procedure leads to a variable loss of SH groups.

In order to investigate whether this loss of SH groups is responsible for the lack of EGTA-sensitizing activity we have prepared tropomyosin *B* by the Bailey method[5] with 0·5 mM dithiothreitol[6] present in all preparative solutions. A parallel preparation was carried out in which the protecting reagent for sulphydryl groups was absent. Crude tropomyosin extract and the protein obtained after Bailey's first, second and third purification cycle were assayed for their effect on the contractile response of synthetic actomyosin (Fig. 1).

In the preparation carried out in the presence of the protective reagent for sulphydryl groups (Fig. 1*A*), the EGTA-sensitizing activity of the crude tropomyosin extract increased greatly during the first purification cycle. Further purification cycles caused only a slight increase of the EGTA-sensitizing activity.

In the preparation carried out in the absence of the protecting thiol (Fig. 1*B*), markedly less EGTA-sensitizing activity was present. The activity of the crude extract also increased during the first purification cycle; however, further purification cycles caused a variable decrease of EGTA-sensitizing activity. Dialysis against 0·5 mM

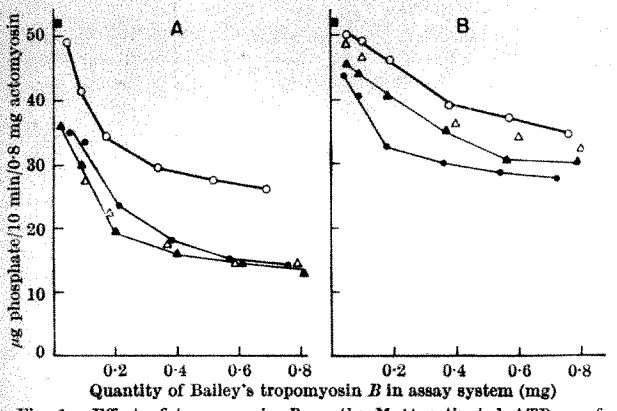


Fig. 1. Effect of tropomyosin *B* on the Mg^{++} activated ATPase of Ca^{++} insensitive synthetic actomyosin. *A*, Preparation carried out in presence of 0·5 mM dithiothreitol. *B*, Same preparation carried out in absence of dithiothreitol. ○, Tropomyosin extract not purified; ●, tropomyosin obtained after one purification cycle; ▲, tropomyosin after two purification cycles; △, tropomyosin after three purification cycles; ■, no tropomyosin present (control experiment). Assay system contained 0·8 mg actomyosin in 2 ml. Reaction medium: 0·06 M potassium chloride, 0·02 M imidazole (*p*H 6·75), 5 mM potassium-oxalate; 5 mM magnesium chloride; 1 mM ATP; 2·0 mM phosphoenol-pyruvate, 0·025 mg/ml. pyruvate kinase; 1 mM EGTA

dithiothreitol increased the EGTA-sensitizing activity to a level similar to that of the protein extracted and purified in the presence of the sulphydryl protecting agent. Dithiothreitol by itself had no effect on the EGTA-sensitivity of synthetic actomyosin.

Comparative assay experiments were carried out on Ebashi's protein (not shown) under identical conditions. The specific activity of the Ebashi preparation[1] corresponded approximately with that of Bailey's tropomyosin obtained after the first purification cycle.

These experiments indicate that EGTA-sensitizing activity requires sulphydryls which are lost during the usual Bailey preparation when these groups are not protected. Spectrophotometric analyses[7] of the sulphydryl content revealed 0·04–0·06 μmoles SH per mg protein for the preparations with high EGTA-sensitizing activity of Fig. 1*A*. Variable lower analytical values were obtained in the corresponding preparations (of Fig. 1*B*) with low EGTA-sensitizing activity.

In apparent conflict with this conclusion are recent findings of EGTA-sensitizing activity in preparations in which the presence of tropomyosin has not been demonstrated[8–11]. Two explanations probably account for these observations: (1) EGTA activating SH-tropomyosin may form complexes with other proteins whereby its characteristics are altered (SH groups in the complexes may be protected); (2) the criteria used for demonstrating tropomyosin were those of *SS*-tropomyosin and not of SH-tropomyosin. A previously implied third explanation[9] that EGTA-sensitization of synthetic actomyosin may not be a specific activity of a specific protein appears unwarranted in the light of the present investigations.

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Ultra-violet Extinction Coefficients of some Muscle Nucleotides and Purines at *p*H 7·0 and in 2 N Hydrochloric Acid

THE nucleotides and purines extracted from muscle were separated on anion-exchange columns and estimated by the methods of Hurlbert *et al.*[1], as modified by Kassemsarn *et al.*[2] and Jones[3]. Fractions of eluate containing the nucleotide or purine peaks were identified by their absorbances at 260 mμ. After bulking the fractions in each peak, the solutions were made 2 N with respect to hydrochloric acid, and quantitatively determined by measuring the extinction of the solutions in the ultra-violet region.

Conflicting values have been given in the literature for the millimolar extinction coefficients of two of the muscle nucleotides—adenosine-5′-diphosphate (ADP) and adenosine-5′-monophosphate (AMP)—and for three of their degradation products—inosine monophosphate (IMP), inosine, and hypoxanthine[4–7]. Thus, for AMP, E_{max} (at 258 mμ) at *p*H 7·0 is given as 14·0 by Bendall and Davey[5] whereas according to Bock *et al.*[4] this value is 15·4 (at 259 mμ). The ultra-violet spectra of these substances were therefore examined.

5′ AMP (monohydrate) and 5′ IMP (disodium heptahydrate), inosine and hypoxanthine (supplied by Koch-Light and Co., Colnbrook), were used in 5×10^{-5} M solution, in both 2 N hydrochloride and at *p*H 7·0 (phosphate buffer).

Measurements of absorbances over the wave-length range 230–280 mμ were made, against the appropriate blanks, in 1 cm silica cells, with a Hilger 'Uvispek' spectrophotometer. The millimolar extinctions obtained are given in Tables 1 and 2.

The extinction values obtained at *p*H 7·0 for 5′ IMP and 5′ AMP agree closely with those given by Bendall and Davey[5]. Values for 5′ AMP are very similar at *p*H 7·0 and in 2 N hydrochloric acid. The same *p*H change, however, exerts a very significant effect on the ultra-violet spectra of 5′ IMP, inosine and hypoxanthine.

The millimolar E_{max} for inosine, at *p*H 7·0 and in 2 N hydrochloric acid, are respectively 12·4 (at 249 mμ) and 11·3 (at 250 mμ). The value for hypoxanthine at *p*H 7·0 is 10·5 (at 249 mμ), and in 2 N hydrochloric acid this becomes 11·0 (at 247 mμ).

5′ IMP may not be completely separated from 5′ ADP on the ion-exchange column. These nucleotides may be quantitatively determined, when present together, by measuring the extinction of the bulked solution at the two wave-lengths, 265 mμ and 275 mμ. Assuming that the extinction values for 5′ ADP are not significantly

Table 1

Wave-length (mμ)	5′ AMP		Wave-length in mμ	5′ IMP	
	*p*H 7·0	In 2 N HCl		*p*H 7·0	In 2 N HCl
230	1·9	3·3	230	4·4	3·1
240	5·2	6·0	240	9·8	7·6
250	11·1	11·5	245	11·8	9·5
255	13·5	13·8	248	12·3	10·2
257	14·1	14·2	250	12·3	10·4
258	14·3	14·2	252	11·6	10·5
259	14·3	14·1	253	—	10·4
260	14·3	14·0	255	9·9	10·1
261	14·2	—	260	7·3	8·6
265	13·0	12·7	265	5·3	6·5
270	9·4	10·0	270	4·1	3·9
275	5·0	6·2	275	2·9	1·8
280	1·9	3·3	280	1·9	0·7

Table 2

Wave-length (mμ)	5′ Inosine		Hypoxanthine	
	*p*H 7·0	In 2 N HCl	*p*H 7·0	In 2 N HCl
230	5·3	4·8	4·2	5·7
240	10·1	8·8	8·2	9·6
245	11·9	10·5	9·9	10·8
247	12·2	—	10·4	11·0
248	12·3	11·2	10·5	11·0
249	12·4	11·3	10·5	10·9
250	12·2	11·3	10·5	10·8
251	12·0	11·3	10·4	10·7
255	9·9	10·8	9·5	9·6
260	7·3	9·3	7·8	7·6
265	5·4	7·0	5·6	5·3
270	4·1	4·6	3·4	2·7
280	1·8	1·1	0·6	0·5

different from those of 5′ AMP at these wave-lengths[4,7], the millimolar concentrations of 5′ IMP and 5′ ADP may be obtained from the equations:

$$E_{265} = 12{\cdot}7\,x + 6{\cdot}5\,y$$
$$E_{275} = 6{\cdot}2\,x + 1{\cdot}8\,y$$

where E_{265} and E_{275} represent the absorbances of the solution (in 2 N hydrochloric acid) at 265 and 275 mμ, and where x and y represent the millimolar fractions of 5′ ADP and 5′ IMP present in the solution.

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Enzymatic Determination in Serum of Lathyritic and Prednisone-treated Lathyritic Rats

While a remarkably raised serum-level of transaminases and aldolase is found in dermatomyositis, such augmentation, as a rule, is not found in cases of severe muscular involvement of scleroderma and disseminated lupus erythematosus (acutus)[1–3].

With this in mind, we investigated whether, in an acute experimental collagenose, that is lathyrism, the enzymatic activity in serum of glutamic-oxalacetic-transaminase (GOT), glutamic-pyruvic-transaminase (GPT), aldolase (ALD) and lactic-dehydrogenase (LDH) would be changed by an uptake of β-amino-proprionitrile. Using the procedure of Holzmann *et al.*[4], eighteen albino rats on standard altromin diet were divided into three groups. Group 1 (six animals) received 40 mg β-amino-proprionitrile daily; group 2 (six animals) received 40 mg β-amino-proprionitrile plus 0·5 mg prednisone daily; group 3 (six animals) served as a control. After 17 days the serum enzymatic activity was measured, using the 'Biochemica-test' combinations (Messrs. Boehringer, Mannheim).

As can be seen from Table 1, in serum of lathyritic rats, the activity of the transaminases, but not that of ALD or LDH, is raised in comparison to normals. The findings were more significant in the case of GPT than in that of GOT, whereas in the prednisone-treated lathyritic animals the transaminase-levels were again within the normal range. The single and probably non-specific intensification of the transaminases which was detected is probably due to primary lathyrogenic effects of β-amino-proprionitrile on the mesenchyme and may result from generally increased permeability of cells[5,6]. Moreover, an elective augmentation of these enzymes by raised suppliance of substrate[5] as a consequence of enhanced collagen destruction[4,7] can also be assumed. Furthermore, such mechanisms are in agreement with the control of elevated transaminase-values by corticosteroids[8,9].

The finding of heightened activity of transaminases in lathyritic rats confirms the clinical belief that these enzymatic activities are mainly related to acute disintegration of collagen, for example, in dermatomyositis, and are far less closely related to chronic collagen diseases such as scleroderma.

Table 1. Enzyme-levels in Rats

	Group 1	Group 2	Group 3
LDH			
Average	1,498	1,403	1,518
Standard deviation	±105	±115	±86
Statistical significance		–	
ALD			
Average	17·1	16·9	16·6
Standard deviation	±0·9	±1·0	±1·2
Statistical significance		–	
GOT			
Average	72·1	56·7	59·5
Standard deviation	±7·2	±9·1	±4·0
Statistical significance		Significant at the 1 per cent level	Significant at the 1 per cent level
GPT			
Average	27·5	18·0	17·6
Standard deviation	±4·1	±2·5	±1·8
Statistical significance		Significant at the 1 per cent level	Significant at the 1 per cent level

Enzymatic activities in mE./ml. serum. Statistical significance was examined using the *t*-test.

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High Nucleo-cytoplasmic Concentration Gradient of Chloride in Rat Liver

Previously, a high nucleo-cytoplasmic concentration gradient of sodium was found in rat liver and other tissues[1,2]. Since the concentration of potassium also was higher in the nucleus than in the cytoplasm, about 500 μequiv./g dry weight of anions should exist in the nucleus if the assumption is correct that nucleic acids and basic proteins approximately neutralize each other. This communication presents data which demonstrate a high concentration of chloride in the nucleus of the rat-liver cell.

Nuclei were prepared by the non-aqueous technique[3]. Analyses for chloride were performed, after digestion with 0·1 N nitric acid, by mercurimetric titration[4] in a recording Zeiss spectrophotometer; the end-point was evaluated by graphical means. By this method 104 per cent of the chloride which was added to the tissue fractions was 'recovered'. None of the materials and solvents which were used for the isolation of nuclei raised the blank values above the limit of error. Tissue preparations which were freed from lipids by exposure to the solvents of the non-aqueous procedure did not lose or gain any chloride.

The results (Table 1) demonstrate a concentration gradient of chloride of about 10 between cytoplasm and nucleus. This gradient compares well with that of intra-

Table 1. Concentration of Chloride in Rat-liver Whole Tissue and Nuclei

Tissue fraction	Experimental conditions	RNA/DNA	Cl^- (μeq./g) Dry weight	Cl^- (μeq./g) Fresh weight	No. of analyses
Whole tissue*	1	2·9	135	41	14
Cytoplasm†	1	—	35	10	—
Nuclei*	1	0·21	204	95	18
Whole tissue	2	2·0	42	—	2
Nuclei	2	0·50	68	—	2
Whole tissue	3	2·7	270	—	2
Nuclei	3	0·41	347	—	2

Experimental conditions: (1) analysed as obtained; (2) after perfusion of the livers of 40 animals *in vivo* with 0·30 M sucrose; (3) after perfusion of the livers of 40 animals *in vivo* with 0·15 M lithium chloride.
* These data represent the average of ten different batches of tissue preparations obtained from 40–50 animals each.
† Derived data from analyses of whole tissue and nuclei, after corrections for the chloride content of blood (10 per cent of fresh weight), of extracellular fluid (15 per cent of fresh weight), and of nuclei (6·6 per cent of fresh weight)[2].

cellular sodium[2]. Most of the nuclear chloride was removed by liver perfusion *in vivo* with a salt-free solution (experiment 2) and the concentration was considerably raised by perfusion *in vivo* with a chloride solution (experiment 3). Furthermore, after *in vitro* incubation of nuclei for 30 min (50 mg dry weight in 5 ml. 0·075 M SO_4^{2-}, containing Na^+ and/or K^+ in different proportions), more than 90 per cent of the chloride originally contained in the nuclei was recovered in the incubation medium.

Existing data[2] suggest that there are about 160 μequiv. Na^+/g dry weight and 320 μequiv. K^+/g dry weight in rat-liver nuclei, as compared with about 200 μequiv. Cl^-/g dry weight (Table 1). Therefore, about 40 per cent of nuclear alkali cations are neutralized by chloride. The deficit of anions which still remains is not covered by sulphate; analyses of the aforementioned liver fractions for total sulphur by the method of Tölg[5] revealed 24 μequiv./g dry weight in whole tissue and 4 μequiv./g dry weight in nuclei. Sulphate would only account for a fraction of the total sulphur content.

Nuclear chloride may be a component of the sodium space of the nucleus[6], the function of which, however, is not as yet fully understood. Anyway, a distinct cellular site of high chloride concentration exists which is very similar to the concentration of chloride in the extracellular fluid. Information concerning the size and the significance of the chloride space in tissues and also the chloride potentials can be deduced from the data given in Table 1, which goes to show that cellular chloride is not distributed uniformly.

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Calf Thymus and Chicken Erythrocytes Nucleoproteins

We have prepared a large series of nucleoproteins (DNP) from calf thymus by the method of Zubay and Doty[1] and, unlike these authors, have obtained amounts of gel (gDNP) that varied from 0 to 80 per cent. We were thus surprised to find that extraction of DNP from chicken erythrocytes by the same procedure regularly gave high percentages of gel; of six preparations, five gave 90 per cent or more gDNP.

The most important difference between the two series of preparations, aside from the origin of the starting materials, was the way in which they were obtained. The chicken blood was collected directly in the sodium chloride-versene used for washing the nuclei, but thymus could not be processed immediately; it was frozen at variable times after the death of the animal, and frequently processed after more or less prolonged sto in a refrigerator. Furthermore, the percentage of DN gel was higher when enzymatic activity was stopped rapidly, as shown by the results with erythrocytes. This observation suggested that the soluble DNP (sDNP) might represent degraded material. To determine the nature of this degradation we prepared a new series of calf thymus nucleoproteins reducing the time before the tissue was frozen, in order to approach the conditions used in collecting the erythrocytes. We also kept the thymu for varying periods of time before starting the preparation, and noted the amounts of gDNP and sDNP obtained. We then undertook an analysis of the two types of DNP. Great care was taken to avoid mechanical degradation when the DNA was being extracted; its molecular weight was determined by light scattering in M sodium chloride.

Preparations made from thymus frozen 10–15 min after the animal had died produced between 92 and 98 per cent of gDNP. The DNA extracted from these DNP preparations had a molecular weight of 10·5–15·5 million.

Of three other preparations made from the same thymus, one, *V*123, had been homogenized 15 h earlier in 0·15 M sodium chloride and left at 0° C. All gave high percentages of gel, 97, 96 and 92 per cent; but the molecular weight of the DNA from gel *V*123 was only 6·5 million, whereas the other two gave values of 10·9 million.

Three preparations made from three different lots of thymus yielded variable amounts of sDNP and gDNP. Thymus *V*127, left 15 h at 23° C before processing, gave 42·4 per cent gel and 57·6 per cent soluble DNP, the molecular weight of the DNA extracted from DNP 127 gel was 8·2 million while that of *V*127 soluble DNA was 5·1 million. Thymus *V*128, stored in a freezer for 3 weeks, gave 77·6 per cent gel, and *V*266, frozen for 2 months, gave 76·3 per cent. The molecular weights of the gel DNAs were, respectively, 6·7 and 7 million; those of the DNAs from the two sDNP only 2·8 and 2·4 million. (The DNAs extracted from two lots of poorly washed[2] erythrocyte nuclei, which had been allowed to stand for 24 h at 0° C, also had very low molecular weights, 0·9 and 1 million.) Thus, in the sDNP that we have examined the molecular weight of the DNA has always been lower than that of the DNA from the corresponding gel, so that the sDNP must have undergone some nuclease action.

Chemical analysis of the sDNP and gDNP from thymus gave mean nitrogen : phosphorus ratios of 4·15 for the gels and 3·80 for the soluble fractions; a gDNP seems to be richer in protein than the corresponding sDNP. The lability of the sDNP was shown by its precipitation in 0·15 M sodium chloride; soluble DNP left 2–2·5 times as many small and dialysable protein fragments in solution. This suggests that the protein of the sDNP has also been attacked enzymatically[3,4] and that the resulting fragments are easily detached. In a DNP gel the protein fraction responsible for the constitution of a network between DNA molecules probably remains intact.

It should be emphasized that we have found no difference in the nitrogen : phosphorus ratio of the s and g fractions of chicken erythrocyte DNP and that this ratio is lower than that of the thymus gels. This suggests that the gDNP of thymus may be richer in some particular histone, perhaps active in gene regulation, that is responsible for the state of the gel, is more labile than other histone fractions, and is removed from sDNP. We are continuing these investigations in order to verify this hypothesis.

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croscopic Localization of Amines entral Nervous Tissue

have been detected in the central eans of a fluoresc chnique[1], and made regard tent of the prese nerv

Several techniques for fine-structural norepinephrine localization have been reported in adrenomedullary tissue[4–7]. One of these[4,5] has been used to detect amines in the central nervous system. This communication reports some results obtained with a group of Syrian hamsters.

Adult Syrian hamsters were perfused, by way of the ascending aorti, with cold (4° C) 4 per cent glutaraldehyde in 0·1 M sodium cacodylate buffer (*p*H 7·21) for 40–60 min at a pressure of 100–120 mm mercury. The brains were removed following perfusion, hypothalami dissected out, tissue diced and placed in fresh cold fixative for 1 h. Cubes of brain material were rinsed overnight in cold (4° C) sodium cacodylate buffer (*p*H 7·21) which contained 4 per cent sucrose. Cubes were then incubated for 24 h at 4° C in a solution of 2·5 per cent potassium dichromate and 1 per cent sodium sulphate in a 0·2 M acetate buffer (*p*H 4·1) (ref. 8). Half the incubated blocks were later treated with 1 per cent osmium tetroxide[9] for 90 min. All tissues were dehydrated in graded alcohols and embedded in 'Epon'. Sections were prepared using an LKB 'Ultratome' and examined on an RCA '*EMU-3F*' electron microscope.

Fig. 1 illustrates hypothalamic material which was incubated in dichromate following glutaraldehyde fixation. This procedure shows only the reaction sites of the amines. Granules of this nature were seen throughout the tissue. Figs. 2 and 3 show tissues which, in order to provide identification of surrounding structures, have been treated with osmium tetroxide following dichromate incubation. Fig. 2 demonstrates granules of varying density within vesicles together with smaller vesicles which appear to be empty. The empty vesicles may represent the site of storage of another type of transmitter or they may be vesicles which at one time or another contained granules. Fig. 3 shows electron-dense granules which are different from those in Fig. 2. The granules in Fig. 3 may be in the process of storing or releasing amine, but it is possible that the different appearance might be due to osmotic changes induced during the process of preparing the tissue.

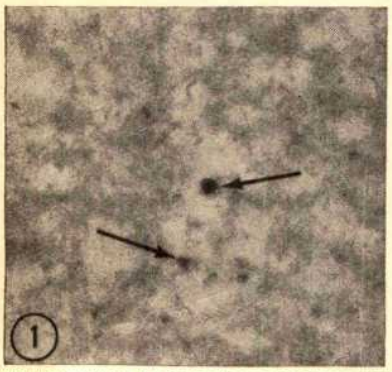


Fig. 1. Hamster hypothalamic tissue: 4 per cent glutaraldehyde fixation with potassium dichromate incubation. Arrows point to granular sites of amine localization. (× 30,000)

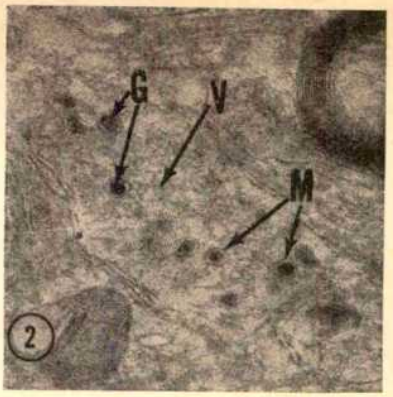


Fig. 2. Hamster hypothalamic tissue: prepared as in Fig. 1 but also treated with osmium tetroxide. Amine granules (*G*) of varying densities surrounded by membranes (*M*) are present in the nerve terminal. Vesicles (*V*) which appear to be empty are also present. (× 30,000)

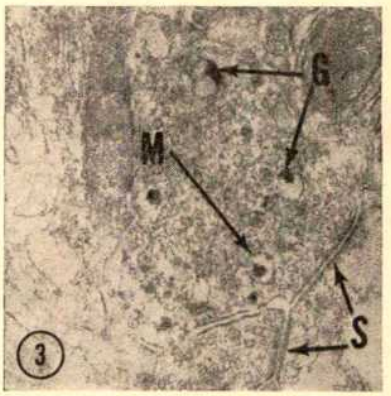


Fig. 3. Hamster hypothalamic tissue prepared as in Fig. 2. Some granules (*G*) have assumed an eccentric location within the granule membrane (*M*). Synapses (*S*) are also evident at this terminal. (× 30,000)

Although the reaction is known to localize norepinephrine at the fine-structural level, it is possible that other biologically occurring amines which possess unsubstituted amino groups may also be demonstrated. *In vitro* investigations were performed and revealed that both dopamine and 5-hydroxytryptamine (5HT), both with unsubstituted amino groups, can also be demonstrated by means of this technique. *In vitro* experiments were conducted using amine concentrations ranging from 6×10^{-3} M to 6×10^{-4} M. Amines tested were epinephrine, norepinephrine, dopamine, 5HT, dihydroxyphenylalanine (DOPA), phenylalanine and tyrosine.

Table 1

a. Various amine solutions treated with 4 per cent glutaraldehyde (*p*H 7·2) followed by addition of potassium dichromate (*p*H 4·1) solution

	Amines						
	Norepinephrine	Dopamine	5HT	Epinephrine	DOPA	Phenylalanine	Tyrosine
Glutaraldehyde	(+)	(+)	(+)	(−)	(−)	(−)	(−)
Glutaraldehyde + dichromate	(++)	(++)		(−)	(−)	(−)	(−)

b. Norepinephrine (6×10^{-3} M) treated with [illegible] glutaraldehyde and potassium dichromate (*p*H 4·1) solution

	araldehyde concentration			
0·4–0·01 M	8	5×10^{-3} M	3×10^{-3} M	2×10^{-3} M	1×10^{-3} M
(+)		(+) *	(−)	(−)	(−)
(++)		(++)* 5/6	(−) 1/2	(−) 1/3	(−) 1/6

[illegible]ipitate.
[illegible]nes norepinephrine in test-tube.

Glutaraldehyde concentrations ranged from 0·4 M to 1×10^{-3} M. Reactions were carried out at 4° C, 25° C and 37° C. Aliquots (3 ml.) of the various amine and glutaraldehyde concentrations were mixed and allowed to stand for 2 h, then 3 ml. of the dichromate solution was added and the test-tubes left to stand for an additional 24 h.

Table 1 summarizes the results of these *in vitro* investigations: *a* illustrates the results of treatment of various amines with 4 per cent glutaraldehyde; only those amines with an unsubstituted amino group reacted with glutaraldehyde and later produced insoluble precipitates with the dichromate solution; *b* shows the results of treatment of 6×10^{-3} norepinephrine with various concentrations of glutaraldehyde. It is evident that, when the glutaraldehyde/norepinephrine (G/N) ratio of molecules approaches 1/2, or less, the limit of the reaction is reached. Reaction occurs with excess glutaraldehyde, but when there is excess norepinephrine, no precipitates are formed. This was evident from *in vitro* pilot experiments when it was found that 6·25 per cent glutaraldehyde was less effective than 4 per cent in producing precipitates. It should also be pointed out that reaction rates increased when the temperature was increased.

Tramezzani *et al.*[7] recently postulated that a Schiff monobase is formed by the condensation of glutaraldehyde with the unsubstituted amino group of norepinephrine and that the condensation product reduces ammoniacal silver, thus providing a means of detecting norepinephrine using a light or electron microscope. In the present investigation, formation of the Schiff base was evident and the precipitate was the result of reduction of the dichromate solution to chromium dioxide, which is a brownish-black precipitate recovered from the bottom of the test-tubes. One question which existed regarding the initial reaction was that because glutaraldehyde is a dialdehyde it may possibly react with two molecules of norepinephrine and thus there would exist a disubstituted aldehyde rather than a Schiff monobase with reducing properties. The graded concentrations of glutaraldehyde helped in resolving this question, as it was found that one molecule of glutaraldehyde will react with two molecules of norepinephrine only if there are not sufficient glutaraldehyde molecules present in solution. When there is an excess of amine molecules, both aldehyde groups of glutaraldehyde become complexed, no Schiff monobase is formed and the solution loses its reducing properties. Thus, the reaction proceeds only when there is an excess of glutaraldehyde.

Earlier work using the light microscope in differentiating epinephrine and norepinephrine cells in adrenomedullary tissue[8] revealed that dichromate was essential for the reaction. If the tissue was first placed in formaldehyde, the dichromate reaction was inhibited. In the present investigation, amine solutions were treated with both 10 per cent and buffered 4 per cent (*p*H 7·2) formaldehyde until the amine solution became pink. It was assumed at this point that the reaction in which the isoquinoline derivative of Falck[10] was formed had occurred. Treatment of these solutions with glutaraldehyde produced no evidence of Schiff base formation and no precipitates were formed when dichromate solution was added. The addition of dichromate preceding glutaraldehyde also prevented formation of a precipitate, although the solutions became quite dark in colour.

It is evident from these experiments that the unsubstituted amino group of the compounds tested is important in the completion of the reactions and the production of the insoluble, electron-dense precipitates. It should be pointed out th[illegible] the present tech[illegible] nique can demo[illegible] norepinephri[illegible] from th[illegible]

manner similar to that of the *in vitro* investigations, then the amino groups are free in tissue to react and, therefore, not involved in the natural binding process.

I thank Dr. J. F. Diehl, Department of Biochemistry, the University of Arkansas Medical Center, for his advice, and Mrs. Nancy L. Bell and Mrs. Brenda H. Ryker for their technical assistance. This work was supported by U.S. Public Health Service grants *FR*–05050–01 (general research grant) and *NB*–05093–01 (National Institute of Neurological Diseases and Blindness).

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Electrophoresis of the Soluble Proteins of Wheat Leaf

THERE have been several reports of gel electrophoretic separations of leaf proteins. Four components were reported for cabbage leaf[1], seven bands for rice leaf[2], and about six bands for bean leaf[3]. Bean leaf proteins have also been studied by Racusen and Calvanico[4] using preparative gel electrophoresis. Steward and Barber[5] obtained good electrophoretic fractionation of the soluble proteins of *Neurospora crassa*, but observed that extracts from tissues of higher plants yielded bands which were less discrete against a diffuse background. The present paper describes the electrophoresis of the soluble proteins of wheat leaf on 7·5 per cent polyacrylamide gel using a discontinuous *tris* chloride–*tris* glycinate buffer system. The method used gave a well-defined pattern and separated more components than reported previously for leaf tissues.

First and second leaves of 30-day-old wheat plants were ground at 4° C with two parts by weight of sand (acid washed) and one part of 0·05 M phosphate buffer (*p*H 7·5) containing 12·5 per cent glucose. The extract, freed of sand and fibre by centrifugal filtration, was centrifuged at 144,000*g* for 30 min. The clear amber supernatant (containing approximately 8 mg protein/ml.) was used for electrophoresis without further treatment.

Disc electrophoresis was carried out as described by Ornstein and Davis[6] except that the sample (300 μg protein) was applied to the top of the polyacrylamide column by layering under the electrode buffer. Electrophoresis was carried out at room temperature (about 25° C) at 3 m.amp per column for 1 h. Gels were stained with amido black 10*B*[6], and destained electrophoretically at 10 m.amp per column for 1 h. Fig. 1 shows the pattern obtained for *Triticum dicoccum* leaf proteins. The accompanying scan was performed on a 'Photovolt' densitometer (Model 530) equipped with a narrow slit (0·1 mm) and set on response 12.

The use of [illegible]henol blue to mark the chloride–glycine bou[illegible] unnecessary with the leaf extract [illegible]d by a sharp amber band. Staining [illegible] was also associated with the [illegible]ed by comparing its mobility [illegible] particular band (comparative [illegible]r over half of the applied [illegible]nds to Fraction I, reported [illegible]though Fraction I was re-

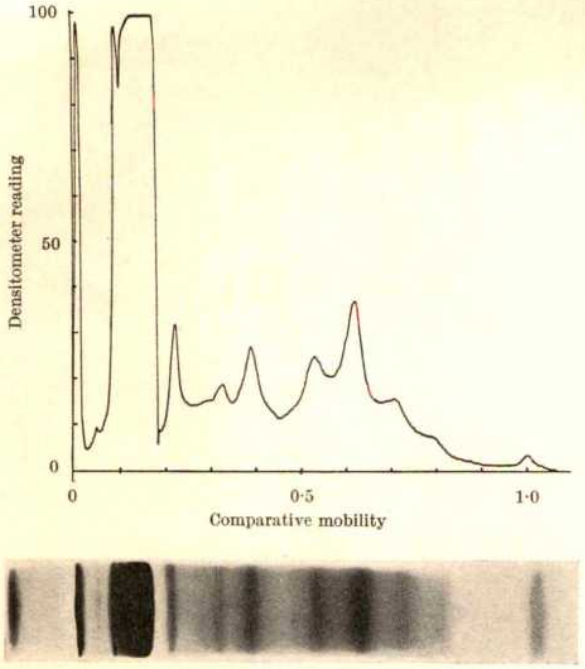


Fig. 1. Electrophoretic separation and corresponding densitometer tracing of the soluble leaf proteins of *Triticum dicoccum*. Direction of migration is left to right. The large pore spacer gel protrudes to the left of the origin

ported[7] to have a higher mobility than the other leaf proteins, its relatively large size (molecular weight of 375,000 (ref. 8)) was probably responsible for its low mobility in 7·5 per cent polyacrylamide (pore size 50 Å (ref. 6)).

At present fifteen to eighteen bands can be distinguished and this may be improved by current investigations designed to refine the technique. The method is being used to investigate the role of leaf proteins in rust infection of wheat plants.

One of us (C. W. W.) wishes to acknowledge the award of a Commonwealth Research Studentship.

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Available Lysine Content of Dried Milk

The heat treatment to which milk is subjected during drying causes some combination between the ε-amino groups of lysine and lactose, which renders such lysine unavailable for nutritional purposes. Since drying conditions on rollers are more severe than [illegible] a spray chamber it is possible that there is less available [illegible] in roller-dried than in spray-dried powder. A ser[illegible]lable lysine determinations was made on both [illegible] number of factories to discover [illegible] this essential amino-acid and to [illegible] are sufficient to cause any def[illegible] ditions of feeding.

Four different batches of [illegible] tories were tested in duplica[illegible] fluorobenzene method as modified by Rao *et al.*[2], and correcting for the loss due to the acid hydrolysis in the presence of lactose. The results are shown in Table 1, together with figures for raw bulk milk. By comparing the results for liquid and dried milk an estimate can be made of the average percentage of lysine destroyed during production in each case.

Table 1. Available Lysine Content of Liquid and Dried Milk

Factory	Available lysine (g/16 g N) Maximum	Minimum	Mean	Average percentage destruction of lysine
		Liquid milk		
—	7·5	7·4	7·4	—
		Spray-dried milk		
A	7·3	6·9	7·1	4·1
B	7·6	7·4	7·4	Nil
C	7·3	6·9	7·1	4·1
		Roller-dried milk		
A	6·5	5·8	6·2	16·2
D	7·3	7·1	7·2	2·7
E	7·4	6·9	7·2	2·7
F	6·5	6·0	6·2	16·2
G	7·3	6·2	6·7	9·5

Table 2. Comparison of the Available Lysine of Human Milk and of Reconstituted Full-cream Roller-dried Powders

	Available lysine (g/16 g N)	Protein (per cent)	Available lysine (mg/100 ml.)
Human milk (ref. 3)	6·2	1·5	93
Reconstituted full-cream roller powder, best individual sample	7·4	3·3	244
Reconstituted full-cream roller powder, worst individual sample	5·8	3·3	191

Table 3. Daily Requirement of the Infant for Lysine

Minimum weight of lysine required per kg body-weight per day (ref. 4)	103 mg
Weight of lysine supplied per kg body-weight per day by full-cream roller powder (70 g):	
(*a*) Best individual sample	347 mg
(*b*) Worst individual sample	274 mg

The loss of lysine in the spray powders, and in the roller powders from factories *D* and *E*, is very small. The loss is greater in roller powders from factories *A*, *F* and *G*, but is in no case sufficiently high to make these products deficient in this amino-acid when used for infant feeding. This aspect is demonstrated in Tables 2 and 3, where comparisons are made with human milk and with the daily requirements of the infant.

All the individual samples of powder examined are richer sources of lysine than is average human milk, and when fed in the proportions normally recommended for infants they will provide two and a half to three times the minimum daily requirements.

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Production of Peptide Alkaloids in Submerged Culture by a Strain of *Claviceps purpurea* (Fr.) Tul.

The production of lysergic acid α-ethoxy amide in submerged culture by a strain of *Claviceps paspali* Stev. et Hall was reported earlier[1–3]. The present communication describes the production of ergotamine in submerged culture by a strain of *Cl[illegible] purpurea* (Fr.) Tul. isolated [illegible] sclerotium [illegible] on a variety of Triticale [illegible] This strain [illegible] of Bio-[illegible]

The results (Table 2) show that the *in vivo* treatment of the host prolongs the survival of the skin homograft only if 'Urethan' is used at very high doses. The action of 'Urethan' in the experimental conditions reported here may be partly due to an antigenic simplification induced *in vivo* at the level of the grafted tissue.

It may be concluded that our experiments suggest the possibility of attenuation of the reaction to homograft by means of a chemical antigenic deletion.

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Immuno-adsorbents of High Capacity

Preparation of antibodies is greatly facilitated by the use of insoluble immuno-adsorbents. We have found that cross-linkage of soluble antigens with formaldehyde readily yields adsorbents having a high antibody capacity and which are insoluble over a wide range of *p*H. Most experiments have been carried out with an antigen (BSA–CPE) prepared by coupling the diazotized *p*-amino-phenylether of choline (CPE; $NH_2C_6H_4OCH_2CH_2\overset{+}{N}(CH_3)_3$) to bovine serum albumin (10 mols/mole). An insoluble polymer of the BSA–CPE was prepared by slowly adding an equal volume of formaldehyde solution (40 per cent; *p*H 3·1) to a stirred 20 per cent solution of the antigen in 0·15 M sodium chloride. The reaction was allowed to proceed for 1 h at 25° C before the insoluble floccules of antigen polymer were separated by centrifuging. The floccules were washed exhaustively to remove excess formaldehyde with a solution containing 2 M sodium chloride and 0·01 M ammonium chloride adjusted to *p*H 9·0 with ammonium hydroxide, and finally with 0·15 M sodium chloride.

Antibody was extracted from rabbit antisera (1·5 mg antibody/ml.) to BSA–CPE by stirring 250 ml. serum (*p*H 7·0) with 1 g of the polymerized antigen for 30 min at 20° C. The polymer was removed by centrifugation and washed with 0·15 M sodium chloride (*p*H 7·0) until the washings contained less than 10 μg/ml. protein. Washing was then continued with 2 M sodium chloride. The antibody was dissociated from the insoluble antigen by the competitive hapten choline (2 M; *p*H 7) by stirring with successive portions for 15 min at 25° C. Choline was removed by passing through 'Sephadex *G*-75' equilibrated with 0·015 M ammonium acetate. The choline-free antibody solutions were lyophilized and dissolved in a small volume of 0·15 M sodium chloride at *p*H 7·0. The small amount of insoluble protein was removed by centrifugation. The successive elutions by 25, 50 and 50 ml. of choline chloride contained 103, 47 and 15 mg of protein respectively. The total yield of 165 mg represented 71 per cent of the antibody extracted by the antigen polymer and an overall yield of 47 per cent for the single extraction. The purified antibody was 84 per cent precipitable by soluble BSA–CPE. Prolonging the time of contact between antiserum and the polymer increased the overall yield to 80 per cent. The capacity of the antigen polymer was about 300 mg antibody/g. This capacity was somewhat lower than that of the cross-linked protein polymers prepared by Pressman *et al.*[1].

This method has also been used successfully with other determinants, and polymerized BSA has been used to remove anti-BSA antibodies from sera. Dissociation in acid may be substituted for hapten competition.

The method described here would seem to offer a simple and general method for preparing highly purified antibodies against protein-linked determinants and may have a limited application in isolating antibodies to native proteins.

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Stimulation of Phagocytosis in Relation to the Mechanism of Action of Adjuvants

Antigens in emulsions of water-in-oil are known to enhance antibody response more effectively than when dissolved or suspended in saline[1], but the mechanism of action of the adjuvant remains unsettled. Freund[2] postulated that absorption, destruction and elimination of antigen are retarded by emulsions of water-in-oil with or without the presence of mycobacteria. Recently, however, Dresser[3,4] found that subcutaneous injection of Freund's adjuvant without antigen enhanced the antibody response against bovine γ-globulin given to mice intravenously: this suggests that the adjuvant exerts a non-specific influence on the immune mechanism. Further, it has been demonstrated that Freund's adjuvant activates the immune machinery in the early phase of the primary immune response preceding the specific response against an antigen[5]. It has also been shown that phagocytosis of the antigen is a crucial step in the induction of the primary immune response[6,7].

Recently we have found that oestrogens have an adjuvant effect on the primary immune response in mice[8]; and previously we have shown that stimulation of the reticulo-endothelial system by the administration of oestrogen raises general body defence as indicated by increased phagocytic activity, raised serum γ-globulin, and increased protection of experimental animals against virulent infections[9]. The present investigation has been designed to ascertain whether stimulation of the phagocytic activity of the reticulo-endothelial system is part of the mechanism of action of Freund's adjuvant.

Intact male white mice (*T.O.* Swiss strain) of 20–25 g body-weight were used throughout. Groups of five animals were used to assess the effect on the reticulo-endothelial system of each dose of adjuvant. Each animal received a subcutaneous or intraperitoneal injection (Table 1) of Freund's incomplete or complete adjuvant (Difco Laboratories) once daily for 3 days in the doses shown. Phagocytic activity was assessed in these animals on the fourth day after treatment had started by measuring the rate of clearance of a known amount of a specially prepared carbon suspension from the circulating blood[10]. For each dose-response experiment five animals were used as controls; they received no treatment and were assessed by the carbon method on the same day as the treated animals. To investigate the speed of action and duration of effect of Freund's adjuvant on the reticulo-endothelial system (Table 2), groups of five animals received a single intraperitoneal injection of 0·2 ml. adjuvant; and on each successive day thereafter, up to the fourth day, phagocytic activity was assessed by the carbon method. Five untreated animals were used daily as controls.

The results are shown in Tables 1 and 2. The index of total-body phagocytic activity is denoted in the tables by the symbol *K*. The *K* value indicates the rate of removal

Table 1. INFLUENCE OF ERYTHROID MARROW ACTIVITY ON THE PLASMA DISAPPEARANCE ($t_{\frac{1}{2}}$) OF INJECTED ERYTHROPOIETIN DOGS

	No. of animals	Reticulocytes in blood (%)	Erythropoietin plasma clearance $t_{\frac{1}{2}}$ (h)
Erythropoietically normal dogs	5	0·2 ± 0·08*	7·3 ± 0·8*
Erythropoietically depressed dogs	3	0·05 ± 0·02	8·1 ± 1·4
Erythropoietically stimulated dogs	3	7·2 ± 2 54	8·8 ± 1·1

* Standard error of the mean.

pressed (post hypoxia) dogs after exchange transfusion with 'anaemic plasma' from previously bled donor dogs, in an attempt to assess these hypotheses.

In order to obtain high concentrations of erythropoietin in donor dog plasma, normal mongrel dogs were bled once daily for 4 days until the haematocrit was 7–11 per cent. Blood volume was maintained by replacing blood drawn with an equal volume of 6 per cent dextran in saline. The erythropoietic marrow of three dogs was stimulated by exposure to a simulated altitude of 18,000 ft. for 10 days. The rate of removal of intravenously injected erythropoietin was determined without removing the dogs from the hypoxic environment. Erythropoiesis was suppressed in dogs by first making them polycythaemic by exposure to hypoxia in a decompression chamber and then returning to sea-level. Three dogs were kept continuously at a simulated altitude of 14,000–21,000 ft. for 90 days (14,000 ft. for the first 2 weeks, increased to 21,000 ft. for the remainder of the exposure). To ensure adequate iron for haemoglobin synthesis, 100 mg of 'Inferon' was given to the dogs before they were exposed to hypoxia. The dogs were then 'returned' to sea-level conditions and the study was commenced on the sixth day after hypoxia. At this point erythropoiesis had become almost completely suppressed, as was judged by the near absence of reticulocytes in the peripheral blood.

The rate of removal of erythropoietin from the plasma of normal dogs, erythropoietically stimulated and erythropoietically depressed, was determined by following the rate of loss after partial replacement of the dogs' plasma with plasma from severely anaemic (bled) donors.

The erythropoietic activity of samples was measured by assay of iron-59 incorporated in the red blood cells, using hypertransfused polycythaemic mice, as described by DeGowin *et al.*[4].

When the curve of plasma erythropoietin disappearance was derived on semilog paper by plotting the amount of erythropoietin remaining in plasma (log scale) against time after exchange transfusion (linear scale), only one component was apparent, which was practically linear to the point where it could no longer be detected (15–24 h). Table 1 shows the average erythropoietin plasma clearance ($t_{\frac{1}{2}}$) of the three groups of animals studied. In the three groups the average half time of disappearance was almost identical. These results agree with those from Naets and Wittek[5], who were unable to detect any effect of erythroid hyperplasia on utilization of erythropoietin, and suggest that there is no correlation between the erythroid activity of the bone marrow and the rate of loss of the hormone from the plasma compartment.

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IMMUNOLOGY

Chemical Deletion of Histocompatibility Antigens—Homograft Survival of Rat Skin treated with 'Urethan' *in vitro*

THE prolongation of the survival of homografts has been achieved by treating the recipient host with large doses of drugs which interfere with the immune response[1]. Effective agents are, however, often toxic, so that their use in man is limited.

In recent years evidence has been accumulated in our laboratory that tumour cells treated with various chemical agents *in vitro* lose membrane antigens[2,3]. 'Urethan' has proved to be one of the most effective and least toxic of the compounds so far tested[4].

Because an 'antigenic deletion' at the level of histocompatibility antigens could prevent the early immunological response against a grafted organ, experiments have been carried out to obtain a prolongation of skin homograft survival by pretreating the tissue with chemical agents *in vitro*. Preliminary results have shown that the first-set rejection of skin homografts in the rat can be attenuated if the skin is treated with 'Urethan' *in vitro* before grafting onto the recipient host[5]. This communication reports additional experiments carried out to confirm our previous results.

Adult, randomly bred 2-month-old rats of Sprague-Dawley and Long-Evans strains, weighing about 200 g, were used.

In the first set of experiments, skin from the same donor animal was divided into two strips of equal size (3 × 4 cm). One of them was incubated with 2 per cent 'Urethan' in isotonic phosphate buffer (IPB). The other, the control, was incubated with IPB without 'Urethan'. Both incubations were carried out for 24 h at 37° C. The skin was then grafted onto an animal of the same strain chosen at random, using the technique previously described[6].

The results (Table 1) show that treatment with 'Urethan' is effective in prolonging the survival of the skin homograft. Autotransplants of 'Urethan'-treated skin take very easily and live practically indefinitely, which shows that the treatment does not damage the tissue.

In a second set of experiments, a 'classic' treatment of the recipient host with 'Urethan' was carried out. Cole and Davis[7] observed that 'Urethan', as well as radiation, was able to prolong the survival of skin homografts. We therefore decided to compare the activity of graded doses of 'Urethan' with the results obtained with the pretreatment of the skin *in vitro*.

Fifty rats of Sprague–Dawley strain were divided into five groups of ten animals each. After grafting, recipients were given either 'Urethan' (in doses of 150, 300, 450 and 600 mg/kg respectively) or saline (controls). Two-thirds of the dose was given in the evening and one-third in the morning so as to avoid food intake deficiencies induced by the sedative effect of 'Urethan'.

Table 1. EFFECT OF PRETREATMENT WITH 'URETHAN' *in vitro* ON SURVIVAL OF HOMOGRAFTS

Strain	No. of animals	Treatment	Mean survival time (± *S.E.*) (days)
Sprague-Dawley	12	– (Control)	6·08 ± 0·37
Sprague-Dawley	12	'Urethan' 20 mg/ml	11·8 ± 0·48
Long-Evans	10	– (Control)	6 2 ± 0·29
Long-Evans	14	'Urethan' 20 mg/ml.	10·38 ± 0·51

Vitality of the 'takes' was controlled by injecting the host *in vivo* with disulphyne blue and observing the uptake of the dye and discoloration of the graft

Table 2. EFFECT OF 'URETHAN' INJECTED IN THE HOST ON SURVIVAL OF SKIN HOMOGRAFTS

Strain	No of animals	Treatment	Mean survival time (± *S.E*) (days)
Sprague-Dawley	10	– (Control)	6·1 ± 0·47
Sprague-Dawley	10	'Urethan' 150 mg/kg	6 6 ± 0 38
Sprague-Dawley	10	'Urethan' 300 mg/kg	8 6 ± 0·29
Sprague-Dawley	10	'Urethan' 450 mg/kg	9·4 ± 0·73
Sprague-Dawley	10	'Urethan' 600 mg/kg	13·3 ± 0 36

ear) over a period of 2 weeks. The doses were increased from 0·25 ml. to 2·00 ml. of venom solution. The injected rabbits were able to survive amounts at least as great as five times the lethal dose of venom solution. Precipitin assays in test-tubes and on agar plates were positive for the whole venom as well as for the proteolytic and unidentified electrophoretic fractions.

The venom did not show any activity for coagulant or anticoagulant factor, histamine, histamine-liberating substances or any ileum contracting agent, acetylcholine, acetylcholinesterase, acetylcholinesterase inhibitor, phospholipase *A*, ATP phosphohydrolase, ribonuclease, NAD nucleosidase, D- and L-amino-acid oxidase.

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Failure of Methylcellulose to alter the Growth of Walker Tumour 256 in Rats

Methylcellulose is a long-chained methyl ether of cellulose. Recent reports have associated regression of the transplantable Murphy–Sturm lymphosarcoma in rats with treatment by intraperitoneal injections of methylcellulose[1–4]. In the present investigation the same type of rats and treatment were used with the Walker tumour 256. As will be shown, no beneficial effect was noted in the parameters studied, namely, size of the tumour and longevity of the rats.

Adult female Sprague–Dawley rats averaging 140 g were divided into three groups of twenty each; size and age were similar. The methylcellulose was prepared as a 2·5 per cent aqueous suspension of 400 CPS methylcellulose. Intraperitoneal injections of 2 c.c. of the suspension were made three times per week (Monday, Wednesday, Friday)[1], starting 10 days before the subcutaneous transplantation of the tumour as a cell-suspension. Injections of methylcellulose continued so long as the animals remained alive in Group *A*, and for only two injections after tumour transplantation in group *B*. Group *C* received tumour but no methylcellulose. The tumour was measured every second day and the product of the longest and shortest dimension recorded.

Fig. 1 summarizes the findings; each point represents the average of the size of the tumours in the group.

In our laboratory, the Sprague–Dawley host has shown some degree of natural resistance to the Murphy–Sturm lymphosarcoma, but not to the Walker tumour. We have observed 10–20 per cent complete spontaneous regression of the Murphy–Sturm tumour, in various experiments; however, complete spontaneous regression of the Walker tumour has not been seen. We feel that methylcellulose may enhance a natural resistance already present in the host and which is sufficient in the case of the Murphy–Sturm lymphosarcoma to tip the balance in favour of the host. However, in the case of the Walker tumour any natural resistance which may exist is not strong enough to be expressed spontaneously and cannot be potentiated to that point by methylcellulose. The mechanism of action of methylcellulose is not clear, but, the Murphy–Sturm tumour being a homograft, an enhancement of the homograft rejection mechanism may be involved. The Walker tumour 256 is also a homograft.

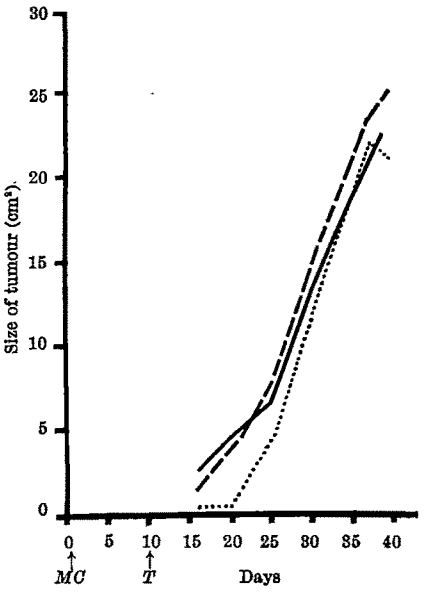


Fig. 1. Comparison of average size of tumours in methylcellulose-treated and non-treated groups. ——, Continuous treatment with methylcellulose (Group *A*). - - - -, Treatment with methylcellulose stopped after two injections post tumour transplantation (Group *B*). . . . . , Control, no methylcellulose (Group *C*). Arrow *MC* indicates start of methylcellulose injections. Arrow *T* indicates transplantation of Walker tumour

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HAEMATOLOGY

Influence of the Erythroid Activity of the Bone Marrow on the Plasma Disappearance of Injected Erythropoietin in Dogs

The finding that plasma erythropoietin titre in patients with an aplastic or hypoplastic bone marrow was higher than in patients with haemolytic anaemias despite similar haemoglobin concentration led Stohlman to postulate that the plasma erythropoietin level is related not only to the severity of the stimulus but also to the degree of erythroid activity of the bone marrow[1]. Jacobson *et al.*[2] and Hammond *et al.*[3] have suggested that erythropoietin may be consumed or degraded by actively proliferating erythrocytic elements.

This report presents data on the rate of disappearance of erythropoietin from plasma of normal, erythropoietically stimulated (acute hypoxia) and erythropoietically de-

After this initial potentiation the antagonism to the effect of the sympathomimetic now appears and after large doses of prostaglandin it is of a progressive and irreversible nature.

To investigate the possibility that prostaglandins may occupy the adrenergic receptors, 'receptor protection' experiments were performed on the rat vas deferens. After obtaining regular cumulative dose-response curves to phenylephrine, one vas was exposed to PGE_1 alone and the other to PGE_1 in the presence of a high concentration of phenylephrine. Fig. 2 shows the results of such an experiment. Clearly, exposure to PGE_1 in the presence of a high concentration of phenylephrine confers no protection from the inhibitory effect of PGE_1. Indeed, the reverse is apparent. The presence of phenylephrine during the exposure to PGE_1 increases the rate of development of the inhibitory effect of the prostaglandin. It is suggested that a possible explanation for these observations is that the prostaglandin molecule does not itself occupy the adrenergic receptor but binds the sympathomimetic to the receptor. Repeated application of the sympathomimetic after exposure to prostaglandin would lead to a progressive elimination of receptor sites, accounting for the progressive depression in the maximal response of the preparation to the sympathomimetic. This mechanism, by increasing the affinity of the sympathomimetic for the adrenergic receptor, may also explain the initial potentiation of the effects of sympathomimetics after exposure to prostaglandins. Further work is in progress to examine this possibility.

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PHARMACOLOGY

Venom of the *Megalopyge urens* (Berg) Caterpillar

The caterpillar of *Megalopyge urens* (Berg), commonly known as '*Bicho peludo*', is abundant in southern Uruguay in some years during February and March (Fig. 1). Its sting is feared because of the intense pain experienced by the victims. The black rigid pinking hairs (1 mm long) emerge from the papillae of the caterpillar among longer apatulated and white feathery hairs. There are several reports on the effect of *M. urens* venom in man and animals[1-4]. This work is concerned with the enzymatic and immunological properties of the venom and the electrophoretic separation of its different components.

All the experiments reported here were carried out with a venom solution obtained by irritating clean caterpillars in saline solution with a glass rod (one caterpillar per ml. saline). The venomous solution had a protein concentration varying from 0·3 mg per ml. to 0·6 mg per ml. and was stable for 1–2 weeks in the refrigerator. Concentration by vacuum evaporation or lyophilization of this solution led to loss of activity. The venom was of globulin nature.

Direct haemolytic activity was investigated *in vitro* according to Ganguly[5] and was moderately positive for human and dog red cells, slightly positive for rat and negative for guinea-pig red cells. Hyaluronate lyase activity was demonstrated in the venom by the Jaques bio-assay[6] and by a modification[7] of Mayer's turbidimetric method[8] (Fig. 2). The proteolytic enzyme was determined by digestion of casein[9], by Anson's assay for trypsin[10] and by digestion of 20 per cent gelatine containing 1 : 10,000 merthiolate at laboratory temperature for 45 h (Fig. 3).

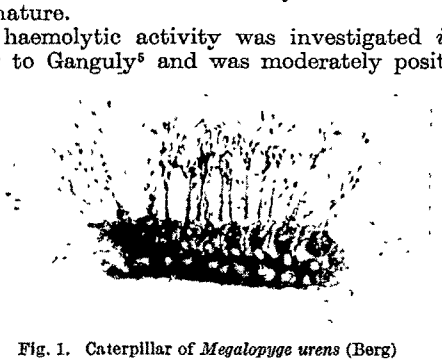


Fig. 1. Caterpillar of *Megalopyge urens* (Berg)

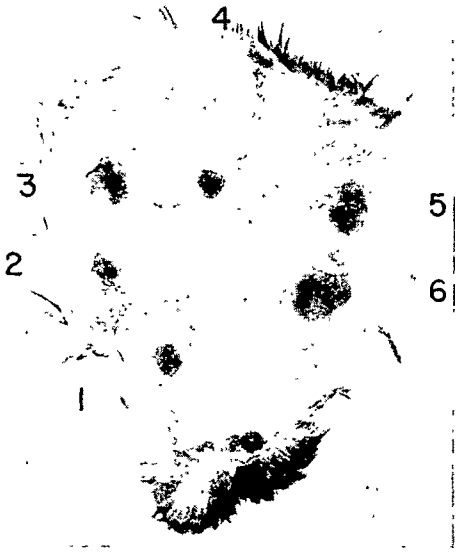


Fig. 2. Jaques's bio-assay for hyaluronate lyase with solution of venom from the *Megalopyge urens* (Berg) caterpillar: (1) 8 μl. *M. urens* venom, (2) blank; (3) 2 μg hyaluronate lyase; (4) 20 μg crystalline *Bothrops jararaca* venom; (5) 40 μl. 10-day-old *M. urens* venom; (6) 40 μl. fresh *M. urens* venom



Fig 3. Gelatine digestion by solution of venom from the *Megalopyge urens* (Berg) caterpillar: (1) blank; (2) 24-day-old *M. urens* venom; (3) 15-day-old *M. urens* venom; (4) fresh *M. urens* venom; (5) concentrated *M. urens* venom; (6) 0·5 mg/ml. crystalline *Bothrops jararaca* venom

The venom was analysed by filter-paper electrophoresis using a Spinco apparatus (model *R*) and 470 filter-paper (Schleicher and Schüll), a veronal buffer (*p*H 8·6), and ionic strength of 0·075. Separation was continued at 5 V/cm for 8 h. The strips were cut into thirty-two segments each 1 cm long, and these were carefully eluted with saline in separated test-tubes and the bands detected by the method of Lowry *et al.*[11] in a portion of the contents of each tube. The venom protein was separated into four fractions: 1, (fastest anodic mobility, 9 cm) with haemolytic activity only; 2, (anodic mobility, 5 cm) with hyaluronate lyase activity alone; 3, (origin) with proteolytic activity together with a very low haemolysin effect; 4, (cathodic mobility, 7 cm) unidentified protein.

Rabbits weighing 2 kg were immunized against the total venom with nine intravenous injections (by way of the

a direct effect which may be synergistic (tracheal chain preparation) or antagonistic (dioestrous guinea-pig and rat uterus, intestine) to the effect of the sympathomimetic substance. In these preparations, the doses of prostaglandins used were such that no direct effect on the tone of the preparation was apparent. In the guinea-pig uterus, for example, bath concentrations of $PGF_{2\alpha}$ as low as 10^{-12} g/ml. showed antagonism to the effects of noradrenaline, although a direct stimulatory effect of $PGF_{2\alpha}$ does not appear until bath concentrations of 10^{-8} g/ml. are reached. Moreover, the long-term effect of prostaglandins on responses to sympathomimetics, even after brief exposure to low concentrations, clearly distinguishes direct and indirect effects. In the rat vas deferens, no direct effect of prostaglandins is evident at concentrations up to 5×10^{-6} g/ml. PGE_1 although antagonism to the effect of sympathomimetics can be demonstrated at concentrations as low as 10^{-9} g/ml. For this reason the rat vas deferens was chosen for 'receptor protection' studies.

It was found that irrespective of the nature of the direct effect of the prostaglandin (stimulation, inhibition, or no effect), and irrespective of the nature of the response of the preparation to sympathomimetics (stimulation or inhibition), the net effect of the prostaglandin was to depress the response of the tissue to sympathomimetics.

Fig. 1 shows dose-response curves of the vasa deferentia of a rat where one is stimulated by adrenaline and the other by carbamyl-choline. One curve represents the effect of the stimulant before exposure to prostaglandin and the other the effect of the stimulant 90 min after exposure to PGE_1 for 5 min at a concentration of 4×10^{-6} g/ml. The responses to carbamyl-choline are potentiated. The dose-response curve shows a shift to the left and the maximal response of the preparation to carbamyl-choline is increased. The dose-response curve for adrenaline, however, shows a shift to the right with a depression of the maximal response. The extent of this depression increased with time and without further shift of the lower part of the curve, and after a further 40 min the maximal response was only 58 per cent of the original value. This effect on the dose-response curve is also characteristic of the action of PGE_1 on the responses of the vas to phenylephrine and noradrenaline. The antagonism cannot be reversed by increasing the bath concentration of the agonist, even up to molar concentrations.

Two further features of the effect of prostaglandins are apparent. The first response of the preparation to the sympathomimetic after exposure to prostaglandin may be potentiated (Curve *B* (ii) in Fig. 2) and this initial potentiation is seen in preparations which are relaxed by the sympathomimetic as well as in those which are stimulated.

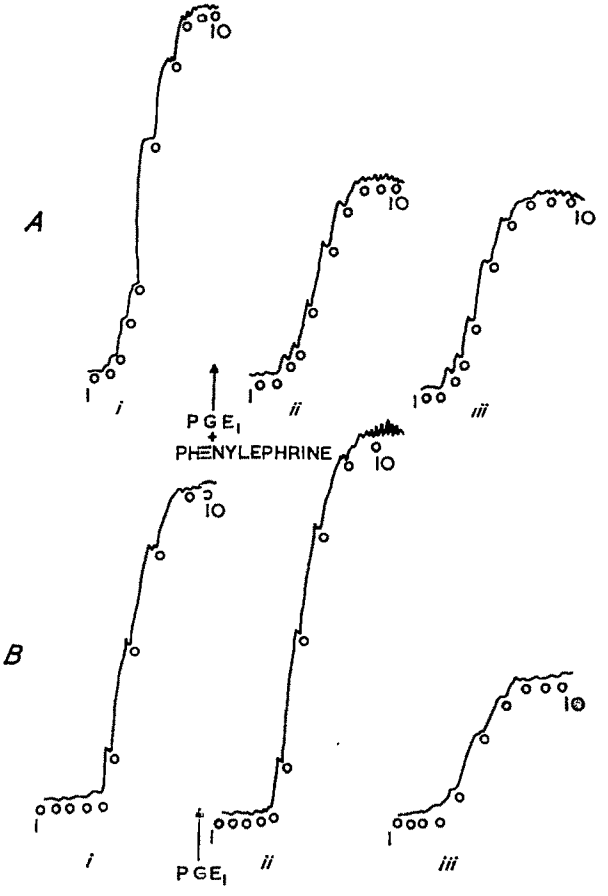


Fig. 2. Tracings of cumulative dose-response curves of the rat vas deferens stimulated by phenylephrine. The concentration of phenylephrine was increased by steps of (log 10)/3 after closing the tap leading to the organ chamber. The circles represent the points at which phenylephrine was added. The label 1 corresponds to a bath concentration of 10^{-7} M; label 10 to 3×10^{-6} M. Both preparations were exposed to PGE_1 (4×10^{-7} g/ml.) for 5 min, one in the presence of 10^{-5} M phenylephrine (*A*) and one in the absence of phenylephrine (*B*). Curves *A* (ii) and *B* (ii) were made 100 min after exposure to PGE_1, curves *A* (iii) and *B* (iii) were made 160 min after exposure to PGE_1

Table 1. Effects of Prostaglandins and Sympathomimetics on Various Tissues

Preparation*	Prostaglandin used†	Sympathomimetic and its effect	Effect of prostaglandin on response to sympathomimetic
Rat vas deferens (20)	PGE_1 (*nil*) $PGF_{2\alpha}$ (*nil*)	Phenylephrine (+) Noradrenaline (+) Adrenaline (+) Isopropylnoradrenaline (+)	Inhibition Inhibition Inhibition Inhibition
Rat colon (6)	PGE_1 (+) $PGF_{2\alpha}$ (+) $PGF_{1\beta}$	Noradrenaline (−) Adrenaline (−)	Inhibition Inhibition
Rat fundic strip (5)	PGE_1 (+) $PGF_{2\alpha}$ (+) $PGF_{1\beta}$	Noradrenaline (−)	Inhibition
Guinea-pig uterus (i) Dioestrous (stimulated by vasopressin) (3)	$PGF_{2\alpha}$ (+)	Noradrenaline (−)	Inhibition
(ii) Oestrous (in presence of a β blocking agent) (3)	$PGF_{2\alpha}$ (+) PGE_1 (+)	Noradrenaline (+)	Inhibition
Rat uterus (electrically stimulated) (6)	$PGF_{2\alpha}$ (+) PGE_1 (+) $PGF_{1\beta}$	Noradrenaline (−) Adrenaline (−)	Inhibition Inhibition
Guinea-pig tracheal chain (contracted by carbamyl-choline chloride 10^{-7} M–10^{-6} M) (3)	PGE_1 (−) $PGF_{2\alpha}$	Noradrenaline (−) Isopropylnoradrenaline (−)	Inhibition Inhibition

* Number of experiments in parentheses.
† Nature of direct effect in parentheses.

It is concluded that chloride ions are actively transported across the frog corneas and this ionic transport represents the only source of current across this membrane, and probably it is directly related to corneal hydration and transparency. The ATPase found in the frog corneas would seem to be different from that found in sodium transporting systems[6] and might not be related to the chloride transport found. Though this does not mean that chloride transport is not ATP dependent, it is possible that another mechanism might be involved in the transport of chloride across biological membranes.

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Vanadium and Dental Caries

THE effects of trace elements other than fluoride on dental caries have recently received increased attention. The results of numerous investigations, reviewed elsewhere[1], have indicated that selenium is capable of increasing the susceptibility to caries, particularly when it is consumed during the formation of the teeth and incorporated into their structure. On the other hand, molybdenum appears to have a beneficial effect on caries[2].

In recent years, vanadium has been investigated as a caries-inhibiting trace element. Vanadium was found to decrease caries when given to hamsters and rats either during the time of tooth formation or post-developmentally[3,4]. However, further animal experiments failed to substantiate these findings[5,6].

The only investigation of the effect of vanadium on caries in man was conducted in Wyoming among children[7]. This State was selected because it contains widespread deposits of vanadium. For ascertaining the dietary intake of vanadium by the children, its concentration in water samples was determined. This was based on the premise that water is the main vehicle for vanadium ingestion in man, as is the case with fluoride. The majority of the water samples contained minute amounts of vanadium ranging from 0·03 to 0·09 p.p.m. with a few above the latter level. It was concluded that the consumption of water containing vanadium as low as 0·07–0·09 p.p.m. was responsible for decreasing dental caries in children as compared with those drinking water with vanadium at levels of 0·03–0·06 p.p.m.

In the light of recent findings, it appears that, in general, water does not constitute an important source of vanadium and that its concentration cannot serve as a reliable criterion of the total amount of this element ingested daily. An investigation of 194 public water supplies in cities located in forty-nine States was conducted by the U.S. Public Health Service. It revealed the presence of minute amounts of vanadium in the samples ranging from 0·0004 to 0·07 p.p.m. with a mean content of 0·006 p.p.m.[8]. In a second investigation by the U.S. Public Health Service, it was shown that from a total of 247 water samples taken from five major rivers (Ohio, Mississippi, Colorado, Missouri, Columbia) and the Great Lakes, vanadium was detected in only five samples[9]. No vanadium was found in the samples from the Ohio and Mississippi Rivers and the Great Lakes.

It is concluded that in epidemiological studies on the association between vanadium intake and caries, the concentration of this element in water alone cannot be relied on as an index reflecting the total amount ingested daily. It is known that the same is true with regard to selenium, even in seleniferous regions[10]. Other methods should be used for assessing the dietary vanadium intake in population groups.

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Antagonism by Prostaglandins of the Responses of Various Smooth Muscle Preparations to Sympathomimetics

PROSTAGLANDINS are known to produce two types of effects on smooth muscle. First, they may produce direct and relatively short-lived effects, such as stimulation of the isolated uterus or relaxation of the isolated tracheal chain preparation. Secondly, in doses too low to produce a direct effect, they may produce a long-term potentiation of the effects of other stimulants. These two effects have been investigated in the isolated guinea-pig uterus[1]. The long-term potentiation of the effects of other stimulants which is induced by brief exposure of the guinea-pig uterus to low concentrations of PGE_1 or PGE_2 appears to be non-specific with respect to the potentiated stimulant, and can be demonstrated with a variety of stimulants including vasopressin, oxytocin and histamine, and also with electrical field stimulation[2]. The potentiating effect, however, is fairly specific to prostaglandins of the PGE series. This type of effect is not restricted to the guinea-pig uterus and can be demonstrated on the isolated rat colon or vas deferens (Fig. 1).

Another indirect long-term effect of prostaglandins of both the PGF and the PGE series is described here, and consists of a depression of the responses of various isolated smooth muscle preparations to sympathomimetic substances—to adrenaline, noradrenaline, phenylephrine, and isopropyl noradrenaline.

Some tissues in which this effect has been examined are shown in Table 1. In some of these, prostaglandins have

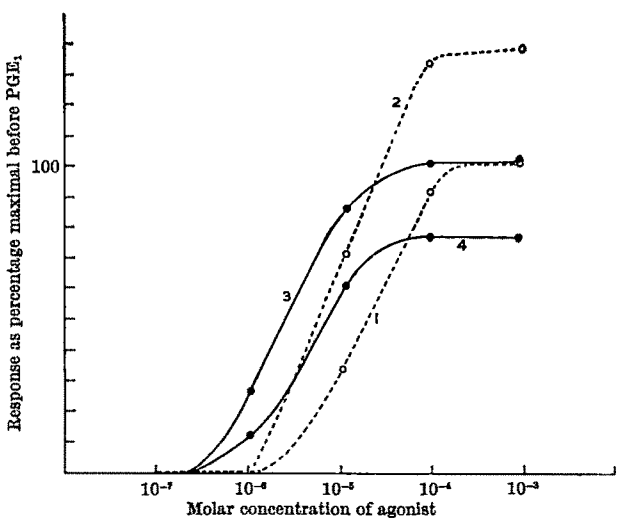


Fig. 1. Cumulative *in vitro* dose-response curves of rat vas deferens to carbamyl-choline (interrupted line) and adrenaline (solid line). Curves 1 and 3 were obtained before exposure to prostaglandin. Curves 2 and 4 were obtained 90 min after exposure for 5 min to PGE_1, at 4×10^{-6} g/ml.

PHYSIOLOGY

Asthma and Testosterone

It was previously shown[1,2] that testosterone is a powerful inhibitor of histamine and SRS* responses, and in view of the varying age incidence of status asthmaticus in the two sexes it was thought testosterone levels might have aetiological significance. Dutch workers have thought along similar lines[3]. Accordingly blood levels were measured in male and female subjects (by courtesy of Dr. J. Coghlan and Prof. Hudson) immediately after a severe attack. In two males the levels were 0·87 and 1·03 μg/100 ml. (high and one above normal: range 0·37–1·00) while in four females the levels were 0·13, 0·16, 0·19, and 0·29 μg/100 ml. (above normal: range 0·04–0·12). In another asthmatic subject, in the interval between attacks, the level was 0·24 μg/100 ml. (male), which is significantly low. I am now investigating the possibility that the level may be low in asthmatics, but that as a reaction to the stress of an attack, possibly associated with anoxia and hypercapnia, the levels rise high temporarily. Corticoid and also oestrin levels will be measured, as sometimes oestrins enhance histamine responses.

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* SRS, Slow-reacting substance.

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Active Transport of Chloride across the Cornea

The presence of an active transport of ions across the cornea has been postulated[1–3] in connexion with the loss of transparency of this membrane when it swells, after reduction of its metabolic rate or injury. The swelling and opacity, especially after exposure to cold solutions, is reversible and this has prompted the notion that an active ionic transport is performed by the cells of the epithelium or endothelium, dragging water out of the stroma to preserve the normal orientation of the fibrils.

The cornea of the American bull frog (*Rana catesbiana*) maintains a potential difference of 15–60 mV and produces short-circuit currents of 6–30 μamp/cm², when mounted as a membrane in Ussing's modified type of chamber[4]. The aqueous or endothelial side is positive and the tear or epithelial side is negative. The potential and the short-circuit current are mainly dependent on the presence of chloride ions in the aqueous side. As can be seen in Fig. 1, when all the chloride ions are replaced by sulphates in the fluid[5] bathing the corneas, the short-circuit current is markedly reduced; it recovers to control-levels, however, when chloride ions are again present in the fluid. The same effect is obtained if methyl-sulphate ions are used to replace the chlorides. The measurement of chloride fluxes with chlorine-36 in short-circuited corneas, as shown in Table 1, indicates that there is a net transport of chloride from the aqueous towards the tear side of the cornea. From 95 to 100 per cent of the short-circuit current measured could be computed as due to the chloride net transport, as shown at the bottom of Table 1. Sodium fluxes measured with sodium-22 indicate that no net transport of this cation is performed, since both unidirectional fluxes are similar. In these corneas chloride is secreted towards the outside, and then possibly sodium ions are dragged along passively, due to the potential difference. This salt movement could then induce water to follow it, keeping the hydration of the cornea at the normal level, and consequently preserving its transparency.

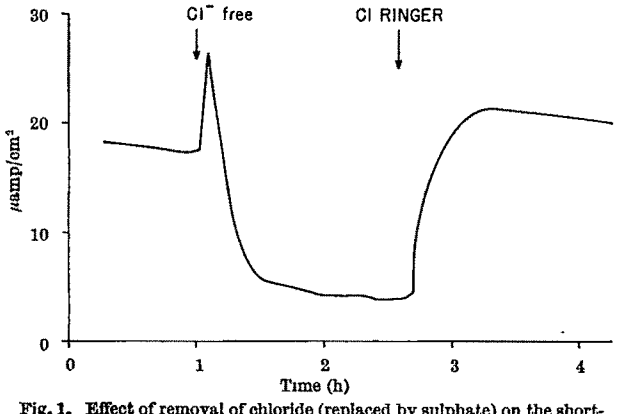


Fig. 1. Effect of removal of chloride (replaced by sulphate) on the short-circuit current produced by the isolated frog corneas

Table 1. Chloride Fluxes across Isolated Frog Cornea

Aqueous to tear side		Tear to aqueous side	
μEquiv./h/cm²	Short-circuit current (μamp/cm²)	μEquiv./h/cm²	Short-circuit current (μamp/cm²)
0·535	13·1	0·188	14·0
0·780	23·3	0·288	10 3
0 803	18·4	0·086	15 0
0 740	14·0	0·153	33·0
1·075	11·4	0·162	19·0
1·060	25·8	0·237	9 6
0 513	15·1	0 402	21·3
0·952	21·6	0 074	17·6
1·185	30 8	0·187	15 5
Mean 0 849	19·3	0 197	17·25

Chloride net flux = 0 849–0·197 = 0·652 μequiv./h/cm² Current measured = 18 2 μamp/cm². Current from chlorine transport = 17·5 μamp/cm².

When the chloride concentration is increased or decreased in steps of 10–20 mM of chloride in the inside, while regular fluid is kept in the outside or tear side, a saturation-type curve is obtained as the chloride concentration is plotted against the stable short-circuit current attained after each replacement. In double reciprocal plottings, straight lines are obtained as shown in Fig. 2, indicating saturation-type kinetics.

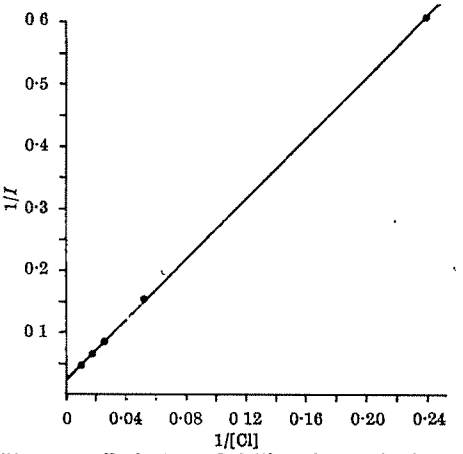


Fig. 2. Lineweaver–Burke type of plotting of short-circuit current (*I*) against chloride concentration in the fluid bathing the aqueous side of the frog cornea

The short-circuit current is sensitive to ouabain, but only at high concentrations, 10^{-4} M, and a definite drop of the current is observed only after a delay of about 1 h when the glucoside is added in the inside or aqueous side of the corneas. Though sodium ions move only passively across the corneas, the presence of these cations is necessary inside to maintain the potential.

Homogenates of the corneas exhibit ATPase activity in the presence of magnesium and in the absence of calcium. This magnesium-activated ATPase activity does not, however, show any affinity for sodium or potassium. The ATPase splitting activity is found in the microsomal as well as in the mitochondrial fraction of these corneas, and does not show any sodium, potassium or chlorine affinity. Ouabain does not affect the ATPase splitting activity of the preparations.

colonies grown on agar are grey-white and show in cross-section a typical plectenchymatic structure like that of a true sclerotium. As with *Claviceps paspali*, the mycelium of this strain grown in submerged culture has a cellular structure similar to that of a natural sclerotium. Ergotamine production in submerged culture occurred in a culture medium of high osmotic pressure of the following composition: Mannitol 20 per cent; peptone-Difco 3 per cent; tap water; final *p*H 6·2. The culture medium was sterilized for 30 min at 110° C. Cotton-wool plugged 500-ml. Erlenmeyer flasks containing 100 ml. of culture medium were used on a rotary shaker at 24° C (200 r.p.m., eccentric throw 10 cm). After 8–10 days the total alkaloid content from the broth and mycelium ranged from 800 to 1,400 μg/ml. (calculated as ergotamine). Chromatographic analysis of the alkaloids extracted from the culture medium showed ergotamine to be the principal component present.

I thank Prof. E. B. Chain for his continued interest in this work.

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Further Proof of Direct Oxygen Transfer by Carotenoids in Respiration and Photosynthesis

The hypothesis that carotenoids participate in oxygen transfer in photosynthesis was first put forward by Warburg and Krippahl[1], then by Cholnoky *et al.*[2]. Sapožnikov[3] observed increased production of violaxanthin (a diepoxide of lutein) in darkness with a corresponding decrease in lutein production. In illuminated medium the opposite reaction occurred. Hydroxylamine, a well-known inhibitor of photosynthesis, has been shown[4] to retard this light–darkness reaction. Blass, Anderson and Calvin[5] confirmed the work of Sapožnikov, but, although they observed the decrease of violaxanthin in light, they found no corresponding rise in the concentration of lutein. In these experiments, however, nearly equal specific radioactivity of lutein and violaxanthin was noted. This fact suggests an interrelationship between these two carotenoids in photosynthesis and respiration respectively. Recently, Saakov[6] gave a complete scheme of the biosynthesis of carotenoids and of their oxido-reduction reactions in photosynthesis.

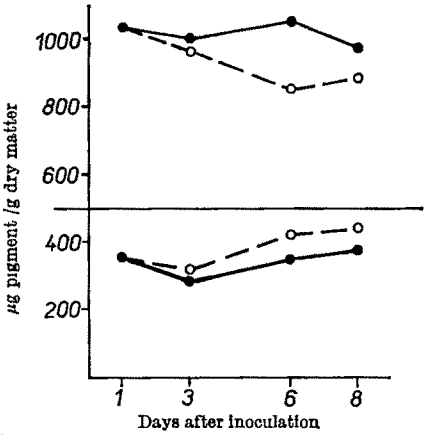


Fig. 1. Increase in violaxanthin content with corresponding decrease in lutein content in barley after infection with powdery mildew. Mean values of at least three determinations; maximal standard deviation for violaxanthin (bottom graphs): ±0·009; for lutein (top graphs): ±0·043. ●——●, Control plants; ○ — — ○, infected plants

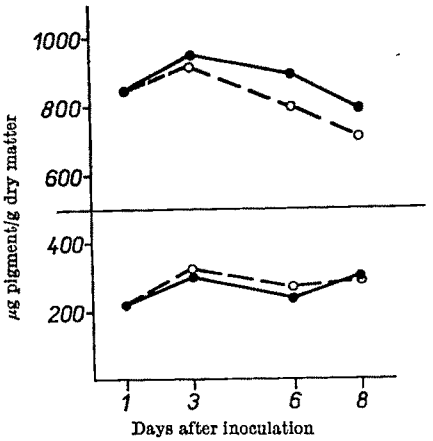


Fig. 2. Increase in neoxanthin content and decrease in β-carotene content in barley after infection with powdery mildew. Mean values of at least three determinations; maximal standard deviation for neoxanthin (bottom graphs): ±0 004; for β-carotene (top graphs): ±0 021. ●——●, Control plants; ○ — — ○, infected plants

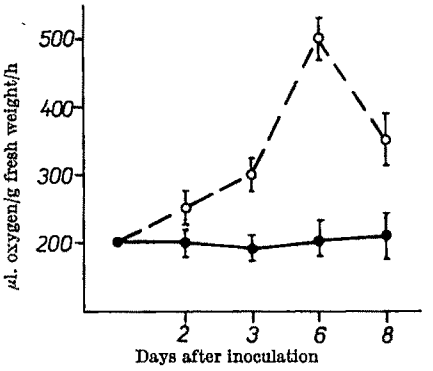


Fig. 3. Rise of respiration of barley after infection with powdery mildew. ●——●, Control plants; ○ — — ○, infected plants

During our investigations[7] of barley infected with powdery mildew (*Erysiphe graminis f.sp. hordei* Marchal, strain C_6) under standard conditions we observed increased content of violaxanthin immediately after infection with the fungus up to the sporulation time of the parasite. Together with the increase of violaxanthin content a corresponding decrease in lutein content was observed (Fig. 1). Increase of oxidized carotenoid with a corresponding drop in the content of the reduced form took place in the same phase of the disease, in which increased respiration of the host occurs. (Pigment analyses were made spectrophotometrically after thin-layer chromatography on silica gel.)

It may be mentioned that a decrease of β-carotene (mono-hydroxy-β-carotene) and a slight increase of neoxanthin (mono-epoxy-lutein) were also found during the period of increased respiration (Fig. 2).

These results may be regarded as further proof of direct oxygen transfer by carotenoids in photosynthesis and respiration.

I thank Mr. F. Frič for his assistance.

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Table 1. Effect of Freund's Incomplete and Complete Adjuvant on the Phagocytic Activity of the Reticulo-endothelial System in Intact Male *T.O.* Swiss Mice

Daily dose of adjuvant for 3 days (ml.)	No. of animals in each group	Route of administration	Animals given incomplete adjuvant			Animals given complete adjuvant		
			Mean phagocytic index (*K* value)	Mean *Kr* ± *S.E.*	Probability *P* in relation to distribution of '*t*'	Mean phagocytic index (*K* value)	Mean *Kr* ± *S.E.*	Probability *P* in relation to distribution of '*t*'
Experiment 1								
0·05	5	S.c.	18	56 ± 3·66	> 0·05	25	41 ± 2·10	0·02/0·01
0·10	5	S.c.	23	43 ± 2·10	0·05/0·02	28	35 ± 1·78	< 0·001
0·15	5	S.c.	24	43 ± 3·20	0·05/0·02	30	32 ± 2·48	< 0·001
0·20	5	S.c.	24	41 ± 1·60	0·01/0·001	35	29 ± 1·88	< 0·001
Untreated controls	5	—	19	53 ± 2·88	—	19	53 ± 2·88	—
Experiment 2								
0·05	5	I.p.	29	35 ± 2·23	0·01/0·001	30	33 ± 1·48	0·01/0·001
0·10	5	I.p.	29	35 ± 2·78	0·01/0·001	37	27 ± 1·11	< 0·001
0·15	5	I.p.	28	35 ± 0·86	0·01/0·001	37	27 ± 2·12	< 0·001
0·20	5	I.p.	27	39 ± 3·78	0·05/0·02	48	22 ± 1·00	< 0·001
Untreated controls	5	—	20	52 ± 3·61	—	20	52 ± 3·61	—

Table 2. Speed of Action and Duration of Effect of Freund's Incomplete and Complete Adjuvant on the Phagocytic Activity of the Reticulo-endothelial System in Intact Male *T.O.* Swiss Mice

Days after injection	Untreated daily controls		Animals given incomplete adjuvant			Animals given complete adjuvant		
	Mean phagocytic index (*K* value)	Mean *Kr* ± *S.E.*	Mean phagocytic index (*K* value)	Mean *Kr* ± *S.E.*	Probability *P* in relation to distribution of '*t*'	Mean phagocytic index (*K* value)	Mean *Kr* ± *S.E.*	Probability *P* in relation to distribution of '*t*'
1	20	51 ± 2·68	26	40 ± 2·22	0·02/0·01	31	32 ± 1·73	< 0·001
2	20	53 ± 2·97	25	41 ± 1·87	0·01/0·001	28	37 ± 3·21	0·01/0·001
3	19	58 ± 3·66	18	58 ± 3·41	> 0·05	21	49 ± 2·59	> 0·05
4	19	55 ± 2·76	19	52 ± 3·33	> 0·05	20	51 ± 3·62	> 0·05

Each animal received a single dose of 0·2 ml. adjuvant intraperitoneally.

of the carbon from the blood stream by the reticulo-endothelial cells: although useful for comparison with other published work, it is not a linear index and it is more correct to express phagocytic activity as the reciprocal of *K*, since this gives a linear dose-response relationship ($Kr = 1/K \times 10^3$) and allows statistical analysis of the results[11].

It can be seen (Table 1) that both incomplete and complete Freund's adjuvant stimulate the phagocytic activity of the reticulo-endothelial system, but the complete adjuvant is a more powerful stimulant of phagocytosis than the incomplete. The levels of phagocytic stimulation produced by the incomplete adjuvant are similar after subcutaneous or intraperitoneal injection, but the complete adjuvant produces higher levels of phagocytic activity when given intraperitoneally rather than subcutaneously. It thus appears that the water-in-oil emulsion constituting the incomplete adjuvant acts as a weak stimulant of phagocytosis, and that the stronger reticulo-endothelial system stimulation produced by the complete adjuvant is due to the added *Mycobacterium butyricum*. These findings also suggest that the rate of absorption of the water-in-oil emulsion is the same following subcutaneous or intraperitoneal injection, but that the absorption of *M. butyricum* or its active principle is more effective intraperitoneally.

When a single intraperitoneal injection of 0·20 ml. of Freund's incomplete or complete adjuvant was given, it can be seen (Table 2) that, in both cases, phagocytic activity was significantly stimulated on the first day after injection, and that it remained stimulated on the second day, but declined to control level thereafter. It has been reported that Freund's adjuvant is most effective when administered at the same time as the antigen[12], although Muramatsu[5] has stated that it is most effective when given 5 days before the antigen. The present results suggest that the antigen should be given about the same time as the adjuvant.

A vast literature has accumulated over the past forty years on the nature and activity of substances capable of enhancing the immune response. This effect in the case of mineral particulate carriers and water-in-oil emulsions is generally accepted. More recently, bacterial endotoxins, lipopolysaccharide, lipid *A*, zymosan and oestrogen, all of which stimulate the phagocytic activity of the reticulo-endothelial system[9,13–15], have been shown to enhance markedly the primary antibody response to a variety of antigens[6,13,16,17]. The present results show that a water-in-oil emulsion type of adjuvant also stimulates the phagocytic activity of the reticulo-endothelial system and, since it has been demonstrated that phagocytosis of antigen is an essential step in the induction of the primary immune response[6,7], it seems likely that stimulation of phagocytosis is an important part of the mechanism of action of most adjuvants in enhancing antibody response.

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HISTOCHEMISTRY

Rapid Staining of Juxtaglomerular Cell Granules and Renal Tubular Mitochondria for Light Microscopy

The methods in use for staining renal tissue for assessment of the juxtaglomerular index require either prolonged fixation or staining or both. We wish to report a technique which has the advantages of rapidity, staining contrast of the juxtaglomerular granules adequate for counting under

the light microscope, and the simultaneous preservation and staining of renal tubular mitochondria.

The stains most frequently employed for assessment of juxtaglomerular granulation are neutral dyes such as ethyl violet-biebrich scarlet (Bowie's stain)[1] or crystal violet-orange *G* (ref. 2). The reports of Wilson[3], Hartroft and Hartroft[4], Pitcock and Hartroft[1], Friedberg[5], and our own preliminary experiments with neutral dyes, indicate that the adequate demonstration of juxtaglomerular granules is a lengthy procedure occupying anywhere from 2 to 7 days, and that it is largely dependent on the use of fixatives containing potassium dichromate, followed by prolonged post-chroming. Staining alone requires at least 10–12 h. These methods of staining secretory granules, as well as the use of post-chroming in the demonstration of mitochondria for light microscopy, have been attributed to the action of potassium dichromate in rendering a wide variety of unsaturated lipids insoluble in lipid solvents[6].

We have carried out investigations into the staining of juxtaglomerular granules and renal tubular mitochondria in formalin-fixed tissue, using a procedure which avoids entirely the use of concentrated ethyl alcohol and xylene. Adult male white mice were killed with a blow on the head and their kidneys removed rapidly. Slices about 3 mm in thickness were fixed for 24 h in buffered 10 per cent formalin (*p*H 7·3) and the tissue was processed and embedded in polyethylene glycol ('Carbowax') and a polyvinylacetate resin according to a modification[7] of the method of Reid and Taylor[8]. Sections were cut at 2μ and 3μ and mounted on glass slides. The embedding medium was readily dissolved from the sections by brief immersion in methyl alcohol. Slides were stained for 2–2·5 min in a solution consisting of 25 drops of 1 per cent crystal violet or ethyl violet in 100 ml. of 20 per cent ethyl alcohol, followed by 5–10 sec in 100 ml. distilled water containing forty drops of 1 per cent neutral red or safranin *O*. The sections were rinsed in cold water and mounted directly using a water-soluble mounting medium.

This procedure results in considerably less tissue shrinkage than in control tissues embedded in paraffin and processed in a routine fashion, as judged by the size of the glomeruli and tubules and by the width of the intertubular tissue. Juxtaglomerular granules are stained an intense purple and are readily discernible as dark clumps at a magnification of × 80. The tubules are demonstrated chiefly through staining of the mitochondria which are easily recognized as blue-mauve rod-like filaments. Those in the distal tubules appear a deeper blue than those in the proximal tubules. Nuclei are stained red, and red blood corpuscles blue.

This method permits extremely rapid demonstration of the granulated cells of the juxtaglomerular apparatus, as well as the macula densa, since the cells of this structure are endowed with distinctly fewer mitochondria than the rest of the distal tubule and hence stand out in contrast (Fig. 1). The simultaneous rapid staining of renal tubular mitochondria offers unique opportunities for further studies in renal pathology and tends to confirm the preservation of phospholipid membrane systems by the present technique.

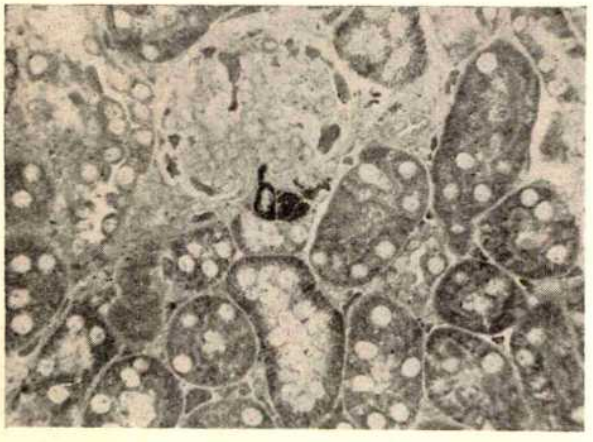


Fig. 1. Photomicrograph showing staining of *JG* granules and tubular mitochondria. Note paucity of mitochondria in macula densa. (Crystal violet–neutral red × *c.* 285)

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Localization of Catecholamines in Brown Fat

The role of interscapular brown fat in non-shivering heat production is well established in new-born rabbits[1], new-born guinea-pigs[2], and during arousal from hibernation[3]. Heat is produced by the local oxidation of long-chain fatty acids. Regulation of heat production by the autonomic nervous system is strongly suggested by the fact that intravenous infusion of noradrenaline in physiological amounts stimulates heat production, and this heat production is abolished by pronethalol[1]. Stimulation of the cervical sympathetic chain in new-born rabbits causes a local increase in brown fat temperature above the deep-body temperature[4]. Fat cells contain a lipase which can be activated by catecholamines[5]. Brown fat from the interscapular region of adult rats contains a large amount of noradrenaline, which disappears rapidly after denervation or treatment with reserpine[6]. Brown fat is also richly innervated with non-medullated autonomic nerve fibres terminating in relation to fat cells[7].

Recent advances in the fluorescent method for the demonstration of catecholamines have opened new possibilities for their histological localization. Interscapular brown fat was examined from normal new-born rabbits, aged 24–48 h, and from littermates which had been given reserpine (0·2 mg/kg) 24 h previously. These tissues were then processed according to the techniques of Falck and Hillarp[8]. Sodium borohydride was used as a specific inhibitor for catecholamine fluorescence[9].

The characteristic green fluorescence of primary amines was found to be strongly present in the cytoplasm, but not in the cell nucleus of brown fat cells (Figs. 1 and 2). Individual nerve fibres showing green fluorescence were also found running between brown fat cells. Sodium borohydride treatment abolished this fluorescence. Moreover, both intracellular fluorescence and fluorescence of nerve fibres was very weak or absent in reserpinized animals.

In view of the previous work[6], showing a high concentration of noradrenaline in brown fat, the green fluorescence disappearing after reserpine treatment may be taken to indicate the presence of noradrenaline, both in autonomic nerve fibres and within brown fat cells. This would appear to be the first demonstration of the presence of catecholamines within brown fat cells.

Catecholamines have been shown to activate a lipase *in vitro* in adipose tissue with cyclic 3′5′-AMP probably acting as intermediate[5]. The observed cytoplasmic localization of a catecholamine, presumably noradrenaline,

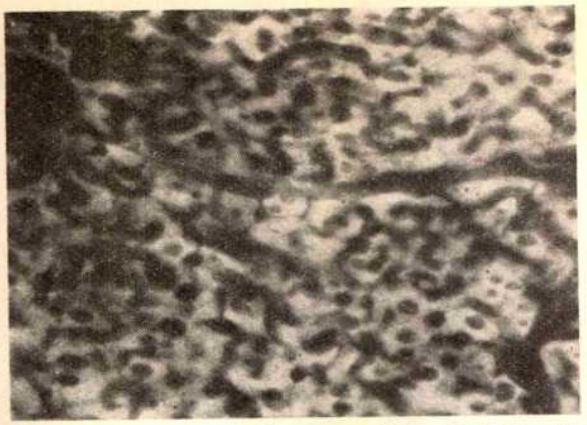


Fig. 1. Low-power view of the fluorescence of brown fat cells from the interscapular deposit in a new-born rabbit. (Falck-Hillarp method, ×*c.* 170)

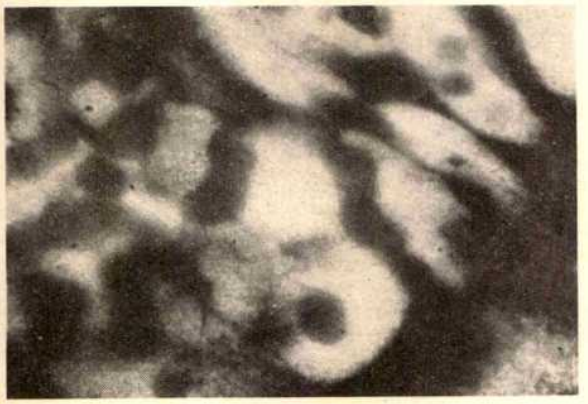


Fig. 2. The cytoplasm of the cells of brown fat shows a green fluorescence, whereas the nucleus does not fluoresce. (Falck-Hillarp method, × 550)

is clearly appropriate for activation of this lipase in the vicinity of fat droplets, leading to hydrolysis of triglyceride and subsequent oxidation of long-chain fatty acids.

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Fatty Acid Composition of Triglycerides from Adipose Tissue

Brown adipose tissue has recently been shown to be the major site of non-shivering heat production in new-born rabbits[1] and to participate in heat production in the human new-born infant in a cool environment[2]. The major lipid in brown adipose tissue is triglyceride, which is present in numerous small droplets throughout the cytoplasm, and heat is produced by the local oxidation of this triglyceride after lipolysis[1]. White adipose tissue contains only a single large fat droplet, is almost entirely triglyceride[1] and its main function is the synthesis and storage of triglycerides and the release of free fatty acids into the blood stream when required. In view of these differences in function we have compared the fatty acid composition of triglycerides from brown and white adipose tissue in new-born and adult rabbits and in samples of brown and white adipose tissue obtained at autopsy in human new-born infants. The fatty acid composition of adipose tissue triglycerides is not significantly different in biopsy and autopsy samples[3].

Lipids were extracted from adipose tissue samples by the method of Folch, Lees and Sloane-Stanley[4], dried over anhydrous sodium sulphate, and the triglycerides isolated by means of thin-layer chromatography using Kieselgel *G* (Merck) activated at 105° C for 1 h and petroleum ether (40°–60° C)–diethyl ether–acetic acid (80:20:1) as solvent system. Fatty acids were methylated in 5 per cent hydrochloric acid in super dry methanol as described by Stoffel, Chu and Ahrens[5] and were identified by gas liquid chromatography using a 'Pan' chromatograph (Pye) with an argon ionization detector and polar (10 per cent polyethylene glycol adipate) and non-polar (5 per cent Apiezon grease *L*) stationary phases and comparison of retention times with known standards. Results are expressed as a percentage of total fatty acids detected.

Table 1 shows the results obtained. Substantial amounts of linoleic and linolenic acids are present in triglycerides of both brown and white adipose tissue from unsuckled new-born rabbits, suggesting transplacental passage of these essential fatty acids. The only significant difference between brown and white adipose tissue in the new-born rabbit appears to be a larger proportion of stearic acid and a lower proportion of palmitic acid in brown adipose tissue. No major differences exist between triglycerides of white adipose tissue from new-born and adult rabbits, apart from the increased palmitoleic in the new-born rabbit. Triglycerides from adipose tissue of both types from human new-born infants also contain linoleic and linolenic acids, but in smaller amounts. There is no significant difference in fatty acid composition of triglycerides from brown and white adipose tissue in the human new-born infant.

Comparisons of the fatty acid composition of brown and white adipose tissue triglycerides have been made in the adult rat[6] and the hibernating and non-hibernating bat[7]. In the rat, saturated fatty acids (myristic, palmitic and stearic) accounted for more of the triglyceride of brown adipose tissue than in white adipose tissue, but the differences were not large[5]. In the bat, both hibernating and non-hibernating, oleic acid was the major fraction,

Table 1. Fatty Acid Composition of Adipose Tissue Triglycerides

Source	Type	Percentage of total fatty acids							
		Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Other
Interscapular adipose tissue (new-born rabbit)	Brown	2·2 ± 0·5	32·7 ± 2·2	5·8 ± 0·8	12·7 ± 1·2	27·3 ± 1·0	14·6 ± 1·8	3·5 ± 0·8	0·7
Inguinal adipose tissue (new-born rabbit)	White	2·5 ± 0·3	39·8 ± 2·1	6·4 ± 0·6	6·2 ± 0·2	29·3 ± 1·9	11·8 ± 1·0	2·4 ± 0·2	1·0
Interscapular adipose tissue (adult rabbit)	White	2·4 ± 0·5	42·0 ± 5·6	3·5 ± 0·7	6·1 ± 2·0	25·3 ± 2·2	12·4 ± 1·3	2·9 ± 0·3	4·9
Axillary adipose tissue (new-born human)	Brown	3·3 ± 0·9	46·7 ± 2·4	9·0 ± 0·7	5·3 ± 1·3	32·2 ± 2·8	1·5 ± 0·3	2·0 ± 1·0	—
Subcutaneous abdominal adipose tissue (new-born human)	White	4·0 ± 2·0	49·0 ± 7·0	10·0 ± 3·2	4·0 ± 2·0	29·2 ± 5·4	3·2 ± 1·8	0·6 ± 0·4	—

Results are given as mean ± *S.E.* ($n = 5$)

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the concentration being three to four times greater than palmitic acid[6]. Human new-born adipose tissue triglyceride, both brown and white, contains more palmitic acid and less oleic acid than that reported by others[3,8–10] who have examined mixed lipids from adult subcutaneous white adipose tissue. Dietary differences may also account for these differences[10]. The distribution of fatty acids is closely similar to that reported for subcutaneous adipose tissue from three full-term human infants[8]. Triglycerides from human new-born adipose tissue differ from the rabbit chiefly in the much lower proportion of linoleic acid. This may reflect a difference in placental permeability to long-chain fatty acids. It is clear that the differences in function of brown and white adipose tissue are not accompanied by any consistent differences in the fatty acid composition of their triglycerides.

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Collagen, Elastic Tissue and Resorcin Fuchsin

In recent years several investigations[1–3] have thrown doubt on the specificity of resorcin fuchsin as a reliable stain for structures containing the protein elastin. These investigations have also indicated that some alteration in the state of collagen may cause the latter to react with this stain. Partridge[4] has suggested that this alteration is denaturation and the increase in reactivity which occurs with this change allows the protein to react with phenolic dyes. However, although resorcin fuchsin contains a phenol, the actual basic dyes used in the stain are not phenolic. Furthermore, it can be shown that certain fibres in connective tissue which stain with resorcin fuchsin but which are demonstrably different from the elastic tissue of the aorta, cannot be distinguished from normal collagen using the basic dyes from resorcin fuchsin without additives. Since denatured collagen has an isoelectric point of 4·8 compared with 7·5 (ref. 5) for the normal protein a simple basic dye should distinguish between the two. Such a result could not be obtained in the connective tissues mentioned. It follows, therefore, that connective tissue contains either three types of fibre reactive to resorcin fuchsin, namely, elastic tissue, denatured collagen and a previously undescribed fibre (Fig. 1), or some other aspect of collagen denaturation must be considered in accounting for the similarity in the staining reactions of elastic tissue and denatured collagen. It is the purpose of this communication to suggest that the loss of crystalline structure that occurs when collagen is denatured[6] accounts for this similarity and disposes of the need to postulate any other resorcin positive fibre in connective tissue.

Fig. 1 shows an aggregation of resorcin-positive fibres taken from the soft tissue beneath a denture that had been worn for many years. No such differential staining pattern could be observed when only the basic dyes, basic fuchsin or crystal violet, in aqueous solution were used to stain serial sections. In each case the addition of resorcin fuchsin caused a tangled pattern of dark staining fibres to appear resembling the appearance of Fig. 1. If, however, the elastic laminae of a young aorta were exposed to a simple solution of either of the two dyes for at least 4 h these structures could be clearly differentiated from the intervening collagen layers although the contrast was markedly inferior to that obtained with resorcin fuchsin. In addition to this difference these two tissues showed a difference in their relative affinity for iron. Ferric chloride is one of the additives in resorcin fuchsin, and Fig. 2 shows the result of soaking a section of aorta in 0·1 per cent ferric chloride followed by treatment with potassium ferrocyanide. The resorcin-positive elastic fibres in the aorta show no affinity for iron; the resorcin-positive fibres in the oral connective tissue showed an affinity equal to that of normal collagen, that is, no differential pattern could be obtained in the latter tissue.

Resorcin fuchsin contains, apart from the two basic dyes mentioned and the ferric chloride, resorcinol, ethyl alcohol and hydrochloric acid. Water is also present, as the alcohol used is industrial (70 per cent). Further variation of these components resulted in the observation that the behaviour of the whole stain could be duplicated by two different combinations of these additives. These two combinations, in the aorta, improved considerably the distinction that could be obtained using basic dyes only and in the dental tissue caused a differential pattern to appear similar to that obtained using resorcin fuchsin.

The first combination found to duplicate the behaviour of resorcin fuchsin was simply the addition of ferric chloride to an aqueous solution of basic fuchsin. These two components, when used separately, show up differences as already discussed between resorcin-positive fibres in the aorta and the experimental tissue; when combined, this property is lost. A probable explanation of this result may be based on the competition that exists between iron and basic fuchsin for the available carboxyl groups in a tissue section. According to Wigglesworth[7] iron co-ordinates with these groups in an extremely rapid reaction which takes preference over that with the positive charges of a basic dye. Although collagen reacts much more intensely with acid dyes under histological conditions, there is some reaction with basic dyes due to the fact that while the prevailing or net charge is positive, negative groups, mainly carboxyls, are present. The number of carboxyls in elastin is much smaller and the differentiation obtained between collagen and elastin by a simple basic dye in the aorta must be due to the differing modes of attachment, ionic or long-range in the case of the dye and collagen, short range (Van der Waals and/or hydrogen bonding) in the case of the dye and elastin. The effect of iron would be expected to improve differentiation between collagen and elastin by depressing the intensity of the collagen staining with basic dyes. This result is obtained in the aorta. In the experimental dental

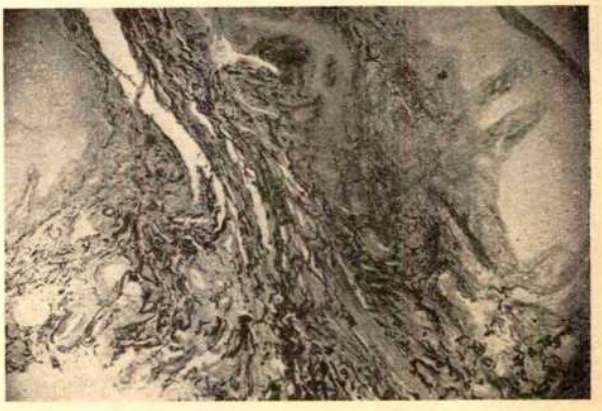


Fig. 1

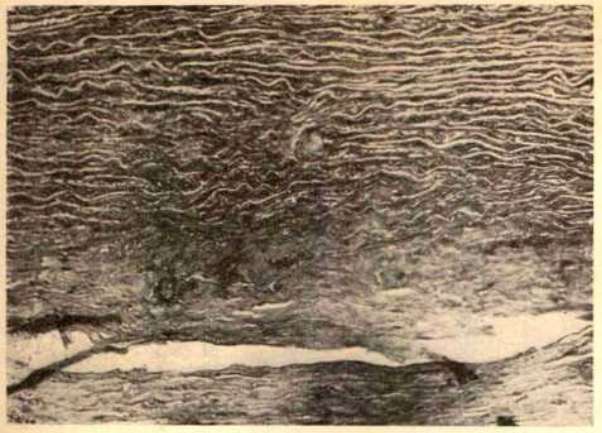


Fig. 2

tissue, however, fibres shown to be resorcin positive in serial sections are outlined by basic fuchsin combined with ferric chloride. A pattern only slightly inferior to that shown in Fig. 1 was obtained if the section was left in the staining bath for at least 4 h, a period well in excess of the 10 min usually required to colour a connective tissue section with basic fuchsin dissolved in water. In view of this time factor and the virtual elimination under these conditions of any reaction between charged groups, it is suggested that short-range forces become more prominent than is usual in simple staining procedures. Since short-range dye attachment[8] is governed more by variations in the degree of crystallinity at the surface of a dyeing substrate than charge distribution, it may be that the differential pattern in the dental tissue is the result of the basic fuchsin outlining the difference between amorphous and crystalline collagen. The long staining time supports this contention; short-range dye attachment takes much longer to reach equilibrium than long-range.

The second combination of additives found to duplicate the behaviour of resorcin fuchsin was a system composed of basic fuchsin, resorcinol and alcohol. The results obtained with this system were not sensitive to either the presence of water or various proportions of alcohol and water, provided some alcohol was present. Phenol could be substituted for resorcinol, as could pyrogallol, without affecting the result. Phenol, resorcinol and alcohol all have uses in the dyeing industry. Vickerstaff[8] lists phenol and resorcinol as additives that increase the pore size of fabrics, thus aiding short-range dye attachment. These additives are more efficient when the substrate is amorphous, crystalline areas being penetrated, if at all, much more slowly. Organic solvents[9] often facilitate dye attachment particularly in those cases in which the dye and a fibre possess similar charges and water is the main solvent. Provided the added organic solvent is more efficient at solvating the dye than water, the resulting redistribution of solvating forces in the system causes an increase in the intensity of dyeing with only a very small concentration of the organic compound present. The net effect is to decrease the effectiveness of the surface electrical barrier.

The simple experiment of adding alcohol to an aqueous staining solution of basic fuchsin caused an immediate increase in the intensity of the colour deposited on a tissue section and appeared to act in accord with the theory of solvent assisted processes. The presence of a swelling agent and alcohol in a solution of basic fuchsin will cause a double improvement in short-range attachment when a substrate is present and a single improvement in long-range attachment. In this system, therefore, crystalline amorphous differentiation is probably obtained in a manner opposite to that by which it is achieved using ferric chloride, namely the exaggeration of amorphous areas instead of long-range suppression. The combination of these two systems will be complementary since the iron reaction is predominant, limiting the action of alcohol in the complete system to short-range facilitation. The low *p*H in resorcin fuchsin is a further factor in favouring short-range attachment; the carboxyl groups are more effectively eliminated and the *p*H of maximum swelling of collagen is about 2·0 (ref. 10).

In summary, it is suggested that of the two principal changes reported to occur when collagen is denatured, the loss of crystalline structure is more important than the increase in electronegativity in relation to the specificity of resorcin fuchsin. Collagen and elastin should ideally be distinguished under ionic conditions and preferably by a reagent possessing no short-range attraction for either protein. Ferric chloride would seem to be a suitable reagent. Any connective tissue fibre giving a positive reaction to either resorcin fuchsin or any other elastic stain but which shows an attraction for iron similar to that of normal collagen is unlikely to contain a high proportion of the protein elastin.

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PATHOLOGY

Erythrocyte Glucose-6-phosphate Dehydrogenase Activity in Haemoglobin H Disease

The occurrence of erythrocyte glucose-6-phosphate dehydrogenase deficiency in patients with haemoglobin H disease was first reported by Ramot, Sheba, Fisher, Ager and Lehmann[1] and Vella[2]. The former investigators considered the association in their patient to be coincidental, but Vella[2] reported that three of his six patients with haemoglobin H disease were deficient in this enzyme. Using the assay method of Kornberg and Horecker[3] we have determined the erythrocyte glucose-6-phosphate dehydrogenase levels in twenty consecutive, but unrelated, Chinese patients (eight males and twelve females) with haemoglobin H disease and found only one female to be enzyme deficient. The association of these two defects is therefore not so common as would appear from the report by Vella[2]. Although the number of patients investigated is not large, the incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency in haemoglobin H disease is probably not greater than that in the population —5·5 per cent in males[4] and 8·4 per cent in females (using calculations described by Allison[5] and assuming that approximately only 80 per cent of female heterozygotes are identified by the assay procedure[6]).

Table 1 shows that the mean erythrocyte glucose-6-phosphate level was significantly higher ($P < 0.001$) in patients with haemoglobin H disease than in non-anaemic controls (twenty males and twenty females). This is in agreement with Rigas and Koler[7], who reported that erythrocyte glucose-6-phosphate dehydrogenase levels were 2–3 times normal in two patients with haemoglobin

Table 1. Erythrocyte Glucose-6-phosphate Dehydrogenase Activity in Haemoglobin H Disease

Subjects	No.	Erythrocyte glucose-6-phosphate dehydrogenase activity: ΔO.D. per min per g haemoglobin	ΔO.D. per min per 100 ml. erythrocytes	ΔO.D. per min per 10^9 erythrocytes
Controls	40	9·6 ± 1·6 (6·4 – 14·9)	332 ± 52 (222 – 470)	0·290 ± 0·046 (0·194 – 0·449)
Haemoglobin H disease				
(*a*) Non-enzyme deficient	19	23·3 ± 5·1 (13·8 – 33·0)	645 ± 149 (405 – 977)	0·443 ± 0·124 (0·256 – 0·700)
(*b*) Enzyme deficient	1	2·8	79	0·055

Values shown are mean ± standard deviation. The range is in parentheses.

H disease and may be attributed to the presence in these patients of erythrocyte populations with a younger than normal mean erythrocyte age[7,8]. That the present series of patients with haemoglobin H disease had younger circulating erythrocytes can be adduced from their significantly elevated mean reticulocyte count of 3·5 ± 1·8 per cent (normal: 0·2 ± 0·2 per cent).

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Tolerance of Phenylalanine after Intravenous Administration in Phenylketonurics, Heterozygous Carriers, and Normal Adults

Substances which have been introduced into the body by intravenous injection are eliminated from the blood in accordance with a mathematically definable pattern[1]. The results of heterozygote tests in phenylketonuria after oral administration of phenylalanine[2,3], however, do not make directly possible any estimation of the elimination rate from the blood. It is possible that, according to the configuration of the tolerance curves, the elimination of phenylalanine from the blood occurs at the same speed in controls as in heterozygous carriers[4]. It would be difficult to explain this mode if the velocity of elimination were primarily a function of the activity of phenylalanine hydroxylase[4].

The purpose of the investigation described in this communication was to estimate the rate of elimination of phenylalanine from the blood.

We tested seventeen healthy adults, nine heterozygotes, and six phenylketonurics. First we obtained a fasting specimen of capillary blood by means of a finger-prick. We then intravenously injected phenylalanine (sterile solution of 2·5 per cent of L-phenylalanine in water). The dose was about 50 mg per kg body-weight and the injection lasted about 10 min. Thirty minutes following the phenylalanine administration we collected further specimens at half-hourly intervals for a total period of 2·5 h. Assays of L-phenylalanine and L-tyrosine were performed on 0·1 ml. of blood by a modification of the enzymatic micro-method of La Du *et al.*[5] (for details of the modification see ref. 6). The phenylalanine-loading procedure was well tolerated and we did not observe any sympathomimetic response.

The semi-log graph of the phenylalanine concentration between 30 and 150 min after administration presents a straight line which complies with the general formula of a mono-molecular reaction.

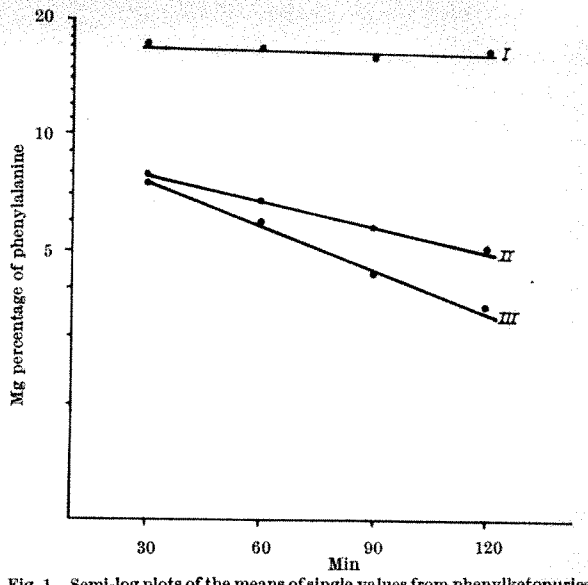


Fig. 1. Semi-log plots of the means of single values from phenylketonurics (I), heterozygotes (II), and normal adults (III). The mean of the fasting values in group I was 7·9 mg per cent; in group II, 1·7 mg per cent; in group III, 1·3 mg per cent

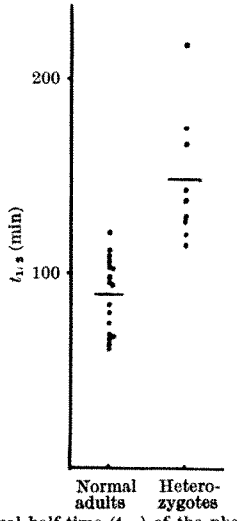


Fig. 2. The biological half-time ($t_{1/2}$) of the phenylalanine elimination from the blood of normal adults and of heterozygous carriers

Table 1 shows the mean time of elimination to the half concentration ($t_{1/2}$) and the mean disappearance constant (k), calculated from the general equation of a mono-molecular reaction.

In Fig. 1 the means of single values from these three groups have been plotted against time on a semi-log basis; Fig. 2 gives the $t_{1/2}$ values for the various tests with normal individuals and heterozygotes.

These results show that the heterozygotes have a significantly smaller rate of elimination than do the normal individuals. In phenylketonurics there was no great decline of phenylalanine during the test. The activity of the phenylalanine hydroxylase is, therefore, in all probability one of the limiting factors of the elimination rate of phenylalanine.

Recently, Nelson[4] raised the question concerning the phenylalanine tolerance tests for heterozygous carriers of

Table 1. Mean Values of $t_{1/2}$ ± Standard Error

	No.	$t_{1/2}$ (min)	k (per cent/min)
Adults	17*	88·7 ± 19·4	0·785 (range 0·573–1·14)
Heterozygotes	9*	148·1 ± 32·6	0·468 (range 0·318–0·602)

* The differences of the mean values of $t_{1/2}$ are significant according to the t-test ($P < 0·001$).

Table 2. MEAN TYROSINE-LEVELS ± STANDARD ERROR IN MG/PER CENT

Time after administration (min)	0	30	60	90	120
Normal adults	0·85 ± 0·60	1·55 ± 0·77	1·93 ± 0·42	2·01 ± 0·53	1·66 ± 0·42
Heterozygotes	0·90 ± 0·33	1·30 ± 0·62	2·00 ± 0·62	1·60 ± 0·07	1·77 ± 0·60
Phenylketonurics	0·10 ± 0·06	0·27 ± 0·26	0·20 ± 0·22	0·24 ± 0·21	0·32 ± 0·32

The standard error in this concentration range is high (ten assays from an individual: 0·83 mg per cent ± 0·49).

phenylketonuria. He suggested that the substantially higher phenylalanine-level in the heterozygote and in the phenylketonuric after oral phenylalanine loading was due to a difference in volume of the distribution of phenylalanine between these two groups. After intravenous injection of phenylalanine we found that the blood level of the control and the heterozygous group increased almost to the same extent. There was a slightly greater increase in blood-level in the case of phenylketonurics, but this increase was probably not a genuine one, as was found when the concentration was calculated at zero time from the semi-log plot.

An increase in the blood tyrosine-level was found in normal individuals as well as in heterozygotes. The dose of phenylalanine administered did not suffice, however, to identify a heterozygous state by an increase in the tyrosine-level (Table 2).

Estimation of the elimination rate after an intravenous load of phenylalanine may serve as a test for heterozygotes. due to the fact that the elimination in heterozygotes and in normal adults proceeds at different speeds. Some overlap, however, between normals and heterozygotes may occur in adults.

Furthermore, during the early days after birth the phenylalanine elimination may be markedly delayed[7]; thus during this period it is not possible to test for heterozygotes by this method.

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RADIOBIOLOGY

Interphase Death and Related Biochemical Radiation Effects in Murine Leukaemia Cells

WITHIN a few hours after X-irradiation with sub-lethal doses, cytological and biochemical changes become demonstrable in the lymphocytes of the thymus and other lymphatic tissues.

The early death of lymphocytes occurring in the period after irradiation when cell division is inhibited (mitotic delay) is often referred to as 'interphase death'[1] as distinguished from cell death associated with mitosis (mitotic death) which occurs in other cell types.

It is frequently difficult to evaluate whether the biochemical disturbances precede the cellular degeneration as reflected by nuclear pyknosis and karyorrhexis. Some of the biochemical radiation effects, for example, the loss of cations from the nucleus[2], the decrease of sulphydryl groups in thymus nuclei[3] and the inhibition of nuclear oxidative phosphorylation[4,5], have been classified among the few early biochemical radiation effects which seem to precede the occurrence of morphological signs of cell damage.

Nuclear oxidative phosphorylation and its radiobiological implications have been the subject of our investigations in the past few years[5,6]. Recently we have investigated nuclear oxidative phosphorylation in three different transplantable lymphosarcomata and one myeloid leukaemia in mice. Certain findings concerning the cytological and biochemical effects of X-irradiation in these tumours and in rat thymus will be presented briefly here. Other aspects of these investigations have already been published[7].

The experiments were carried out with three different transplantable lymphosarcomata from the *C57BL/Rij*, *CBA/Rij* and (*CBA/Rij* × *C57BL/Rij*)F_1 mouse, respectively, and a myeloid leukaemia from the *RF* mouse. Only those leukaemic spleens were used in which the majority of cells consisted of leukaemic ones. Oxidative phosphorylation was measured at intervals after whole-body irradiation in the nuclei and in the mitochondria isolated from the leukaemic spleens and normal thymus tissue. The mode of cell death was identified in routinely stained histological sections from the same tissues.

Important differences between the radiation responses of the various cell types were found. According to the biochemical effects observed, the cells can be divided into two groups. In the first group, which includes thymic lymphocytes, myeloid leukaemic cells and *C57BL* lymphosarcoma cells, inhibition of nuclear ATP synthesis is observed within 2 h after irradiation with 900 r. (Fig. 1, group I). In thymus this inhibition is even more pronounced[5] and seems quite distinctly to precede cell death as judged by morphological criteria. In these three cell types mitochondrial phosphorylation is also depressed at an early stage, although the maximum inhibition seems to occur slightly later than that observed with nuclear ATP synthesis.

In the other group consisting of the *CBA* and F_1 lymphosarcoma cells, inhibition of nuclear oxidative phosphorylation develops later, that is between 8 and 24 h after irradiation, concurrently with the appearance of cytological changes. Mitochondrial oxidative phosphorylation is not disturbed in these cells even at the time when cellular disintegration is well advanced (Fig. 1, group II).

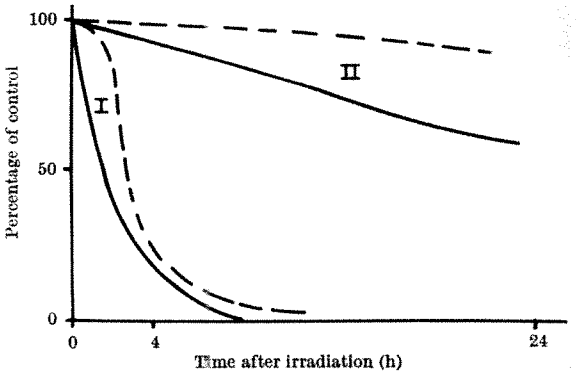


Fig. 1. Effects of 900 r. whole-body irradiation on oxidative phosphorylation. Averages of results obtained with thymus, myeloid leukaemia and *C57BL* lymphosarcoma (I) and with F_1- and *CBA* lymphosarcoma (II). ——, Nuclear oxidative phosphorylation; - - - - -, mitochondrial oxidative phosphorylation

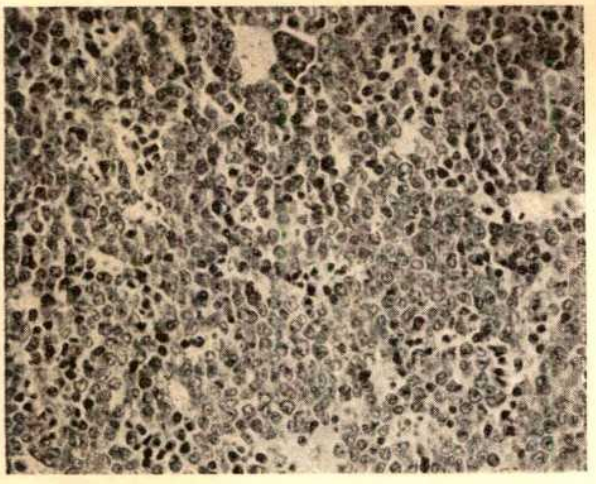


Fig. 2. Interphase death of murine myeloid leukaemia cells. Leukaemic spleen 2 h after 900 r. whole-body irradiation. Note nuclear pyknosis and homogenization of chromatin. Mitotic activity is completely absent. (Haematoxylin-eosin, × 150)

These results suggest that the pattern of biochemical changes seems to be specifically related to the mode of cell death after irradiation. As do normal lymphocytes, many of the myeloid leukaemic cells and *C57BL* lymphoma cells die in interphase (Fig. 2) while interphase death was much less apparent or even absent in the *CBA* and F_1 lymphosarcomata[8].

This relation had led us to postulate a biochemical basis of interphase death: cells in which radiation induces a rapid inhibition of both nuclear and mitochondrial oxidative phosphorylation may die in interphase as a consequence of energy exhaustion. When mitochondrial and nuclear oxidative phosphorylation remains relatively unaffected, the cells survive until the next mitosis.

Of special interest is the finding that interphase death is apparently not restricted to lymphoid cells (normal or leukaemic) but also occurs in an entirely different cell line: a myeloid leukaemia cell.

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BIOLOGY

Secretion of the Thiocyanate Ion by the Nasal Gland of the Adelie Penguin

Analysis of the secretion from the nasal gland of marine birds indicates that the secretory mechanism is highly specific for sodium and/or chloride. Only very small amounts of potassium, magnesium, or sulphate normally appear in the secretion[1]. It was therefore of interest to determine how the secretory mechanism would behave in the presence of an anion which has roughly the same physical–chemical properties as chloride.

Thiocyanate space experiments performed during an investigation of the salt and water metabolism of the Adelie Penguin provided an opportunity to determine to what extent this ion, which does not normally occur in appreciable quantities in the plasma, would be excreted by the nasal gland. Consequently, the secretion from birds which had been given a thiocyanate injection and then 'salt loaded' was analysed for both thiocyanate and chloride.

Table 1 shows the results which were obtained from three sets of samples. It is seen that the concentration of thiocyanate in the nasal gland secretion is 6–7 times as great as the concentration of this ion in the plasma. After thiocyanate analysis, insufficient plasma remained for the chloride analysis. However, data from other experiments on 'salt-loaded' penguins indicate that an estimation of the plasma [Cl^-] at about 115 m.equiv./l. is justified. The ratio of secretion concentration to plasma concentration (S/P ratio) for thiocyanate is then seen to be quite similar in magnitude to that for chloride.

In order to eliminate the possibility that the salt gland was itself producing the thiocyanate ion in the course of its metabolic work, secretion was analysed from salt-loaded but not thiocyanate-dosed birds. In no case was there any indication of the presence of the ion.

The salt-secreting nasal gland has been referred to on occasion as "a gill in the nose". This comparison with the salt-secreting function of the marine teleost gills is not unwarranted. The first studies which demonstrated how marine fish maintain body fluids hyperosmotic to their environment without producing hypertonic urine involved determinations of chloride concentration[2,3]. The cells supposedly performing the transport of the salt from the blood into the sea were consequently termed 'chloride cells'. More recent work on ion transport mechanisms in general indicates that the term 'sodium cells' would be more apt.

Electrophysiological investigations have led to the view that the active transport of sodium chloride probably involves a sodium pumping mechanism and a passive diffusion of chloride. The studies which have been reported from the laboratories of Ussing[4] and Giebisch[5] have shown rather conclusively that it is the movement of sodium ions which constitutes the energy-consuming transport in the frog skin and in the proximal tubules of the kidney of *Necturus*. Hokin and Hokin[6] have presented evidence suggesting not only that it is sodium which is being pumped by the nasal gland of the albatross, but also that it is phosphatidic acid which serves as the cation carrier. Thesleff and Schmidt-Nielsen[7] have attempted a direct attack on the electrophysiology of the salt gland. Owing to the extensive folding of the membranes of the salt gland cells, intracellular potentials could not be reliably determined. It was found, however, that potential positive with respect to the homolateral jugular vein appeared in the duct of the gland at the time of secretion. This they interpreted as indicating that the potential was due to the active transport of the positive sodium ion from gland cell into duct lumen. Strophanthin, known as a sodium pump-blocking agent, blocked secretion and abolished the potential.

Table 1. Secretion of Thiocyanate by the Nasal Gland

Bird No.	Plasma SCN^- conc. (m.equiv./l.)	Secretion SCN^- conc. (m.equiv./l.)	S/P ratio	Secretion Cl^- conc. (m.equiv./l.)	Est. S/P ratio
1	0·70		6·1		
		4·29		748	6·5
		4·09	6·5	714	6·2
	0·63				
		4·05		715	6·2
		3·92		690	6·0
		4·02	6·5	756	6·6
	0·50				
2	0·65		6·6		
		4·27		748	6·5
		4·05	6·2	784	6·8
	0·65				
		3·99		758	6·6
		3·99		754	6·6
		4·02	6·8	614	5·3
	0·59				
3	0·69		7·1		
		4·92		756	6·6
		4·94		750	6·5
		4·10		608	5·3
	0·58		7·1		

In the light of these findings, the secretion of thiocyanate by the nasal gland at S/P ratios comparable with those of chloride is explained as a passive diffusion of the SCN^- ion. The equivalent conductivity of the thiocyanate ion at infinite dilution and 18° C is 57·4 ohm^{-1} cm^{-1} $equiv.^{-1}$ litre while that of chloride is 66·3 (Eichler, ref. 8. Values from the work of Landolt-Börnstein). The difference between the conductances of these two ions is 8·9. Information is not available on the conductance of the anions alone at higher concentrations. The difference in equivalent conductance of potassium thiocyanate and potassium chloride solutions, however, changes only slightly over the range of 0·1 m.equiv./l. to 500 m.equiv./l. (ref. 9). As a first approximation, the electrical requirements of the system could be as satisfactorily met through the diffusion of SCN^- as through that of Cl^-.

A report by Carey and Schmidt-Nielsen[10] indicates that some discrimination may be exercised by the gland on the anionic composition of its secretion. They found that the S/P ratio for iodide was about 2·5 as compared with about 6 for that of chloride in the herring gull. The S/P ratios in the Adelie for both chloride and thiocyanate were mostly slightly greater than 6. It would appear that the hydrated ionic radius is probably not the determining factor since the conductivity of iodide is about the same as that of chloride.

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Strain Differences in Mouse Mammary Tissue Sensitivity to Prolactin and Somatotrophin in Organ Culture

THE lactogenic activities of ovine prolactin and bovine somatotrophin have been investigated in several strains of inbred mice, and a spectrum of responses was reported[1,2]. Prolactin was lactogenic in all strains, with some variations in sensitivity encountered. On the other hand, whereas somatotrophin showed considerable activity on tissues from some strains, other strains were much less reactive, and a single strain was completely unresponsive to the lactogenic effects of this hormone.

Two strains showing the extremes of responsiveness to somatotrophin, *C3H* (highly sensitive to somatotrophin) and *A* (refractory to somatotrophin), were later examined both in mammary transplantation studies[3] and in organ culture[4]. The results of these investigations clearly demonstrate that their differential sensitivity lies in the mammary tissues themselves and thus must reflect strain-specific tissue responsiveness.

Our observations on hormonal sensitivity *in vitro* have now been extended to several strains of mice other than *C3H* and *A*. This communication reports the responsiveness of *DBA*, *C3Hf*, *BALB/c*, and *C57BL* mammary tissues both to prolactin and to somatotrophin in organ culture. Comparison is made with the responses obtained in *C3H* and *A* mammary tissues in organ culture and with the differential strain sensitivity reported previously *in vivo*.

Mammary tissues for explantation were taken from nulliparous pregnant female mice from the following inbred strains: *DBA/2NCrgl*, *C3Hf/Crgl*, *BALB/cCrgl* and *C57BL/Crgl*. All mice were in the second week of pregnancy (10–13 days), at a time when the mammary alveoli are small and non-secretory, with few or no lipid vacuoles, and show no stainable material in the lumina[4].

The organ culture procedure has been described[4,5]. Synthetic basal culture medium 199 was supplemented with insulin, 5 μg/ml., aldosterone, 1 μg/ml., and either ovine prolactin (C. H. Li), 1–4 μg/ml., or bovine somatotrophin (C. H. Li), 2–10 μg/ml. Approximately 8–16 mammary explants from 4–5 mice from each strain were cultured with each concentration of each pituitary hormone. After 5 days in culture the tissues were prepared for histological examination and graded as previously described[4], according to the level of secretory activity attained. Particular attention was directed to the number of explants in each series showing maximal secretory responses: alveolar distension three to four times greater than in the initial control tissues, lipid vacuoles in most alveolar cells, and large amounts of stainable secretion in alveolar lumina[4].

A prolactin concentration of 1 μg/ml., capable of initiating maximal secretion consistently in *C3H* tissues, produced comparable effects in only 75, 60, 47 and 36 per cent of tissues from *DBA*, *BALB/c*, *C3Hf* and *C57BL* mice, respectively. This concentration of prolactin has no secretion-stimulating effect in tissues from *A* mice.

Tissue responsiveness was somewhat greater with 2 μg/ml. of prolactin; with 3 μg/ml., maximal responses were obtained in all *DBA*, *BALB/c*, and *C3Hf* tissues. *C57BL* mammary tissues, on the other hand, showed only 50 per cent maximal secretion even with 3 μg/ml. of prolactin. At 4 μg/ml., the responsiveness was increased to 86 per cent, a result comparable with that obtained in *A* tissues by this concentration of prolactin.

The mouse strains used may thus be ranked in the following order of decreasing sensitivity to prolactin: $C3H > DBA \simeq BALB/c \simeq C3Hf > A \simeq C57BL$. Except for the position of *BALB/c* mice in rank order, these results follow the general pattern observed *in vivo*: $BALB/c > C3H \simeq DBA \simeq C3Hf > A \simeq C57BL$ (taken from Nandi and Bern[1], Table 4). The high sensitivity of *BALB/c* mice to subcutaneous administration of prolactin is interesting, inasmuch as *C3H* tissues show the highest sensitivity *in vitro*. It should be mentioned, however, that prolactin also causes an increase in body-weight of *BALB/c* mice but not of other strains[1]. The relationship between the effects of prolactin on body-weight and mammary gland responsiveness is not at all apparent, but if the two effects are, in fact, causally related, then it is not surprising that *BALB/c* mammary tissues *in vitro* should fail to respond as *in vivo*.

A somatotrophin concentration of 2 μg/ml., capable of initiating consistent maximal secretion in *C3H* tissues, had corresponding effects in 78 per cent and 53 per cent of *DBA* and *C3Hf* tissues, respectively. As in *A* tissues, this concentration of somatotrophin caused no secretory response in *BALB/c* or in *C57BL* tissues. Consistent maximal responses could be obtained in *DBA* tissues by 3 μg/ml., whereas *C3Hf* tissues required at least 5 μg/ml. of somatotrophin for similar responses.

In contrast, 3, 4 and even 5 μg/ml. of somatotrophin had comparatively less secretory effect in tissues from *BALB/c* and *C57BL* mice. The responsiveness of these latter two strains could be increased to 100 per cent and 86 per cent, respectively, by 10 μg/ml. of somatotrophin, a concentration five times that optimally effective in *C3H* mice. Only 40 per cent maximal secretion was obtained in *A* tissues by 10 μg/ml. of somatotrophin. Thus, although *BALB/c* and *C57BL* tissues were somewhat more responsive to somatotrophin than *A* tissues, they were considerably less responsive than tissues from *C3H*, *DBA* and *C3Hf* mice.

In summary, the several mouse strains can be arranged in the following order of decreasing sensitivity to somatotrophin: *C3H* ≅ *DBA* > *C3Hf* > *BALB/c* ≅ *C57BL* ≫ *A*. These results follow the general pattern of responsiveness observed *in vivo*: *C3H* ≅ *DBA* > *C3Hf* ≅ *BALB/c* ≅ *C57BL* ≫ *A* (taken from Nandi and Bern[1], Table 3), and in both situations, mammary tissues from strain *A* mice may be considered refractory to somatotrophin.

It may be noted that although the strain differences in hormonal sensitivity *in vitro* in general compare well with observations *in vivo*, the patterns are not absolutely identical. For example, strains *C3Hf*, *BALB/c*, and *C57BL* mice respond to the same degree to somatotrophin *in vivo*; however, in organ culture *C3Hf* tissues were more responsive to somatotrophin than were tissues from *BALB/c* and *C57BL* mice.

The possibility that contamination with prolactin can account for the lactogenic activity of somatotrophin has already been discussed[4], and the question remains open until absolutely purified somatotrophin preparations are available. However, several other preparations of bovine somatotrophin have since been examined in organ culture and found capable of initiating mammary secretion in *C3H* mice but not in *A* mice[6]. Pigeon-crop and immunological assays of these preparations indicate less than 1 per cent prolactin contamination. The present data, therefore, continue to support the view that bovine somatotrophin possesses lactogenic activity independently of its contamination with prolactin but that this inherent activity is apparently limited to certain strains of mice. Barnawell[7] has found no stimulation of secretion ascribable to bovine somatotrophin itself in cultured mammary tissues from two rat strains, hamster, guinea-pig, rabbit and dog.

Further investigations are now in progress to determine whether somatotrophins from various non-bovine species are also lactogenic in mice.

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Interspecific Spore Inhibition in the Cellular Slime Moulds

Experiments were designed by one of us (H. M. S.) to determine whether or not there was any kind of competition between different species of slime mould when they were inoculated in the same culture dish. If *Escherichia coli* (as a food source) was spread with an inoculation loop over the surface of non-nutrient agar[1] in a Petri dish and approximately equal numbers of spores from two species of slime mould were mixed in one small spot, then invariably only one of the species developed (either in the light or in the dark); the second species appeared to be completely inhibited. Using this method, the following hierarchy of inhibition was established: *Dictyostelium purpureum* (No. 2) > *Polysphondylium violaceum* (No. 6) > *D. discoideum* (No. 1) > *D. mucoroides* (No. 2). This method was also attempted using the wild type of strain No. 6 of *D. mucoroides* and a small mutant derived from the same strain. In this case also, one form (the wild type) completely inhibited the development of the mutant.

In an attempt to make the tests more quantitative, quite conflicting results were obtained and the difference in the techniques provided a clue to the mechanism of competitive inhibitions. With this second method, the spores were suspended in distilled water, counted, centrifuged down and again placed on a small spot as in the previous method. Here, no matter what combination of species was used, equal development was evident in the first few days; there was no inhibition.

The explanation of this apparent conflict came from some experiments that were in progress independently in the same laboratory (C. C.). A few years ago Russell and Bonner[2] showed that densely plated spores had a far lower percentage germination than sparsely plated spores, suggesting the presence of a spore germination inhibitor. This has now been shown to be the case. If spores of *D. discoideum* are washed four or five times with distilled water they will show more than 95 per cent germination at very high densities. It is even possible, after removing the inhibitor from the spores, to concentrate it by boiling, and a very low percentage of spores placed in such a concentrate germinates, even when they are sparsely plated.

From this evidence we may conclude that the germination inhibitor is the effective agent in preventing growth of one species when the spore mixtures are made in dry and concentrated conditions. The inhibition can be removed either in a single species or in mixtures of species by washing or dilution in water. We must presume that the reason for the hierarchy of inhibition is that there is a differential susceptibility to a single inhibitor or possibly different inhibitors.

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Temperature-controlled Meristic Variation in the Salamander *Ambystoma gracile*

The number of meristic parts in fishes (vertebrae, fin rays, scales, etc.) is often susceptible to modification by temperature or other environmental factors to which the developing embryo is exposed. This has been demonstrated experimentally on at least a dozen fish species[1,2]. In reptiles, one experiment[3] showed that the number of scales on a snake could be raised by higher incubation temperature. Serially repeated parts are less variable in amphibians than in fishes, but among the urodeles the number of vertebrae (or of somites) is known to vary intraspecifically among wild populations, and is sometimes used taxonomically. A hereditary basis to the number of trunk segments in *Plethodon cinereus* has been demonstrated by rearing young from different populations under constant conditions[4]. This communication describes the converse experiment, in which urodele eggs from one locality were reared at various temperatures, to determine whether different environments could produce different meristic counts in young having similar genetic background.

Egg masses of the north-western salamander, *Ambystoma gracile* (Baird), were collected by A. L. Hamilton on April 17, 1964, fron Jacobs (Marion) Lake, 7 miles north-north-east of Haney in the lower Fraser River valley of British Columbia. Each egg mass consisted of from 60 to 140 eggs distributed in a clear stiff jelly matrix. Egg masses had been attached to plant stalks a few inches above the mud bottom in 2–5 ft. of standing water, at 3°–4° C. Each batch was probably laid by a single female within the preceding 2 days.

Each egg mass was divided in two, and each half placed in a basket suspended in aerated water maintained at constant temperature and subject to 15 h artificial light. Fig. 1 shows the distribution of halves of three egg masses, *A*, *B* and *C*, at various rearing temperatures. As a guide to the period during which body segmentation is alterable, those eggs from mass *A* which had been placed at 12° C were again divided, and half of them were moved to the 18° C bath 14 days after start of the experiment. At the same time, half those from mass *A* which had been placed at 18° C were moved to the 12° C bath. Resulting mean costal groove counts of these transferred lots (not shown in Fig. 1) were almost identical to the means of those left permanently at the original temperatures, demonstrating that costal groove count is already determined before 2 weeks at either 12° C or 18° C.

After hatching, the larvae were fed pond plankton and brine shrimps. Animals reared at high temperatures were preserved after 75 days. Those at low temperatures were moved to warmer water after several weeks and were preserved after about 200 days. Snout-vent lengths of most larvae were 15–25 mm when preserved.

Total vertebral count is an unsatisfactory taxonomic character in this salamander, as posterior vertebrae are added well after the young are free swimming and feeding. Counts of total vertebrae visible in cleared and alizarin-stained salamanders varied from about 29 at snout-vent length of 14 mm to about 49 at snout-vent length of 30 mm (the latter being a wild 1-year-old specimen). Accordingly, only the trunk segments were investigated. Determination of trunk vertebral count is difficult in young specimens, because the pelvic girdle has as yet no ossified attachment to the vertebral column, and the position of the first haemal arch may be unclear. Fortunately, a high correlation has been shown[5] between the number of trunk vertebrae and the number of costal grooves which mark the myosepta between adjacent muscle segments. In the present experiment enough specimens were prepared in alizarin and potassium hydroxide to establish that trunk vertebrae displayed a pattern of variation similar to that of costal grooves. The latter are most commonly referred to in taxonomic literature, and may be more accurately and conveniently counted in young salamanders.

All those costal grooves were counted which lay behind the posterior margin of the fore-limb and ahead of the anterior margin of the hind-limb, when the limbs were held out perpendicular to the body axis. If more than half a segment width lay ahead of the first counted groove, and more than half a segment width lay behind the last counted groove, the count was increased by a half unit. Costal groove counts were made on both sides of each specimen and averaged. Sample sizes shown in Fig. 1, and used in calculations of probability, refer to the number of specimens, not the number of sides counted.

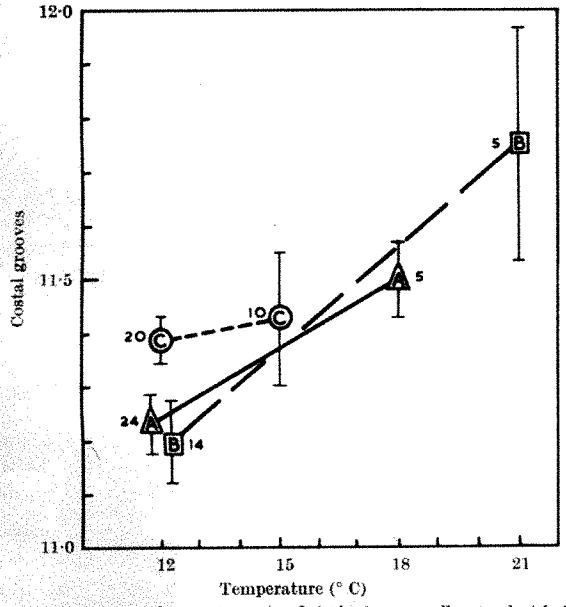


Fig. 1. Mean costal groove counts of *Ambystoma gracile* reared at indicated temperatures. Letters refer to original egg masses. Figures indicate sample size. Vertical bars are ±1 standard error

Individual costal groove counts varied from 10·5 to 13. Combined means were progressively higher at higher rearing temperatures. Within each egg batch, individuals reared at the higher of the two temperatures tested had the higher mean count (Fig. 1). Differences between means for high and low temperature were statistically significant in batches *A* and *B* (*t*-test, $P < 0{\cdot}01$). Also, a significant difference occurred between means from different egg batches reared at the same temperature (between *B* and *C* at 12° C, and also in experiments involving other egg batches not reported here), suggesting genotypic as well as environmentally induced variation.

Because mortalities were in some cases high (20–87 per cent), the possibility that the differences in costal groove count of survivors are due to differential mortality, rather than to phenotypic modification, cannot be dismissed. However, dispersal of counts tended to be high in egg lots with low percentage survival, and low in lots with high survival, which would not be anticipated if temperature were differentially selecting from among a normally distributed assortment of genotypes.

The sub-species *Ambystoma g. gracile* (Baird) used in this experiment is reported to have a modal costal groove count of 10, while the more northerly sub-species *A. g. decorticatum* Cope has 11 or 12 (ref. 6). (The count values given in the literature may not be directly comparable with those given here due to differences in the methods used.) The present demonstration that costal groove number is environmentally labile does not, of course, remove the possibility that there is also a heritable tendency towards higher costal groove counts in the northern sub-species (which displays other, probably genetically determined, differences). However, the likelihood that segmental differences may be phenotypically labile must evidently be borne in mind by urodele taxonomists as well as by fish and reptile taxonomists.

Although environmentally induced changes in meristic series have been documented in many fishes, we are still totally ignorant of their underlying mechanisms. Similar changes can evidently be induced in salamanders. The relative ease of experimental manipulation of urodele eggs[7] (in contrast to teleost eggs) should commend them to experimental embryologists wishing to explore the vexing problems of variability in meristic counts.

[Since preparation of this letter, I have found reported an investigation[8] in which the number of trunk segments of *Triturus vulgaris* was significantly increased by higher rearing temperatures; the number of trunk segments in *Ambystoma mexicanum* was greatest at intermediate temperature with fewer at high and possibly also at low rearing temperatures.]

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Sporogenesis in the Bryophyte *Anthocerosis*: Features shown by Fluorescence Microscopy

Fluorescence microscopy has been found useful in bacterial identification, clinical diagnosis, fluorescent antibody techniques, and general cytological studies[1]. Recently, Shellhorn, Hull and Martin[2] have extended the use of fluorescence microscopy to the detection of fresh and fossil pollen grains by using autofluorescence of exines.

In an investigation of sporogenesis in *Anthoceros hawaiensis* Bartlett and related genera, fluorescence microscopy has proved useful in the detection of incipient deposition of the exosporium in the tetrad immediately following meiosis. In preparing the sporogenous tissue for examination, a median bisection of the living sporophyte was placed in distilled water on a glass slide and covered with a No. 1 cover glass. No fluorochrome was added.

The material was illuminated by an incident ultra-violet light from an Osram '*HB*' 200-W mercury lamp with current supplied from a 'Universal' light source, model 250. Observations were made through a Leitz 'Ortho-Lux' microscope. Filters used during the illumination by ultra-violet light were *BG* 38, *BG* 12 and an ocular blue-absorbing filter.

In Fig. 1, two sporocytes are shown as they appear under incandescent illumination. One sporocyte (top) has just formed tetrads, while the one below contains the four chloroplasts which are prominent in the pre-meiotic condition. The same field (Fig. 1 reversed) is shown in Fig. 2, but under ultra-violet illumination. In Fig. 2, the sporocyte containing the tetrad (top) shows the brightly autofluorescing exosporia of the individual spores; this layer shows a greenish-yellow autofluorescent. The exosporium of developing spores and mature spores shows this same greenish-yellow autofluorescence even after the spore is released from the ruptured sporocyte wall. The sporocyte wall itself is not autofluorescent. In the pre-meiotic sporocyte subjected to fluorescence, only the four chloroplasts can be seen because of the red autofluorescence of chlorophyll.

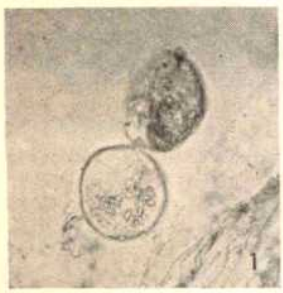


Fig. 1. Two living sporocytes under incandescent illumination. The post-meiotic sporocyte (top) contains a tetrad and the pre-meiotic sporocyte (below) contains four prominent chloroplasts. (× *c.* 770)



Fig. 2. Same field (reversed) as Fig. 1, but under ultra-violet illumination. The strong autofluorescence shows the exosporia of the tetrad in the post-meiotic sporocyte (top); whereas only the four chloroplasts of the pre-meiotic sporocyte (below) show autofluorescence of their chlorophyll. (× *c.* 770)

The phenomenon of autofluorescence in the exosporia of *Anthoceros* suggests that fluorescence microscopy may well be useful in investigations of sporogenesis among other bryophytes. Similar observations of other species of the same genus and representatives of other orders of bryophytes were made. Autofluoresence within the exosporium was found in the following species: *Reboulia hemisphaerica*, *Fossombronia salina Lindb*, *Anthoceros laevis* L., and *Anthoceros punctatus* L. With this technique, distinct developmental changes can easily be observed between the pre-meiotic and post-meiotic conditions. Indeed, fluorescence microscopy may also prove useful in studying exine development in angiospermous species. Exines of *Yucca treculeana* also exhibit bright yellow-green autofluorescence. The similarity of autofluorescence in bryophyte exosporia and pollen exines suggests a homologous chemical composition.

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MICROBIOLOGY

Transformation by Hybrid DNA in *Bacillus subtilis*

Doty *et al.*[1] have reported that when an aqueous solution of DNA is heat-denatured and then annealed by slow cooling, the complementary strands separated by the thermal treatment recombine specifically. By this method, Marmur and Lane[2] made hybrid DNA molecules in which one of the complementary strands derived from the DNA of a streptomycin-resistant strain of *D. pneumoniae*, and the other strand from a wild-type strain. The hybrid molecules thus formed were said to be functional in transforming *D. pneumoniae* to streptomycin resistance.

In certain transformation experiments Herriott[3] used a molecular hybrid of the DNA extracted separately from two strains of *Hemophilus influenzae*, one streptomycin-resistant and the other catomycin-resistant. He found the number of double transformants to be significantly higher (fifty-fold) than with the control—a mixture of the two DNA materials denatured and renatured separately. This result may suggest that both strands of the hybrid molecule are effective in expressing the information into the recipient cell. Different results were obtained by Marmur *et al.*[4] and by Kent *et al.*[5] in *D. pneumoniae*.

Recently, Bresler *et al.*[6] obtained results for *B. subtilis* similar to those of Herriott. Using *his*$^-$ and *try*$^-$ strains as DNA donors, they observed that the hybrid DNA can transform a double auxotrophic strain to a prototrophic one. Moreover, when DNA synthesis by recipient cells is excluded, the hybrid molecules are still able to stimulate the ability of double auxotrophic cells to synthesize histidine and tryptophan. Therefore they assume that both strands of the double helix can serve as templates for messenger RNA synthesis.

We report here some experiments on transformation by hybrid DNA in *B. subtilis* in the system used by Bresler *et al.* The recipient strain was *B. subtilis SB* 25 ($his^-_2try^-_2$)—a double auxotroph for two linked markers[7,8]. In our experimental conditions, a 'cotransfer index' of 0·5 ± 0·03 was calculated. The two donor DNAs were isolated by Marmur's method[9]—one from a *B. subtilis* strain auxotrophic for histidine only (*SB* 25 his^-_2) and the second from a strain requiring tryptophan (*SB* 25 try^-_2). Hybrid DNA was formed by the procedure of Schildkraut *et al.*[10]. (For more details see the legend to Table 1.) In transformation assays the methods of Spizizen[11] and Nester *et al.*[8] were both used successfully. The most reliable results were obtained by a slight modification of the latter (20 μg/ml. of L-histidine in CHT 2 and CHT 10 media).

Table 1 shows that the number of double transformants obtained with the hybrid DNA is significantly higher than with the control mixture. A ratio his^+try^+/his^+ of 1×10^{-2} may be calculated. This value is close to that found by Bresler *et al.* in the same system.

We also undertook some experiments to test the hypothesis that the hybrid DNA is integrated into the host genoma and the markers carried by each strand are both expressed in the recipient cell. The inclusion of a hybrid molecule into the host DNA is only one of the conditions necessary for the formation of double transformants. There must be a subsequent semi-conservative duplication of bacterial DNA which results in two double helices each having only one active genetic marker. To reconstitute the wild-type genotype a crossing-over between *his*⁻ and *try*⁻ loci must occur during the first cellular division. This event is expected to be very rare since *his*⁻ and *try*⁻ loci are closely linked.

Table 1. TRANSFORMATION BY HYBRID DNA COMPARED WITH THAT OF THE CONTROL MIXTURE

Experiment	DNA samples	μg/ml.	Recipient cells/ml. ($his^-try^-_2$)	Number of transformants/ml. $his^+try^+_2$	$his^-try^+_2$	$his^+try^+_2$
1	Control mixture	4	4×10^8	7×10^3	3×10^5	23
	Hybrid mixture	4	4×10^8	$1{\cdot}8 \times 10^4$	3×10^5	190
2	Control mixture	4	4×10^8	7×10^3	3×10^5	20
	Hybrid mixture	4	4×10^8	$1{\cdot}8 \times 10^4$	3×10^5	105

Hybridization procedure: 10 μg of DNA from his^-_2 strain and 10 μg of DNA from try^-_2 strain in 1 ml. of solution containing sodium chloride 0·3 M and sodium citrate 0·03 M were heated at 100° for 10 min. The tube was quickly transferred to a bath held at 67° C. After 2 h the DNA solutions were allowed to cool to room temperature.

Transformation assay: 0·2 ml. of hybrid DNA were added to 0·9 ml. of competent bacteria (*SB* 25 his^-try^-). As a control 0·2 ml. of a mixture of both DNAs, denatured and renatured separately, were added to 0·9 ml. of competent bacteria. The tubes were incubated at 37° C for 30 min with gentle shaking. Diluted samples were plated on minimal agar plates containing 40 μg/ml. of *l*-histidine or 20 μg/ml. of *l*-tryptophan. To score double transformants 1 ml. undiluted sample was plated on minimal agar.

Table 2. CLONAL ANALYSIS OF TRANSFORMED CLONES

Exp.	Total No. of clones examined	Pure clones + −	+ +	Mixed clones + − and + +
1	69	32	36	1
2	164	75	88	1

Exp. 1: A hybrid mixture of the DNA from a prototroph strain with the DNA from try^-_2 strain was used in transforming the competent cells of *SB* 25 his^-try^-. Transformed clones were picked up from a tryptophan-agar plate and streaked on complete medium in order to get about 100 separated colonies. The plates were replicated on minimal medium and minimal medium plus tryptophan.

Exp. 2: A hybrid mixture of the DNA from a prototroph strain with the DNA from his^-_2 strain was used in transforming the competent cells of *SB* 25 his^-try^-. Transformed clones were picked up from a histidine-agar plate and streaked on complete medium in order to obtain about 100 separated colonies. The plates were replicated on minimal medium and minimal medium plus histidine.

The symbol + − stands for a cell transformed for only one marker, + + for a cell transformed for both markers.

The segregation of the markers should be much more frequent. In this case a genetic analysis of the transformed clones should detect a large number of mixed clones, theoretically twice the number of single transformants. To test this possibility, a mixture of the DNA from a prototroph strain with the DNA from a single auxotroph strain (*SB* 25 his^-_2 or try^-_2) was used. *SB* 25 $his^-_2try^-_2$ was transformed with these hybridized DNAs and plated on minimal media supplemented with L-histidine or L-tryptophan, respectively. The clonal analysis of transformed cells (Table 2) shows the extremely low number of mixed clones compared with the other classes of transformants.

We were not able fully to evaluate this result without a previous quantitative control of the hybrid DNA formation. However, our data suggest that only one strand of the hybrid molecule is active in expressing the information into the recipient cell. The experiment of Lacks[12] on the fate of transforming DNA soon after penetration in the *D. pneumoniae* cell, and the results of Fox and Allen[13] on the mechanism of DNA integration in pneumococcal transformation, lend support to this hypothesis. Experiments are in progress in which one of the two donor DNAs is density labelled with deuterium and nitrogen-15, and in which denaturation and renaturation of the mixture are followed in a caesium chloride gradient.

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ENTOMOLOGY

Resistance to a Chemosterilant, Metepa, in *Aedes aegypti* Mosquitoes

THE spectacular eradication of the screw-worm fly *Callitroga hominovorax* (Coquerel) through the release of male flies sexually sterilized by γ-irradiation has stimulated efforts to combat other insect populations by sexual sterilization[1,2]. Sterilization by γ-irradiation has the disadvantages that it may reduce sexual aggressiveness of males, that huge numbers of insects must be laboratory reared for release subsequent to sterilization by the stationary source of radiation, and that the behaviour patterns of laboratory adapted strains may have become altered from those of wild populations[3].

To circumvent these difficulties, intensive efforts have been made to discover chemicals which might be used to sterilize insects in the field[4]; and a number of candidate chemosterilants have been found such as tepa (*tris* (1-aziridinyl) phosphine oxide), metepa (1,2 *tris* [1-(2-methylaziridinyl)] phosphine oxide), and apholate (2,2,4,4,-6,6,-hexa (1-aziridinyl)-2,4,6-phosphine oxide). However, as in the case of insecticides the reduction of the number of insects by means of chemicals may result in the development of resistance to them by Darwinian selection for pre-adaptations[5]. In order to avoid control failures it is advantageous to know whether resistance to a chemical will develop, how quickly and to what magnitude. High insecticide-resistances are usually determined by a single gene-allele for a powerful detoxifying enzyme[6–8]. Such resistances can be directly combated by finding an inhibitor for the detoxifying enzyme[7,8], or by modifying the molecular structure of the chemical to fall outside the range of the enzyme's substrate specificity[9].

Such an incipient resistance to one chemosterilant, aphoxide, has recently been reported in *Aedes aegypti* (Linn.)[10]. This communication reports the outcome of the artificial selection with metepa in *Aedes aegypti*, and furthermore indicates the physiological mechanism of this resistance.

A susceptible laboratory strain of *Aedes aegypti* supplied by Dr. G. B. Craig, University of Notre Dame, was used for this investigation. Larvae were reared at a density of 100–150 in white enamel pans 10 × 12 in. with deep water and fed daily on a coarsely ground mixture of equal parts of 'Kellogg's Concentrate', wheat germ and live yeast. This rearing method provided vigorous larvae of uniform size. The adults were maintained in screened cages 12 × 12 × 12 in. in an insectary maintained at

80° F and 80 per cent relative humidity, and were fed on guinea-pigs and honey. Oviposition was accomplished in a water-filled fingerbowl lined with filter paper. These papers on which the eggs had been laid were hung within the insectary for at least 72 h for conditioning prior to hatching the eggs in deoxygenated water.

Selection for resistance was carried out by exposing large numbers of early fourth instar larvae to the metepa solution until pupation. Adults, without being selected further, were placed in a cage and maintained for egg production. Lots of approximately 50–100 larvae were placed in 250 ml. water at 22° C to which 1 ml. ethanolic solution of metepa was added. The first two generations were selected with 16 p.p.m., the third, fourth and fifth with 32 p.p.m. and the sixth, seventh and eighth with 64 p.p.m.

The principal mode of action of tepa seems to arise from its ability to reduce the egg hatchability through male sterilization[11]. In order to determine the levels of egg hatchability, 200 larvae were exposed to each concentration required. This was done by exposing fifty larvae in a glass jar containing 250 ml. water at 22° C to which 1 ml. ethanolic solution of metepa was added. Eggs from these exposed mosquitoes were conditioned as already described, and the percentage hatch was determined by using 200 eggs per concentration. The whole experiment was repeated with entirely different batches of eggs in order to ensure the constancy of the data.

The parental strain produces a preponderance of non-embryonated eggs, and this is evident in Fig. 1. Selection with metepa for eight generations produced a strain which laid only viable eggs. It is also evident from the figure that the F_8 generation had developed a low measure of resistance.

An attempt was also made to elucidate the biochemical mechanism of metepa-resistance in the resistant F_8 individuals. To 1 ml. of the larval homogenate, containing 20 mg wet weight of larval material per 1 ml. in phosphate buffer at *p*H 7·4, 0·01–0·1 ml. 10 per cent metepa solution in acetone was added. The system in a test-tube was incubated with gentle shaking for 30 min at 30° C. The reaction was stopped by transferring the test-tube into an ice-acetone bath. Unreacted metepa was then extracted with 3 ml. of chloroform and dried over sodium sulphate. After re-extracting the aqueous phase and sodium sulphate with an additional 6 ml. fresh chloroform and readjusting the combined total volume to 10 ml. in a volumetric flask, 0·1 ml. of the final chloroform solution was transferred to another test-tube. The solvent was removed by means of a stream of dry nitrogen, and the phosphorus content of the remainder was assessed by microchemical analysis[12]. Standard samples with known amounts of metepa in the homogenate, as well as the colour controls, were used to determine the actual amount of metepa in those preparations. The remaining samples were also tested by means of paper chromatography[13] to ensure the purity of metepa in the chloroform solution. The result of six independent determinations of metepa breakdown at 1 mg/ml. of substrate concentration indicated that the resistant homogenate had almost three times as much breakdown capacity of metepa as the susceptible one, the breakdown rate being 37·5 ± 1·9 and 12·9 ± 3·6 μg/mg tissue/30 min respectively. The *t*-value between these two sets of data becomes 6·03, and, therefore, the interstrain difference may be considered as a statistically significant one. The rate of breakdown in both the homogenates, however, did not increase beyond these values when the substrate concentration was raised from 1 mg/ml. to 10 mg/ml. This could mean either (1) the enzyme system is saturated at 1 mg/ml., or (2) there could be active absorption or conjugation to eliminate the limited amount of substrate from the solution in both homogenates. In either case the resistant homogenates have a more efficient method of eliminating metepa at lower substrate levels; however, at high levels this inter-strain difference becomes less effective.

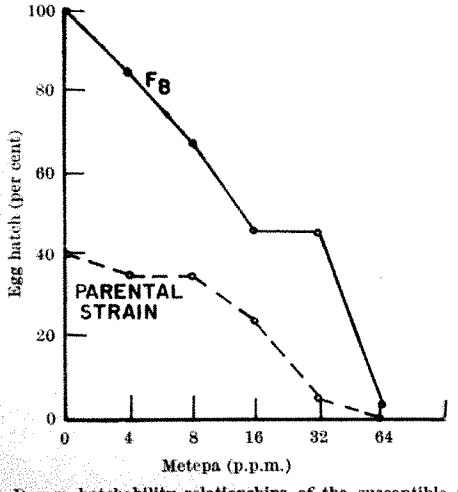


Fig. 1. Dosage–hatchability relationships of the susceptible parental and resistant F_8 strain eggs

Insect populations may respond to selection with every potentiality for resistance that is present within their gene pool. This strain seems to have responded (1) by laying only viable eggs, and (2) by developing an enzymatic degradation system and/or active elimination system for metepa in the body. The former factor seems to play a relatively important part, but the latter factor could also become very important where the selection pressure is very severe and the need of an efficient detoxication system becomes very critical. Our investigation indicates that a population of *Aedes aegypti* may respond to metepa and develop resistance within a few generations when treated at the larval stages. This does not exclude the possibility that field trials with other means of application would produce results entirely different from ours. Nevertheless, as a rule more genes and their combinatory probabilities are available in field populations than in laboratory colonies, and so caution should be applied when carrying out the field control programme, otherwise formidable resistance problems may have to be faced in the future.

We thank Prof. J. B. Kitzmiller for the use of his insectary and laboratory space. This investigation was in part supported by grants from Hatch Project 822 on Biologically Active Substances and from the U.S. Public Health Service, National Institutes of Health (grant *ES*–00067 and *E*–3486).

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VIROLOGY

Isolation of a Poxvirus from Stock Type 12 Adenovirus

Sweet and Hilleman[1] detected *SV40* in seed stocks of adenoviruses, types 1–7, and Gerber *et al.*[2] reported the presence of viable *SV40* in formalinized adenovirus vaccines. Yang and Melnick[3] described *SV40* contamination of both adenovirus stock and vaccine. Such contaminations were attributed to the *SV40* flora in many of the 'normal' monkey kidney cell cultures used for propagation of adenovirus. The report presented here describes the isolation of a poxvirus contaminant from a seed stock of adenovirus type 12. The poxvirus had been propagated by us in undetected form through many passages of the adenovirus in *HEp*-2 cells.

HEp-2 cell cultures, inoculated with stock adenovirus type 12, developed a cytopathogenic effect which was characterized by rounded and clumped cells and well-defined intranuclear inclusion bodies. Monkey kidney cell cultures, inoculated with the same undiluted adenovirus stock, developed a severe toxic reaction with marked cytopathogenic effect and intranuclear inclusion bodies; however, comparatively little infectious virus was produced. When the monkey kidney cell cultures were inoculated with the adenovirus diluted 1 : 10, plaques appeared in the cell sheet which resembled those of poxvirus. DNA-staining cytoplasmic inclusion bodies were observed by acridine orange fluorescence microscopy which were characteristic of those found in poxvirus-infected cells. In monkey kidney cell cultures inoculated with two-fold dilutions of the adenovirus stock (0·1 c.c.), the pox-like plaques appeared when the stock was diluted 1 : 4 or higher. The concentration of the poxvirus was $1{\cdot}6 \times 10^2$ plaque-forming units (P.F.U.) per ml. of adenovirus stock. The contaminating poxvirus (designated *TC*17) was detected in adenovirus stocks prepared on prior occasions from the same virus seed, as well as in the original vial of virus stock which was received from the custodian of the prototype virus. By diluting the adenovirus beyond its end-point in cultures of monkey kidney cells, the poxvirus contaminant was recovered free of adenovirus; and a pool of stock virus was prepared from this for further investigation.

When monolayers of *HEp*-2 cells were inoculated with *TC*17 virus, free of the adenovirus, plaques appeared and infectious virus was produced; however, the plaques appeared more slowly and were smaller and less easily detected than those produced in monolayers of monkey kidney cells. DNA-staining cytoplasmic inclusions were also observed in *HEp*-2 cells infected with *TC*17 virus and examined by acridine orange fluorescence microscopy.

Opaque white pocks were induced by *TC*17 on the chorioallantoic membrane of embryonated eggs. The surface of the pocks often appeared umbilicated. Three-week-old Swiss–Webster mice resisted intracerebral inoculation of 10^3 P.F.U. of *TC*17 virus; however, six of eight mice inoculated intracerebrally with 10^5 P.F.U. died 3 days after inoculation. Neither lesions nor symptoms were observed in mice inoculated intraperitoneally or intradermally with the virus. Rabbits developed a localized papule 2–3 days after intradermal inoculation of the poxvirus, and the lesions evolved through vesicle and pustule stages and were crusted over in 8–9 days.

While diethyl ether had no effect on the virus, it was inactivated by chloroform. Antiserum prepared against *TC*17 virus neutralized both vaccinia and monkeypox viruses, members of the same sub-group of poxviruses[4]. Rectangular or brick-shaped elementary bodies typical of poxviruses, were observed in the cytoplasm of infected cells by electron microscopy.

The biological and physical properties of the contaminating *TC*17 virus identify it with the poxvirus group; and, in view of its close serological relationship to vaccinia and monkeypox, it can be placed in the vaccinia-variola sub-group of poxviruses. This includes the viruses of monkeypox, ectromelia, cowpox, and contagious pustular dermatitis[4].

The discovery of poxvirus in the original stock of adenovirus type 12 and its inapparent but persistent propagations along with adenovirus in *HEp*-2 cells suggests the existence of an interference mechanism. Pereira[6] showed that adenovirus-infected HeLa cells produced a factor which inhibited multiplication of vaccinia virus in HeLa cells. Recent studies of Khoobyarian[7] have indicated that, under certain experimental conditions, the interaction of adenovirus with *RHF*-1 cells would result in cell resistance to vaccinia virus. It is conceivable that some similar mechanism, aided by the low concentration of the poxvirus, permitted it to exist undetected in the adenovirus stock. It is possible that a double infection resulting from the use of contaminated stocks could result in suppression of one, or its survival as an inapparent infection. The second virus would remain hidden until the conditions of propagation were changed in its favour. This may explain the emergence of poxvirus in monkey kidney cells.

The contaminating poxvirus may have originated from an occult infection in an earlier cell culture in which the adenovirus had been propagated or it may have resulted from a laboratory contamination prior to receipt in this laboratory.

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GENETICS

New Method of Isolating the Tetrads of Agarics

HITHERTO the usual technique for isolating tetrads from Agarics has been to detach a piece of gill tissue from a mature sporophore and then, with the aid of a micromanipulator, to pick off whole tetrads directly from the fresh gill. There are three main difficulties inherent in this method: (*a*) because of the high numerical density of tetrads on the gill, it is very difficult to be sure that no extraneous spores have been picked up by the micromanipulator needle until the spores are parked on the germination medium; (*b*) it is impossible to distinguish unripe from ripe tetrads, and since the former are removed from their basidia with great difficulty, many abortive attempts to pick up tetrads are frequently made before a tetrad is successfully removed; (*c*) in the case of *Coprinus*, autolysis of the gill can greatly limit the time available for isolation.

In the course of analysing tetrads from *Coprinus lagopus* (for the purpose of finding new centromere markers to be used for the extension of mapping) a new method has been developed. If a piece of gill tissue is taken from a mature sporophore, placed on a microscope slide and allowed to dry, the spores can be scattered on to the surrounding glass by sweeping the micromanipulator needle across the dried gill. Among the spores on the glass there is nearly always a high proportion of whole tetrads (Fig. 1), and these can very easily be removed from the

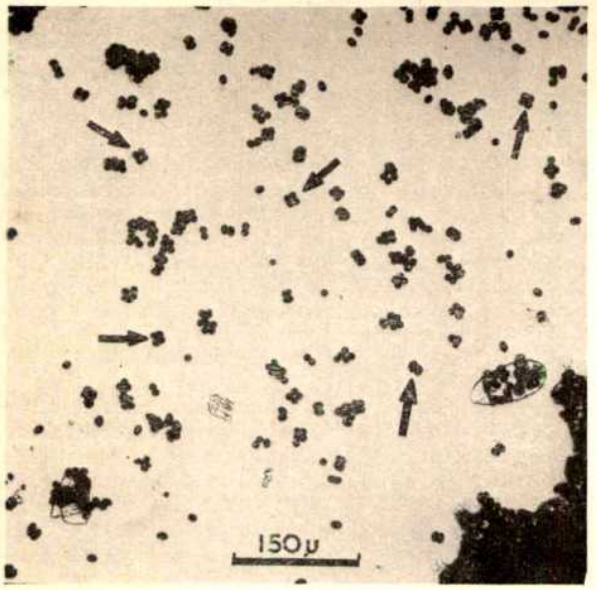


Fig. 1. Photomicrograph of spores scattered from dried gill. There are more than twenty tetrads in this field which are available for picking-off; five of them are arrowed as examples

glass. Drying may be hastened by placing the slide carrying the gill into a Petri dish containing a layer of dry silica gel.

During drying the four spores of each tetrad become aggregated into a single mass so that, when drying is complete, each tetrad tends to behave as a single unit. This presumably accounts for their easy removal as whole tetrads by this somewhat drastic method.

The great advantage of this 'on-glass' technique is that whole tetrads can be stored as such for long periods: in one case tetrads isolated four months after scattering still gave 85 per cent germination. Thus many tetrads can be isolated over a comparatively long period of time as and when it is convenient to do so, with the advantage that all originate from the same fruiting body. Slides carrying scattered spores are best stored over silica gel at room temperature.

That the on-glass technique still provides a random sample of tetrads was demonstrated by a cross in which 104 tetrads were isolated from fresh gill using the old method, and a further 49 isolated from glass. Both sets of data were completely homogeneous.

I thank Prof. N. F. Robertson for providing facilities, and both he and Dr. D. H. Morgan for their continued help and encouragement. This work was performed during the tenure of a D.S.I.R. research studentship.

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AGRICULTURE

Carbon- and Nitrogen-levels of a Medium in Relation to Growth and Nematophagous Activity of *Arthrobotrys oligospora* Fresenius

During the past few years, attempts have been made to enhance the nematode-trapping activity of nematophagous fungi in the soil by adding various carbohydrates[1,2]. As a preliminary investigation to similar experiments, we cultured *Arthrobotrys oligospora* and measured its growth and nematophagous activity on semi-synthetic media having different levels of carbon and nitrogen.

The basic medium had the following ingredients: dipotassium phosphate, 1·0 g; magnesium sulphate, 0·5 g; potassium chloride, 0·5 g; ferrous sulphate, 0·01 g; yeast extract, 3·0 g; agar, 15·0 g; and distilled water to make 1 l. of medium. Dextrose and ammonium nitrate were added to this medium at the rates shown in Table 1. Eight replicate Petri dishes of each medium were inoculated with a culture of *A. oligospora*, which had been growing on Lima bean agar.

After 2 days of incubation at room temperature, the fungal colonies were about 40 mm in diameter. A standard number of free-living nematodes (*Rhabditis* (*Cephaloboides*) *oxycerca* de Man. 1895), which had been surface sterilized in a 1 : 10,000 solution of mercuric chloride and washed thoroughly with sterile water, was then added to each of four of the original eight replicate cultures on each medium. The nematodes were allowed to be trapped for 1 week. The remaining four replicate cultures on each medium were grown for an additional 3 days, after which the diameters of the fungal colonies were measured.

The effect of the levels of dextrose and of ammonium nitrate of the medium on the nematode-trapping activity of *A. oligospora* was determined by transferring the contents of the plates to Baermann funnels and counting the extracted nematodes the next day. The number of nematodes recovered is assumed to be inversely proportional to the number trapped by the fungus.

Table 1. Effect of Carbon- and Nitrogen-levels on Growth and Nematophagous Activity of *Arthrobotrys oligospora*

Carbon- and nitrogen-levels (mg/l.)		Diameter of colony (mm)		Nematodes recovered	
Dextrose	Ammonium nitrate	Mean*	S.D.	Mean†	S.D.
50	5	80	2·6	630	41·3
100	10	78	1·8	711	88·7
200	20	81	0·3	197	64·6
400	40	72	0·2	177	18·9

* Mean of four colony diameters.
† Mean of six counts.

Media low in dextrose and ammonium nitrate supported less nematophagous activity of *A. oligospora* than media containing higher concentrations of these compounds (Table 1). As there was little difference in the amount of radial growth made by the fungus on these media, the effect of the carbon- and nitrogen-levels is assumed to have been on its nematode-trapping activity. The sharp decline in nematophagous activity at a level of dextrose and of ammonium nitrate between 100–200 and 10–20 mg/l., respectively, suggests the existence of a critical point or range in nutritional levels which determines a saprophytic or parasitic mode of existence for the fungus.

These observations cast some doubt on the validity of Duddington's[3] suggestion that nematophagous fungi may be most actively parasitic when grown on media of low nutritional value. Rather, these findings support Cooke's[2] view that nematode-trapping fungi need an organic energy source, other than nematodes, in order to remain in an active nematophagous state. The results of unpublished greenhouse investigations[4] indicate that small quantities of dextrose and ammonium nitrate added to soil enhance the nematode-trapping activity of *A. oligospora*.

This work was financed by the National Research Council of Canada.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 14

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. A. Waldron: "The Explanation of Some Fundamental Phenomena of Modern Physics using a Ballistic Theory of Light".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. S. C. Brown "The Ten-Element Band-Pass Filter Section and Its Applications".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Sky Above, the Mud Below" (colour film).

Monday, March 14—Friday, March 18

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Great Hall of Alexandra Palace, London, N.22)—Eighteenth Technical Exhibition.*

Tuesday, March 15

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C 2), at 5 p.m.—Prof. L. W. Martin "Problems of Nuclear Strategy" (further lecture on March 17).*

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in Lecture Theatre No. 2, Physics Department, Imperial College, London, S.W.7), at 5.30 p.m —Prof. J. C. Shepherdson. "Can Mathematical Proof be Mechanized?"

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. J. C. Anderson "The Science of Materials" (Inaugural Lecture).*

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. C. G. Bernhard (Stockholm). "The Corneal Surface Formation of the Compound Eye and Its Function".*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31-34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. J. Mellaart: "Excavations at Catal Huyuk, 1965".*

Tuesday, March 15—Wednesday, March 16

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "The Planning of Mechanical Engineering Departments in Universities and Colleges of Technology".

PLASTICS INSTITUTE (at the Institution of Electrical Engineers, Savoy Place, London, W.C 2)—Conference on "Flame Resistant Polymers".

Wednesday, March 16

BRITISH POSTGRADUATE MEDICAL FEDERATION, UNIVERSITY OF LONDON (at the Institute of Dental Surgery, Eastman Dental Hospital, Gray's Inn Road, London, W.C.1), at 5.30 p.m.—Prof. D. Slome: "Physiology of Swallowing". (Last of three lectures on "The Scientific Basis of Dentistry".)*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. H. T. Gooding. "Cable Fault Localization on Power Systems".

INSTITUTE OF INFORMATION SCIENTISTS (at The Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. L. Gordon: *"The Economist* Intelligence Unit".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Manson Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 6 30 p.m.—Discussion Meeting on "Technician Training".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Overseas League, Park Place, London, S.W.1), at 6.30 p.m.—Mr. D. Munday. "Costing".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "The Determination of Molecular Weights".

Thursday, March 17

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Meeting for the Election of Fellows. 4.30 p m.—Prof. F. Sondheimer "The Annulenes" (Review Lecture).

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. B. C. Sharman, Miss H. P. Aziz and Miss J. C. Miller. "Developmental Morphology and Anatomy of the Shoot in Cereals, with special reference to Wheat (*Triticum*)".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. H. P. F. Swinnerton-Dyer: "Zeta Functions and Algebraic Geometry".

UNIVERSITY OF LONDON (at Goldsmiths' College, New Cross, London, S E.14), at 5 p m.—Prof R. J. Blin-Stoyle: "The Physical Sciences Today".*

UNIVERSITY OF LONDON (at Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Dr. D. G. Gibson: "Sodium Excretion in Chronic Renal Failure".*

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. Christopher Tugendhat: "Co-ordinating Energy Policy".

CHEMICAL SOCIETY (in the Large Physics Theatre, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. R. M. Barrer, F.R.S. "Some Chemical Aspects of Mineral Synthesis" (Hugo Muller Lecture).

INSTITUTE OF REFRIGERATION (at Lloyds' Register of Shipping, 71 Fenchurch Street, London, E.C.3), at 6 p.m.—Informal Discussion on "Education and Training for Refrigeration Technicians".

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Reactor Maintenance and Inspection".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Mr. D. A. Cahal: "The Assessment of Safety of Drugs".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the Royal Army Medical College, Millbank, London, S.W.1), at 7.30 p.m.—Laboratory Meeting.

Friday, March 18

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 10 a.m.—Dr. D. Burkitt (Makerere): "Recent Developments in Aetiology and Treatment of African Lymphoma".*

INSTITUTE OF NAVIGATION (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 5.30 p.m.—Mr. C. R. Ditchfield: "Microwave Radiometry".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.7), at 7 p.m.—Conversazione.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. L. F. Boyd: "Mission to Mars".

Saturday, March 19

BRITISH INSTITUTE OF RADIOLOGY (at the Middlesex Hospital Medical School, Cleveland Street, London, W 1), at 10 a.m.—Symposium on "Radioisotopes in the Hospital—Applications in Clinical Investigation and Diagnosis".

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr Audrey Butt. "Shamanism—The Techniques and Function of Spirit Contact".*

Monday, March 21

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Discussion Meeting on "Isolation, Identification and Estimation of Organophosphorus Pesticides Residues".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p m.—Mr. H. N. G. King: "Electron Beam Welding and Machining".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Brittle Fracture".

SOCIETY FOR VISITING SCIENTISTS (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7 30 p m.—Discussion Meeting on "The Shape of Ships to Come". Chairman: The Right Hon Lord Geddes, C.B.E. Speakers: Mr. A. Silverleaf, Mr C. S. Cockerell and Sir William Lithgow.

Monday, March 21—Tuesday, March 22

ROYAL PHOTOGRAPHIC SOCIETY OF GREAT BRITAIN (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Symposium on "Light Sources".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

LECTURER (Grade II) IN PURE MATHEMATICS—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (March 14).

ASSISTANT LECTURER (with interests in sedimentology or palaeoecology) IN THE DEPARTMENT OF GEOLOGY—The Secretary, The University, Edinburgh (March 16).

LECTURER IN MECHANICAL ENGINEERING IN THE THERMODYNAMICS AND FLUID MECHANICS DIVISION OF THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester, 1 (March 19).

RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICS for research on aspects of far infra-red instrumentation—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (March 19).

LECTURER or ASSISTANT LECTURER (with a good honours degree in electrical engineering or an honours diploma in technology) IN THE DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. EED (March 21).

POSTDOCTORAL RESEARCH FELLOW (preferably with some familiarity with solid state theory) IN THE DEPARTMENT OF APPLIED MATHEMATICS AND MATHEMATICAL PHYSICS, to work on the theory of semiconductor lasers and to establish contact with government research establishments working in this area—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff, South Wales (March 21).

ASSISTANT IN RESEARCH (with an honours degree in engineering or an appropriate science, or a postgraduate qualification in agricultural engineering or farm mechanization) IN AGRICULTURAL ENGINEERING—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (March 23).

ASSISTANT LECTURER or LECTURER (competent to teach genetics at the undergraduate and postgraduate levels although research interests may be in some other field) IN ZOOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (March 25).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in one of the following fields: genetics, biochemistry, developmental biology and experimental morphology, embryology, mycology, entomology and experimental animal ecology) IN THE DEPARTMENT OF BIOLOGY—The Secretary, University of Lancaster, Bailrigg House, Lancaster (March 26).

LECTURER IN RADIOLOGY (joint appointment with the Northern Ireland Hospitals Authority)—The Secretary, The Queen's University, Belfast, Northern Ireland (March 26).

LECTURER or ASSISTANT LECTURER (with postgraduate experience in genetics and biometry) IN AGRICULTURAL BOTANY—The Registrar, University College of Wales, Aberystwyth (March 26).

JUNIOR RESEARCH FELLOW IN BIOLOGICAL SCIENCE, or related subjects—The Secretary, Balliol College, Oxford (March 28).

LECTURER IN GEOPHYSICS IN THE DEPARTMENT OF EARTH SCIENCES—The Registrar, The University, Leeds, 2 (March 28).

LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS—The Secretary of the Appointments Committee of the Faculty of Mathematics, University of Cambridge, Silver Street, Cambridge (March 30).

ASSOCIATE PROFESSOR OF BIOCHEMISTRY—Prof. G. E. Connell, Chairman, Department of Biochemistry, University of Toronto, Toronto 5, Ontario, Canada (March 31).

CHAIR IN STRUCTURAL ENGINEERING IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, Manchester College of Science and Technology, Manchester, 1 (March 31).

LECTURER (Grade II) IN THE DEPARTMENT OF THEORETICAL MECHANICS—The Registrar, University of Bristol, The Senate House, Bristol, 2 (March 31).

LECTURER (with special qualifications in the teaching of educational sociology, and preferably teaching experience in other subjects) IN EDUCATION—The Registrar, King's College (University of London), Strand, London, W C.2 (March 31).

RESEARCH ASSISTANT (with a degree or equivalent qualification in physics or applied science, and preferably experience in practical electronics) IN COSMIC RAY PHYSICS, to join a small group working on an experiment to detect cosmic ray neutrinos deep in the gold mines of Southern India—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 31).

UNIVERSITY LECTURER and a UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ANIMAL PATHOLOGY IN THE SCHOOL OF VETERINARY MEDICINE—T. D. Kellaway, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge (March 31).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (normally below the age of 30, and preferably with a Ph.D. or equivalent research experience) IN BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 1).

LECTURER (with a degree in geography and good experience in the teaching of geography) IN EDUCATION—The Deputy Secretary, The University, Southampton (April 1).

LECTURER (with a higher degree in biochemistry and some experience in teaching and research in this subject) IN BIOCHEMISTRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, April 1).

SECOND CHAIR IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Deputy Secretary, The University, Southampton (April 1).

SENIOR LECTURER IN PURE MATHEMATICS—The Registrar, The University, Hull (April 1).

CHAIR OF AGRICULTURAL ENGINEERING—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (April 2).

ASSISTANT LECTURER or LECTURER (preferably with a particular interest in spectroscopy or reaction kinetics) IN PHYSICAL CHEMISTRY; and an ASSISTANT LECTURER or LECTURER (preferably with a particular interest in stereochemistry or synthetic chemistry) IN ORGANIC CHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea (April 4)

LECTURER or ASSISTANT LECTURER (preferably with qualifications in biogeography, plants or soils) IN THE DEPARTMENT OF GEOGRAPHY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 5).

LECTURER or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (April 18).

CHAIR OF ELECTRICAL ENGINEERING; and CHAIR OF MECHANICAL ENGINEERING at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, April 30).

HEAD OF THE BIOLOGY DEPARTMENT—The Headmaster, Latymer Upper School, London, W.6.

HEAD (preferably specialist in botany or zoology) OF THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar, Goldsmith's College (University of London), New Cross, London, S.E.14.

LECTURER or SENIOR LECTURER (with a special interest in applied mathematics) IN MATHEMATICS—The Registrar, Goldsmith's College (University of London), New Cross, London, S.E.14.

LECTURER (with veterinary or biological qualifications) IN EXPERIMENTAL VETERINARY SCIENCE WITH DUTIES AS ANIMAL CURATOR—The Secretary, Postgraduate Medical School, Du Cane Road, London, W.12, quoting Ref. 11/1.

MASTER (with good qualifications in mathematics) TO SHARE THE VI FORM AND UNIVERSITY SCHOLARSHIP TEACHING IN THIS SUBJECT; and a MASTER TO TEACH PHYSICS IN THE MAIN SCHOOL AND IN THE VI FORM—The Head Master, Mill Hill School, London, N.W.7.

POSTDOCTORAL FELLOW IN RADIOFREQUENCY SPECTROSCOPY IN THE DEPARTMENT OF CHEMISTRY, for research in nuclear magnetic double resonance in collaboration with Dr. Edward W. Randall—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1.

RESEARCH ASSOCIATE (with a good first degree, a diploma in technology or Grad. R.I.C.) IN THE DEPARTMENT OF CHEMISTRY AND BIOLOGY, to investigate the surface properties of active carbons produced by low-temperature coal carbonization processes—The Principal, Nottingham Regional College of Technology, Burton Street, Nottingham.

SENIOR TECHNICIAN and CHIEF TECHNICIAN IN THE DEPARTMENT OF MEDICINE, Royal Veterinary College, for work associated with the clinical and laboratory investigation into conditions affecting farm animals—Prof. F. R. Bell, Royal Veterinary College (University of London), Hawkshead Lane, North Mimms, Hatfield, Herts.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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REALISM OR DISENCHANTMENT?

IS science an election issue, or should it be? The British general election, to be decided on March 31, has obliquely raised this question, for the appeal of the Labour Government to the electorate differs from that which the same body of men made eighteen months ago in its almost conspicuous neglect of science. In 1964 the Labour Party made a memorable, and even startling, declaration that it would harness science and technology to the causes of social and industrial change in such a way as to transform the United Kingdom. Now, only a little later, science and technology are mere second cousins—though, to be fair, not quite poor relations—of the familiar and time-worn ingredients of election manifestos. What has gone wrong? Has a mere eighteen months been enough to persuade the politicians that science does not work? Was the last election manifesto just an exercise in public relations? Or is it that elected governments are less able than parties seeking power to give hostages to fortune by identifying in public the mainsprings of change?

It would be a pity if the youthful brashness of the manifesto on which the present British Government was elected had now been entirely replaced by disillusionment. There have been disappointments, especially for the Government and its supporters, but there have been modest successes as well. The new machinery for financing research in universities is working well. Good ideas do not for long go begging for support. There is a comfortable feeling that the Science Research Council, which dispenses the money, and the Council on Scientific Policy, which advises the Minister of Education and Science, is taking prudent thought not merely for next year but for that following as well.

There is a sadder tale to tell of the Government's handling of technology, even if it may not be that tale of indecision and ineptitude which it is easiest to spin. The difficulty is not that the Ministry of Technology has been ineffectual, but that it has been asked to accomplish the impossible. The objective was to use science and technology with open eyes to modernize British industry and to increase enormously the prosperity of the British economy. The trouble is that science and technology are necessary but not sufficient ingredients of such a revolution.

This is why the Ministry of Technology has often seemed more like a home for vested interests than an instrument of government. Compelled as it has been by narrow terms of reference to find technological solutions to pressing problems, the Ministry has rediscovered a number of uncommonly old-fashioned remedies. Thus it justified the decision to support a massive programme of research and development in computers—not necessarily unwise—in language smacking of protection and the Corn Laws. It argued that the prosperity of Britain demands an independent and viable computer industry, when, on the face of things at least, it might have been wiser to use the money for training computer programmers, for buying imported machines, and for supporting research and development in the next great technological leap forward—whatever that may be. Similarly, in its quite proper attempt to awaken the United Kingdom to the importance of technology, the Ministry has often tended to exaggerate the curiously British gulf between science and technology. It has, for example, smiled uncritically on schemes for teaching a kind of engineering in British schools without recognizing that the difficulty of recruiting able men to engineering may spring from too much and not too little specialized instruction.

Elsewhere, the Ministry of Technology has done useful work. In attempting to stimulate innovation in industry by the judicious placing of development contracts with commercial and academic institutions, the Ministry has usefully elaborated a mechanism sketched out by the old Department of Scientific and Industrial Research. It has also found it to be much more difficult than expected sensibly to invest large sums of money in this way. The National Research and Development Corporation has begun to make use of its increased capital, but here, too, progress has been slower than expected. And there has been no sign, so far, that the Ministry has been able to persuade the Government as a whole to discriminate between the sheep and the goats in industry by deliberately giving its custom to the efficient companies. Indeed, now as when the election manifesto of 1964 appeared, there is no good reason to think that such a device could have more than a marginal effect in changing the complicated pattern of industry.

It is not, of course, surprising that achievement should so often have fallen short of expectation. British industry may be less advanced and less enterprising than it could be, but the reasons are not simply technological. The chronic shortage of investment capital frequently limits the growth for new enterprises, for example. Defects of administration and management are often as cramping as a failure fully to exploit the potentialities of modern technology. The lack of a common sense of the excitement of modern technology, and of its potentiality for innovation, induces British industry to aim at over-modest goals. There is no cause for hoping that the policies which the Ministry of Technology has been pursuing, whatever their merits in isolation, could overcome all these impediments to change.

This is why it would be foolish if British politicians were to allow the frustrations of the past few months to bury entirely their once-proud hopes for science and technology. In reality they—and their electors—can only benefit from a more measured view of what science and technology can accomplish in the modern world. A great deal needs to be done if governments everywhere are to make full use of these instruments of change. In Britain, there is a lot of tedious detail to be dealt with. The separation between science and technology in government needs to be looked at again, and ideally eliminated. Education needs more attention, and more money. The Government has to accustom itself to more money, and more imaginative support, for scientific research and technological development. Administrators must be won over. These, and the host of other jobs which must be tackled, will not be completed overnight. Science and technology is not a magic wand, but a part—and an

important part—of the fabric of modern society. It must not be dropped from the election manifestos, but given a sober place in them. In this sense it is a virtue that in Britain the manifestos make less of science and technology in 1966 than they did in 1964.

RATE FOR THE JOB

THE American Institute of Physics is uncommonly well informed about the jobs its members do, and about the salaries they are paid. The latest batch of figures, made public in *Physics Today* for January, will as usual comfort those struggling for a Ph.D. with the knowledge that their efforts (if successful) are likely to add something like 5,000 dollars to an annual salary in industrial research and development. In 1964 the median starting salary for Ph.D. physicists in industry appears to have been 12,600 dollars, compared with 8,800 dollars for those starting with a master's degree and 7,500 dollars straight after graduation. The initial value of a Ph.D. seems to be equivalent to ten years of plodding up the promotion ladder, and this advantage appears to persist throughout a physicist's career. Whatever his length of service, a Ph.D. can expect to earn 4,000 dollars more each year in industrial research in the United States than somebody less qualified. But the same industrial jobs are curiously unprogressive. The median salary of those with a Ph.D. and twenty years experience (in 1964) was a mere 18,200 dollars, or not quite half as much again as the salary paid to raw recruits. To do better than this, a man must move into what the American Institute of Physics calls "management and administration", which brings an extra 6,000 dollars or so a year to those with long experience. By contrast, academic life offers a lower starting salary but faster promotion; after twenty years, the median salary of academics is 16,600 dollars, or roughly 90 per cent of the comparable salary in industry.

The sociology of physics described by these statistics will allow students to know what to expect. The more mercenary young people may even be tempted to let their academic interests be guided by the knowledge that fluid physics and solid-state physics carried the highest salaries in 1964, and that work on fundamental particles was the most impoverished specialty of all except for theoretical physics. But there are other inferences to be made. High starting salaries and slow promotions reflect the competition for young people leaving universities and the comparative rapidity with which their bargaining power diminishes with age. The profession of physics has something in common with professional football, where a man must reckon that his earning power will disappear altogether by forty. This suggests that university departments might deliberately set out to provide their students with the means of moving out of research into administration half-way through an industrial career, possibly by coupling academic instruction with more deliberate training in the techniques of management and administration. Something like a union of the Massachusetts Institute of Technology and the Harvard Business School might be a fruitful innovation. Meanwhile potential immigrants to the United States will note that starting salaries in physics will not maintain a second car except where the other costs of living are exceptionally low. And physicists sticking to their last should not expect to move into the Waldorf-Astoria for what are called the sunset years.

PEACEFUL USES OF ATOMIC ENERGY

Proceedings of the Third International Conference on the Peaceful Uses of Atomic Energy

Held in Geneva, August 31–September 9, 1964. Vol. 1: Progress in Atomic Energy. Pp. xxxii+477. (New York: United Nations, 1965.) 12.50 dollars.

THE record of the first International Conference on the Peaceful Uses of Atomic Energy, 1955, was published in sixteen volumes; the record of the 1958 Conference grew to forty volumes and many who went to Geneva that year felt that a useful limit had, in fact, been exceeded; six years elapsed before the third Conference, held under the presidency of Prof. Emelyanov, and this 1964 meeting was confined almost entirely to nuclear reactor technology, which has nearly grown to manhood in the 25 years since fission was discovered. The record of the conference has shrunk once more to sixteen volumes embracing 750 papers. In addition to reactor technology, review papers were given on the application of radioactive tracer elements and of large radiation sources to the physical and life sciences. These brought the reactor specialists up to date in the wider applications of atomic energy, and the session on fusion research recounted the progress of this "promise of the twenty-first century" since it received its copious baptism in the 1958 Conference.

The first volume of the *Proceedings of the Third International Conference on the Peaceful Uses of Atomic Energy* embraces the opening and closing addresses, economic studies of the world's needs for energy and the extent to which these might be supplied by nuclear power, some papers on nuclear projects which have been handled on an international collaborative basis, a very important group of papers on technical and economic aspects of reactors, and finally an evening lecture delivered by Prof. Seaborg, in which he summarized the Conference.

As the president said in his opening remarks, "The question to-day is not whether nuclear fission can be regarded as a practical source of power, the question to-day is how to extract this energy as cheaply as possible, and increase its importance in the energy balance sheet". We have proved reserves of uranium for several hundreds of years, and if we go on using organic fuels for power generation, the chemical industry will soon be deprived of this most important source of raw material (his countryman, Mendelejeff, also said this very strongly in 1876, but then there was no alternative fuel in sight). How soon the organic fuels will run out cannot accurately be stated, but a century seems to be the upper limit of time. How cheaply nuclear power can be produced on a very large scale is the subject of several papers.

The British and Canadian reactors have been characterized by high capital costs and low fuel costs, the American by lower capital costs and higher fuel costs, so the former have been particularly affected by the upward swing in interest rates in recent years. Capital costs of all reactors have fallen dramatically with increase in unit size. It has also been found possible to up-rate many reactors, and fuel costs have fallen, with the overall result that new British and American reactors are becoming competitive with modern coal stations. Within a year of the end of the Geneva Conference, the commercial form of the *AGR* Reactor offered to the Central Electricity Generating Board for base-load operation gives the appearance of producing electric power more cheaply than could be produced by coal stations built at the pithead to operate about 1970, while even in 1964 the American 600-MW reactor to be built at Oyster Creek also appeared to be—on rather favourable calculations—cheaper than a coal station at that site. It is interesting to read that in the United States "whether nuclear reactor break-through in costs will be permanent depends as much on actions of competing fuels as on improvements

in the nuclear art; costs of fossil fuels have been declining under the impetus of competition". The Canadian costs forecast for the next large heavy-water reactor are based on a very low fuel cost and much cheaper heavy-water, and they appear to rival American costs per unit of electricity generated, but exact comparisons are difficult; as Prof. Seaborg said in his summary, greater efforts towards standardization of the bases for computing nuclear power costs would probably have been welcomed in the Conference. It must also be remembered that computed costs must be related to system performance: thus, the cost of taking a reactor off the system for a few weeks in order to change its fuel in the middle of the high winter peak load would depend on the spare capacity available, and we read that the overall gross margin in the United States was 27 per cent in 1963, far higher than the margin available in Great Britain. Several papers deal with costs overseas, such as in India, but these cannot easily be related directly back to the Canadian or American costs, as the stations are of smaller output though of later date; both types, however, appear to be cheaper than the United Kingdom gas-cooled types.

The generation of reactors using natural or slightly enriched uranium now being planned leads smoothly into the 1980's. By that date the several larger breeder reactors now being designed should be operating and should be yielding realistic estimates of future capital costs and fuel costs; it should also be possible to assess the impact of the whole fuel cycle on costs for advanced converter reactors. Before that date, large quantities of plutonium will have been made and decisions will be required about its processing. The Canadian authors suggest that as the uranium-oxide cost is not a very important component of the cost of electricity, particularly in the kind of reactor developed in Canada, poorer sources of uranium ores could be used; even sea water could supply the uranium oxide at only five times the price of the cheapest fuel now mined and, on that basis, there is fuel for centuries: one might thus avoid the development of fast reactors. Another development which will be well investigated in the next decade is the advanced form of converter reactor which economizes in neutron losses and so extracts energy from a few per cent of the heavy uranium isotope. By 1980 many of these matters will be resolved and there are so many variants involved that there is little fear of true duplication occurring in different countries. Prof. Perrin, however, who was president in 1958, sounded a word of warning this time; so much information about power stations and their economic assessment is being kept secret for commercial reasons that this could be very prejudicial to international co-operation.

There are several accounts of adventures being undertaken on a collaborative basis between two or more nations; the *Dragon*, *Vulcain*, and other reactors are described, and Prof. Smyth reviews the development and work of the International Atomic Energy Authority, which is striving for full accountancy of fissile materials in power reactors in the interests of peace. In the 1955 Conference, contributors from very many nations hopefully looked forward to atomic energy to solve their fuel problems; the 1958 Conference showed that there was a long haul ahead; the 1964 Conference is full of optimism again, but as Emelyanov said in his closing remarks, "This is based on a great confidence that nuclear will be the cheapest source of energy" and, indeed, the sole source capable of meeting the requirements of the world.

Prof. Seaborg also spoke of some of the exciting new applications for reactors, such as desalination of sea water ("What is a source of interest to-day could become to-morrow's necessity"), and briefly touched on the few papers given in the short session devoted to fusion research, "one of the most difficult scientific and engineering problems ever encountered". Advances since 1958 have been made in predicting plasma behaviour with good agreement between theory and experiment, in contrast to the situation which prevailed 6 years ago, but "we cannot be sure that fusion can be developed": whether the £30 million per annum being spent on this subject throughout the world is enough just now was questioned.

The volume is full of an immense amount of information well compressed and will be a valuable source book for several years to come. T. E. ALLIBONE

THE NEWTONIAN REVOLUTION

Newtonian Studies

By Alexandre Koyré. Pp. viii + 288. (London: Chapman and Hall, Ltd., 1965.) 50*s*. net.

ALEXANDRE KOYRÉ, who died in Paris in April 1964, started his career as a philosopher, with a particular interest in the philosophy of religion, but later developed a special taste for the history and philosophy of science. On the religious side, he wrote mainly on St. Anselm, various German scholars and mystics of around the sixteenth century, and Descartes. On the scientific side, he dealt mostly with the period which began with Copernicus and ended with Newton. In both contexts, Koyré encountered the work of Descartes, on whom he became a leading authority. At the time of his death, he was collaborating with Prof. I. B. Cohen of Harvard on a critical edition of the Latin text of Newton's *Principia*.

Newtonian Studies contains seven essays, of which six have previously been published in periodicals; three remain in English, and three which were originally published in French are now translated into English. The titles of the six are: "The Significance of the Newtonian Synthesis"; "Concept and Experience in Newton's Scientific Thought"; "Newton, Galileo, and Plato"; "An Unpublished Letter of Robert Hooke to Isaac Newton"; "Newton's 'Regulae Philosophandi'"; and "Attraction, Newton, and Cotes".

However, more than half the book is devoted to a new essay on Newton and Descartes, with no less than thirteen appendixes. Prof. Koyré's excellent qualifications to write on Newton and Descartes will be evident from the brief sketch of his activities given here.

The new essay deals largely with gravitation, as also do several of the appendixes and reprinted essays. The views of Newton and Descartes, and of various other scientists from Copernicus to Leibniz and Samuel Clarke, are discussed on the basis of numerous quoted passages, usually translated into English when necessary, except when the argument turns on niceties of the original language (mostly Latin). This process brings out very clearly the extraordinary strength of the widespread dissatisfaction with the mere use of the inverse-square law as an instrument of calculation.

Newton's mathematical powers, and especially his formidable ability to solve problems without running into error, far exceeded those of any of his contemporaries, and he had discovered an applied mathematical fact of fundamental importance. To us it seems natural that he should confess that he did not understand why planets and satellites should have inverse-square accelerations. Nearly three centuries later, we can see how difficult it is to go beyond what Newton had discovered. The modern worker in astronomical and astronautical dynamics rarely asserts or denies action at a distance explicitly; he merely computes with differential equations which can scarcely fail to encourage him to think habitually in terms of action at a distance for practical purposes. He does so as a first approximation, and is prepared to apply corrections based on Einstein's general theory of relativity in the rare cases in which the corrections are appreciable.

But any empirical procedure seems to have been widely regarded in Newton's time with great repugnance. Desper-

ate indeed were the attempts to explain gravitation by means of mechanisms, often belonging in a general way to the same order of ideas as the vortices proposed earlier by Descartes.

While gravitation bulks largest in Prof. Koyré's book, other Newtonian fields (optics and theology, for example) are by no means neglected. The reader should not be put off by occasional passages in which a nervous style becomes amusing in its qualifications (see, for example, the long sentence on p. 4 which begins "Besides, all of us, or if not all still most of us, have been born and bred —or better and more exactly, not *born* (as this is impossible) but only *bred*—in the Newtonian or, at least, a semi-Newtonian world . . ."). The book is valuable both as a compendium of information (including its numerous references and its many quoted passages made more conveniently accessible) and as an acute discussion of the ideas of Newton and his various adversaries, critics and apologists.

The book is well produced, the printing is excellent, with few misprints, and there is a useful index. I should have welcomed somewhat larger type, especially since the footnotes, which may be described as never occupying more than 90 per cent of the page, are crammed with detail and often call for careful reading. A. Fletcher

FREUD WRITES TO HIS FRIEND

A Psycho-Analytic Dialogue

The Letters of Sigmund Freud and Karl Abraham, 1907–1926. Edited by Hilda C. Abraham and Ernst L. Freud. Translated by Bernard Marsh and Hilda C. Abraham. (The International Psycho-Analytical Library, No. 68.) Pp. xvii+406. (London: The Hogarth Press, and the Institute of Psycho-Analysis, 1965.) 63*s*. net.

A PSYCHO-ANALYTIC DIALOGUE is an interesting book for those wishing to learn of the earlier happenings in psycho-analysis and the personality of the master and his disciples. It consists of letters from Freud to Abraham over 19 years, translated by two different translators in order to give a feeling of individual style. This has been done so well that the impression is of the originals not having been written in a foreign language at all.

If a science consists of the collection of facts, their examination and the meaning deduced from them, and the prediction of further facts as a result, the psycho-analysis can claim to be a science, or at least the beginning of one. The only thing which it lacks is measurement, which must depend on better means of estimating emotion.

Freud and Abraham spent long hours every day in clinical work, in listening to patients talking without guidance in free association. Indeed, Freud says in one of his letters that he has been doing so for 11 hours a day (page 143) and Abraham for nearly as long. Surely this must be regarded as similar to the collection of facts in a laboratory? The formulation of theories to explain what was found led to the body of ideas which is the basis of psycho-analysis to-day.

The discovery of methods to investigate the mind, leading sometimes to cure of the disease, and the potential fields revealed naturally attracted others who were eager to theorize without the trouble of clinical work. This would have led to a distortion of what Freud felt was the basis of his work and naturally he set his face against it. In his letters to Abraham he sometimes releases his irritation for those intruders and expresses his weariness. (This is not surprising when one learns that he was writing books, such as *Totem and Taboo*, as well as seeing patients all day.)

Freud has sometimes been accused of intrigue, although this seems to be contrary to his personality, but in these letters, when dissidents like Adler, Stekel and Jung are mentioned, he does not plot against them, except only inasmuch as he wishes them to leave the movement. Sometimes his language showed his irritation as when he writes of "the brutal sanctimonious Jung", but his efforts are always towards shedding those who will not conform to clinical findings, not their injury.

It would be absurd to pretend that there is a great deal which is very profound in these letters. They are the exchange of comment between two men, one twenty years senior, who are interested in the same work and the same movement. Yet they seem to throw a light on the sort of people they were and the respect which they felt for each other.

They begin with the exchange of letters devoted purely to analytical matters and end on a much more affectionate note, as if Freud had adopted Abraham as a most intimate friend, and Abraham had reciprocated. All through the correspondence there is this friendly note, but Abraham was by no means a mere affirmative to Freud. Indeed, he disagreed strongly in such matters as the personality of Jung and was eventually proved correct in his estimate. A great deal of the contents of Freud's letters has found its way into the various histories of psycho-analysis, but those interested in the original statements will find it fascinating to know what was occurring and said at the time. No one will learn complete psycho-analytical theory from them (although some can be gleaned), but as a study of two eminent men they are of great interest.

C. Allen

BRAIN AND SELF

The Brain and the Unity of Conscious Experience

(The Nineteenth Arthur Stanley Eddington Memorial Lecture, 15 October, 1965.) By Sir John Eccles. Pp. 45. (London: Cambridge University Press, 1965.) 4*s*. 6*d*. net; 95 cents.

SIR JOHN ECCLES's Eddington Lecture for 1965 entitled *The Brain and the Unity of Conscious Experience* is a most distinguished addition to the series. Three-quarters of it is devoted to brilliant and penetrating survey of the latest neurophysiological evidence linking neuronal activity with the existence of conscious states. Much of the work quoted is as yet unpublished, and very important new advances by Moruzzi, Mountcastle, Libet, Myers and Bremer are outlined. Perhaps particularly outstanding is the recent work of Libet and his colleagues, who have utilized the responses of the somaesthetic area in an attempt to discover the nature of the neuronal activity that leads to conscious experience. It was found that with all conditions of threshold stimulation there is a delay of at least 0·5 sec before the onset of the experienced sensation—a sort of 'incubation period' of a conscious experience at threshold level. In the visual system also, there is evidence that at least 0·2 sec of cortical activity is required before the first threshold flash of light can be detected. There is also an excellent account of Sperry's work on the experiences and behaviour of human split-brain patients. This condition in these patients results from the operation of severing the corpus callosum, the anterior commissure and the massa intermedia—an operation which has been highly successful in some cases of severe and long-standing epilepsy.

The final part of the lecture considers whether the uniqueness of the experiencing self derives from genetic uniqueness. Here, Sir John goes back to some little-known, but important, speculations by the eminent American biologist, H. S. Jennings, published in 1930. Since identical twins have identical gene combinations he concludes that these combinations must obviously be compatible with distinctiveness of experiencing selves. He thus concludes that the uniqueness of the individual self cannot be due solely to the uniqueness of the individual gene combination. He further concludes, by what appears to be sound reasoning, that neither can the

accumulated experiences of a lifetime be invoked as the determining or generating factor of the unique self, though naturally they will enormously modify all the qualities and features of that self. He goes on to say that "it is important to recognize that in the first instance this question of the relation of a self to gene combinations can be asked only by an experiencing self of its own existence. For example, I can ask it in relation to my own self, and I reply that I must face up to the problems of my own personal existence as an experiencing self that is dependent on the functioning of a brain, which I try to understand as a biological mechanism; and that my brain has had a biological origin as a consequence of a gene combination and the ensuing embryological development. My experiencing self is the only reality I know by direct apprehension—all else is second-order or derivative reality. The arguments presented by Jennings preclude me from believing that my experiencing self has an existence that merely is derivative from my brain with its biological origin, and with its development under instructions derived from my genetic inheritance. Nor do I believe with the physicalists that my conscious experiences are nothing but the operation of the physiological mechanisms of my brain. It may be noted in passing that this extraordinary belief cannot be accommodated to the fact that only a minute amount of cortical activity finds expression in conscious experience. Contrary to this physicalist creed, I believe that the prime reality of my experiencing self cannot with propriety be identified with some aspects of its experiences and its imaginings—such as brains and neurones and nerve impulses and even complex spatio-temporal patterns of impulses. The evidence presented in this lecture shows that these events in the material world are a necessary but not sufficient cause for conscious experiences and for my consciously experiencing self".

W. H. Thorpe

DIET AND DENTAL DISEASE

Nutrition and Caries-prevention

Edited by Gunnar Blix. (Symposia of the Swedish Nutrition Foundation, No. 3.) Pp. 130. (Stockholm: Almqvist and Wiksell, 1965.) 35 Sw.kr.

Caries-resistant Teeth

Edited by G. E. W. Wolstenholme and Maeve O'Connor. (Ciba Foundation Symposium.) Pp. xii+338. (London: J. and A. Churchill, Ltd., 1965.) 60*s*.

THE notion that the form and structure of the teeth, and consequently their resistance to disease, might be influenced by the nature of the diet has long been one that has had many attractions for clinicians and research workers alike. With the familiar examples of pathological changes in bone and other tissues resulting from prenatal and postnatal variations in nutrition, it has seemed natural to assume that the principal dental disease, dental caries, might also be related to dietetic factors. This, of course, has proved to be the case, but not quite in the way expected by some of the earlier researchers. They had felt that nutritional deficiencies might well produce structural defects in the developing teeth of such a kind that susceptibility to caries would be increased. This would be comparable to the increased susceptibility to disease, for example tuberculosis, that is shown by other tissues under conditions of dietary deficiency. However, there is much evidence to show that perfect tooth structure is not essential for resistance to caries and, indeed, life-long immunity to the disease may be exhibited in the presence of comparatively gross structural defects, and in conditions of severe malnutrition. In fact, caries is more prevalent in well-nourished individuals, and is particularly a disease of populations whose standards of living are relatively high. Thus we have what once would have seemed a completely paradoxical state of affairs, that those most susceptible to disease appear to be those who are best nourished. However, the light shed by geographical pathology and epidemiological methods on the incidence of cardiovascular disease and its relationship to diet, on periodontal disease as well as on caries and on other conditions, is bringing home to us, though expressed nowadays in modern and more scientific terminology, the truth of our forefathers' belief that one can dig one's grave with one's teeth.

In *Nutrition and Caries-prevention*, the whole subject of the influence of the diet on caries-susceptibility and resistance is very fully discussed. The book is the record of a symposium sponsored by the Swedish Nutrition Foundation, the range of topics covering practically every aspect of the relationship of diet to caries, including the effect of general nutrition on tooth formation and on the saliva and the oral flora, the local effects of nutrition, the effect of additives to foods and the effects of fluorides both in foods and in water.

The Ciba Foundation's *Caries-resistant Teeth* is another symposium, with a closely related content. Here again, the tantalizing question of individual resistance and susceptibility to disease is posed. Why do the teeth of some people suffer from rapid decay, while those of others, living under apparently identical conditions, seem to be extremely resistant? The effect of diet is discussed, though not of course as extensively as in the previous volume. Other topics dealt with include the role of heredity in caries resistance, the physical and chemical constitution of caries-resistant teeth, including their ultrastructure, the effect of trace elements, the role of bacteria and of saliva, and caries resistance in experimental animals.

In both symposia the participants are experts of international reputation. The papers are well documented and full references are given. The discussion following each communication is also reported. The final result is that these two volumes, though quite independently produced, are in fact complementary, and together give an up-to-date, comprehensive and authoritative account of resistance and susceptibility to the commonest chronic disease of man.

R. B. Lucas

"FRESH AND DRAMATIC"

Research Problems in Biology

Investigations for Students. Series 1. Pp. xxxiii+232. Series 2: Pp. xxviii+240. (Prepared under the direction of the American Institute of Biological Sciences—Biological Sciences Curriculum Study.) (London: George G. Harrap and Co., Ltd., 1965.) 16*s*. each volume.

THE biological world is watching the birth pangs of a new stage in school science. If the parents of this scion, so to speak, are the mid-century centres of biological interest and a coolness towards didactic methods, then the midwife is the pressure of practical developments over the whole range of medicine, agriculture, human biology and a considerable proportion of industry. The new concepts growing out of the interaction of all scientific disciplines rest on the labours of our nineteenth-century post-Darwinian scientific ancestors, and have perhaps few but significant origins in the past half-century. DNA was recognized in 1889, Tansleian ecology emerged in the first 20 years of this century, Mendelism only became current coin after 1900 and electron microscopy since about 1940.

It is against the background of these events that the new teaching has to be evaluated, and since 1958 there have been serious attacks on both the contents of courses and the ways of conducting them. What should schools do? Their responsibility for preparing children for adult life forces them to view science as but one of a number of factors concerned. A fundamental problem is to decide

the relative merits of the various subjects in this preparation and then to give a suitable amount of time and resources to each. At the present time, therefore, we would do well to ask ourselves what are the special qualities that the study of science possesses as compared with classics, languages, mathematics, history and so on. In any case, unless a certain level of scholarship is attained and maintained, and in the widest sense of the term, no subject is of lasting value to the child. We do not start our quest for a new sense of direction and purpose without being the victims of previous educational evolution. There exists an army of teachers of varying ages and experiences as well as laboratories and their equipment, examination boards, and places of higher education, and all combine to form the ultimate source of the scientists the nation needs.

Probably the most comprehensive definite steps taken so far to deal with school biology have been the well-known Biological Sciences Curriculum Studies in the United States. We have had the plan of these before us for some three years and the final editions of the books in the scheme are coming to us now, including the two on *Research Problems in Biology* (*Investigations for Schools*). Our United States colleagues brought much wisdom to their great scheme. They realized that there was a growing gap between the new pattern in biology and the traditional teaching in the schools, and set out to bridge it. At the same time they saw that there was not just one way to present the new ideas. They proposed, in their own words, a "fresh and dramatic approach" based on the view of science as enquiry. In Britain we cannot use the United States works exactly as they stand, partly because they employ American examples, partly because our grammar school system is not the same as their high schools, and partly because university entry needs differ. Thus sooner or later the problem has to be tackled in Britain and the main agency is the Nuffield Foundation's School Science Project. All are naturally watching its activities with sympathy and hoping that its eventual plans will be a useful basis for some of our future work.

It is apt to suggest what might be some of the principles to be borne in mind, for only so can one judge the direction affairs take and their relation to the United States effort. Hitherto, one has worked to a published syllabus and been left free to cover the list of subjects in any way one wished. In order that one's pupils, however, should pass in the G.C.E. examinations, a certain type of coaching has been essential for both the written and the practical examinations. Now there are a number of effective ways of teaching science, and a basic problem is to choose which way is the best for one's own temperament and for each class of individual who comes before one, and there is no one best way. Pure chalk and talk are useful sometimes, while an experimental investigation with the results and method to be worked out and with an unknown result are also sometimes useful. It would be clearly utterly wrong and contrary to the free spirit of science and even to essential human freedoms to dictate only one way from a central authority. It would be a poor country which could not raise teachers so trained as to choose the best way in their own circumstances. Nor is it reasonable to expect an adolescent with a basis of just a few weeks of so-called 'open ended' investigation to establish the principles and concepts of science which it has taken generations of the acutest scientific minds of the world to reach. So the Nuffield workers ought rightly to be subject to fierce scrutiny and so should the architects of new General Certificate of Education work, such as the examining boards.

Again one may ask what is to happen after the Nuffield Project stops. Should university departments of education set up centres where research may go on in conjunction with the suggested special development schools, and is it right that only some schools should be chosen for development?

On looking closely at the two United States volumes which have prompted the foregoing review, one must ask what their part is in the entire plan. They are aimed at the gifted student and comprise some eighty problems by experienced research workers over a wide range of biological science. The books are valuable as a source of ideas, but there are difficulties on the practical side. The precise extent of the ignorance the investigations are designed to eradicate is not clearly stated, and an intelligent student may well ask why he should investigate a matter he knows has been solved. The references given may answer such questions but are often to be found only in good scientific libraries which may not be easily accessible to the school. The teacher is often assumed to have a considerable amount of background knowledge which he must impart to the student before any investigation can begin.

Perhaps a more serious matter is the almost total absence of reference to the dangers involved in having or handling pathogenic materials, and to the lack of indication of the necessity for statistical analyses of results and of their effects on the design of experiments.

However, all pioneering has its rigours and none should be daunted by them. Perhaps the chief significance at the present time is a general awareness of the need for this fresh and dramatic approach and of a change of emphasis. Newman wrote "Lead, kindly Light". No doubt in time we shall see by a new light which will guide us along the very strait and narrow and indeed arduous path which is the adventure we call "science".

Lance Kramer

GENERAL BIOLOGY

The Biology of Cells

By Prof. Herbert Stern and Prof. David L. Nanney. Pp. xii+548. (New York and London: John Wiley and Sons, Inc., 1965.) n.p.

The Biology of Organisms

By Prof. William H. Telfer and Prof. Donald Kennedy. Pp. xiii+374. (New York and London: John Wiley and Sons, Inc., 1965.) 53*s*.

The Biology of Cells and *The Biology of Organisms* form part of a trilogy (the third on *The Biology of Populations*) in which an attempt is made to break away from the groupings which have traditionally been made in the teaching of biology. Stern and Nanney introduce their book with a general chapter on cell doctrine. This is followed by a chapter on elementary genetics and then, rather surprisingly, eight chapters deal with elementary physical chemistry and biochemistry. After a chapter on information theory, there follow four very good chapters on molecular genetics. The book ends with a section on the organization of cells. Telfer and Kennedy have made one of the first, if not the first, attempts to write an elementary biology text on organisms as a whole, in which sections are not split into botany, zoology, systematics, histology, physiology and so forth. Their section headings are: the problem of diversity; biology of micro-organisms; multicellular organisms; reproduction and development; differential growth; metabolism of multicellular organisms; and integration. Within these sections, plants, animals, structure, physiology and biochemistry are treated as part of a whole aimed at a general understanding of organisms.

Both these books are independent of others but also of each other; consequently, some students might justifiably be inclined to regard each book as sufficient in itself for their course work. However, that of Telfer and Kennedy has a short bibliography and most of their illustrations have detailed references which could enable a student to read further. Rather few of such pointers are found in Stern and Nanney, and expansion of interests

are correspondingly discouraged. The books are well printed, but the point of the illustrations is often lost by the absence of any information of scale. In the texts there is a surprising reticence over the names of the scientists concerned in the work described, unless they be dead.

The books are intended for a freshman reading biology for the first time, and presumably such a student will have no prejudices as to how biology should be presented, provided that it is interesting and stimulating. These texts are interesting and, although there will be complaints that the organisms themselves have been lost in generalities, the authors do not discourage biological novices with overwhelming masses of detailed unrelated facts, as has so often happened in past elementary biology books. However, students may from time to time feel that they would have liked an explanation of the experimental problems involved in obtaining the facts described—a particularly important point for those familiar with the techniques of physical sciences but unfamiliar with the rather different technical problems of a biologist. Much of the interest and use of these books in Britain, particularly that of Telfer and Kennedy, will be for those of our school science sixth-forms and universities where attempts are being made to replace the traditional presentation of biological (and for that matter medical) curricula. In the design of such courses, these books should be carefully examined.

B. B. Boycott
A. Curtis

CAPTIVE ANIMALS

Animals in Captivity

By Philip Street. Pp. 231+24 photographs. (London: Faber and Faber, Ltd., 1965.) 25*s*. net.

KEEPING wild animals in captivity is an ancient art and it flourished in several long-lost civilizations. *Animals in Captivity* is concerned with its history in modern Europe, and primarily in Britain. The only continental zoo treated in any detail is Hamburg, where the Hagenbecks added immensely to practical knowledge about the keeping of captive animals, especially the larger species. The founder of the family can rightly be regarded as the father of modern zoos and circuses, and if more of his ideas had been adopted much better progress in this field would have been made.

Nearly a century ago, Hagenbeck saw the need and possibility of acclimatization. For monkeys he advocated a cage temperature of around 60° F, with flaps giving access to fresh air; they used them, even with the thermometer below zero. A few pages later Mr. Street quotes from the Centenary History of the Zoological Society of London. One of the big problems awaiting Chalmers Mitchell in 1903, when he began his long and successful tenure, was that keepers insisted that all tropical animals be kept warm, even if it meant sealing all windows. This attitude is still widely persistent.

Whipsnade is rightly portrayed as a trend-setter of great importance, but its original purpose—the provision of a rest centre for London animals—has been superseded by more useful objectives. A forcible change of scene upsets wild animals more than it helps them. Many aspects of the subject are dealt with adequately, but the book disappoints in its major omissions, and it could almost have been written 10 or 20 years ago. The bird chapter makes no mention of 'walk-through' flight aviaries. The names of Hediger and Grzimek cannot be found, but their contributions have been of incalculable value. The former's work on the psychology of animals in zoos is fundamental, while Grzimek has rebuilt the bombed Frankfurt Zoo into one of the world's outstanding collections, with a fresh approach to many housing problems.

Under the heading of health and food the work at the Philadelphia Zoo finds no place, yet the balanced diets devised in the laboratory there have given a completely new shape to longevity and breeding statistics wherever the system has been adopted. These and other recent developments could well have replaced long passages on minor matters. The illustrations, mostly by the author, are excellent, and there is an index, but no bibliography.

George Cansdale

A FOUNDATION STONE OF PHYCOLOGY

The Structure and Reproduction of the Algae

By F. E. Fritsch. Vol. 1: Pp. xvii+791. Vol. 2: Pp. xiv+939. (London: Cambridge University Press, 1965.) 90*s*. net per volume.

IN the twenty years which have elapsed since the publication of the second volume of *The Structure and Reproduction of the Algae*, the study of these plants—phycology as it is now fashionable to call it—has undergone profounder change and greater expansion than it did in the forty years which separated the completion of Fritsch's work from that of its forerunner, Oltmann's *Morphologie und Biologie der Algen*. Chemical investigation and the electron microscope have revealed in their different ways an underlying unity of basic structure and fantastic diversity of detail which in 1945 could be only dimly suspected. Algae, some species of which have for a long time been used as convenient experimental material for investigation of some of the classical problems of plant physiology, now engage the attention of the physiologist and biochemist in a more general way, and a large body of publications has appeared showing that their functioning and metabolism have intrinsic interest as great as that of any group of organisms. Phytoplankton still remains the form of plant life receiving least attention from the botanist, but whole new classes of plankton algae have been defined and important advances have been made in understanding their ecology, much of this depending on culture techniques, the use of which Fritsch himself did so much to encourage. Side by side with these developments, which are largely new since 1945, description of species and elucidation of life-cycles by classical method have been progressing steadily.

Nevertheless, Fritsch's two volumes continue to figure in the reference lists of research papers, ecological and physiological as well as morphological; they are constantly brought out in the practical class as the final arbiter on most points relating to algae; and there is no doubt that this present reprinting is both welcome and justified. Partly this is because the foundations of phycology have always been to a remarkable extent broadly based and have stood the test of time. From the beginning, almost, biochemical characteristics have ranked equally with morphological features in algal taxonomy. Phycology has been fortunate in having had many distinguished exponents who, like Fritsch, remembered the ecology and physiology of the organisms they investigated and encouraged others to investigate these, even though their own primary interest was in other aspects.

In addition to wideness and balance of outlook, Fritsch's writing displays meticulous scholarship. Misprints presumably must occur in a work of this length, but I cannot recollect having found one, or any misquotation of fact or reference.

The Structure and Reproduction of the Algae is undoubtedly out of date in detail, and in particular the physiology which it includes has an old-fashioned look, but an account so soundly constructed will certainly continue to serve as a basic text for a long time. Unfortunately, another Fritsch would be required to revise it adequately.

G. E. Fogg

HELIUM PRODUCTION IN AN ANISOTROPIC BIG-BANG COSMOLOGY

By Dr. S. W. HAWKING and Dr. R. J. TAYLER

Department of Applied Mathematics and Theoretical Physics, Cambridge

THERE has recently been much interest in the helium content of the universe. A variety of observations, mainly on objects in our own Galaxy but some of nearby galaxies, have suggested that the helium abundance relative to hydrogen may be rather uniform with about one helium atom to every eleven hydrogen atoms. If such an abundance were universal, except for local irregularities mainly with a higher helium abundance, it would mean that about 27 per cent by mass of the universe is helium and it would seem plausible that the general helium abundance has been produced cosmologically, and that the local irregularities are due to helium production in stars or similar bodies.

If the universe had a singular origin with high temperature and density ('hot big-bang' theory), some helium would have been produced in the first few minutes. The amount of helium produced depends on the ratio of material energy density to radiation energy density in the early stages of the expansion of the universe but for quite a wide range of matter densities essentially the same amount of helium is produced. In the early calculations of Hayashi[1] and Alpher, Follin and Herman[2], this plateau helium abundance was about 40 per cent by mass. More recent calculations by Hoyle and Tayler[3], Smirnov[4] and Peebles (personal communication, 1965) suggest that the value should be about 35 per cent, but there are still some uncertainties in the theory. It seems somewhat unlikely, however, that the errors in theory and observation are sufficient for the true plateau helium abundance to be lower than the observed abundance.

If the initial matter density is taken low enough it is possible to produce a smaller amount of helium and one which would fit the observations. However, there is a further difficulty. It appears that the expansion of the big-bang fireball should leave a low-temperature thermal radiation field in intergalactic space. The value to be expected for the radiation temperature with the present material density depends on the initial matter density. If the latter were low enough for the initial helium abundance to be 27 per cent by mass or less, it seems that the present-day radiation temperature should be at least 10° K and possibly 20° K. Several measurements have been made recently which suggest that there is a universal radiation field and which are certainly not inconsistent with its being black body at about 3° K (refs. 5–7). It now seems unlikely that the conventional hot big-bang theory will be able to give correct values for the helium abundance and the cosmic radiation temperature.

Although the situation is not quite clear, it seems worth while to consider whether there are any modifications to the theory which retain the essential feature of the hot singular origin to the universe and at the same time offer hope of reconciling the results. Two possibilities immediately present themselves. The amount of helium produced would certainly be affected if the Newtonian gravitational constant were not a true constant but was larger in the early stages of the universe as has been suggested by several authors[8,9]. The other possibility is that the universe has not always been isotropic. Observations of distant galaxies and radio sources suggest that the universe has been isotropic for most of the lifetime of an evolving universe, but there are certainly no observations to show that it was isotropic in its earliest stages. It is a possibility, although perhaps not a very attractive possibility, that the universe was anisotropic when created but that the anisotropy rapidly disappeared in the expansion. It is the purpose of this article to point out that the existence of such an anisotropy, even if it only persisted for a few years, could have had a profound effect on the helium production.

The Early Stages of an Anisotropic Universe

It is well known that, in the early stages of the expansion of a homogeneous, isotropic, hot big-bang universe in which radiation energy density dominates over material energy density, there is a unique relation between temperature and time. Hoyle and Tayler[3] give the relation in the form:

$$T = 1{\cdot}04 \times 10^{10}\, t^{-\frac{1}{2}} \qquad (1)$$

where T is in °K and t in sec. The exact value of the multiplicative constant (1·04) depends on the assumption that the photon field is enhanced by positrons, electrons, neutrinos, antineutrinos, μ-neutrinos and anti μ-neutrinos at high temperatures.

The particular solution (1) of the relativistic equations is that which satisfies the initial conditions, $T \to \infty$ as $t \to 0$. If the universe is initially anisotropic but rapidly tends to isotropy, a solution of the isotropic equations is still required, but it can be the more general one:

$$T = 1{\cdot}04 \times 10^{10}\, (t+\alpha)^{-\frac{1}{2}} \qquad (2)$$

where α is an arbitrary constant.

If the universe is initially anisotropic, with expansion from a two-surface rather than a point, the relation between temperature and time takes a form different from (1):

$$T = \beta t^{-\frac{1}{2}} \qquad (3)$$

where now β is a parameter which depends on the type or degree of anisotropy. In the earliest stages the relation (3) will hold and, when the anisotropy disappears, a relation of the form (2) will be valid. The relations (1), (2) and (3) are shown in Fig. 1.

An approximate estimate of the duration of the anisotropy for given β is obtained as follows. It seems plausible that the change-over from the law (3) to (2) should occur smoothly. This implies, as can be seen from Fig. 1, that α is positive. For given β it is possible to choose a time t_0 and a value of α so that a change-over from (2) to (3) occurs

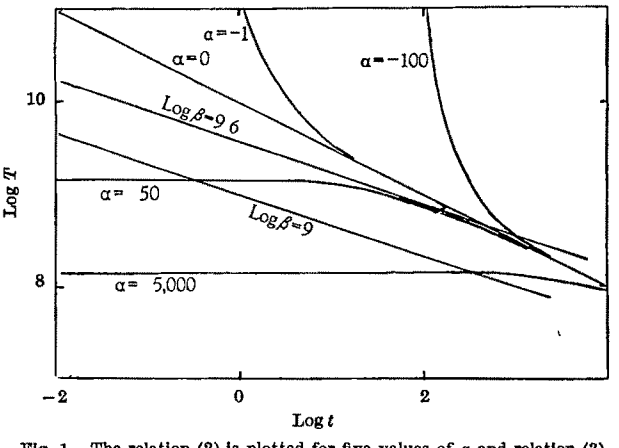


Fig. 1. The relation (2) is plotted for five values of α and relation (3) for two values of log β. The change-over from anisotropy to isotropy is shown for $\alpha \approx 50$, $\log \beta \approx 9{\cdot}6$

with T and dT/dt both continuous. This time t_0 should be a reasonable first approximation to the time at which the anisotropy disappears. A more accurate value will only be found by integrating the anisotropic cosmological equations. This matching of the relations (2) and (3) gives the results:

$$\left.\begin{aligned} t_0 &= \frac{8(1{\cdot}04\times10^{10})^6}{27\beta^6} \\ \alpha &= \tfrac{1}{2}t_0 \end{aligned}\right\} \qquad (4)$$

In Table 1 are shown, for several values of β, t_0 and the times at which $T=10^{10}$ °K and 5×10^8 °K. It can be seen that, even if the anisotropy lasts for only a few hours, the time taken to reach temperatures such as 5×10^8 °K is considerably reduced.

It should be pointed out that, whether the universe is isotropic or anisotropic, provided no nucleon creation occurs, there is the relation:

$$n \propto T^3 \qquad (5)$$

between nucleon number density and temperature. This means that, if we now consider a set of anisotropic universes characterized by the same present values of radiation temperature and matter density, they will have the same matter density at any higher temperature; only the time-scale is different. Let us consider the rate of formation of helium at high temperatures and densities. The first reaction that must occur is:

$$n+p \rightarrow D+\gamma \qquad (6)$$

and to a good approximation we may take the value of $<\sigma v>$ for this reaction to be independent of temperature at the temperatures of interest. The value adopted is 7×10^{-20} (W. A. Fowler, personal communication, 1965). Table 2 shows the characteristic time-scale of this reaction at a temperature of 10^{10} °K for a present-day radiation temperature of 3° K and for several values of the present-day matter density which cover the range of values which is believed plausible. It can be seen that this reaction, which is an essential preliminary to the production of helium, will scarcely go at 10^{10} °K for any of the densities if the anisotropy lasts for 10 years or more; this follows from a comparison of reaction times in Table 2 and evolution times in Table 1. While the universe remains anisotropic the reaction time and evolution time are directly proportional, so that if the reaction will not go at 10^{10} °K it will not go at any other temperature while the anisotropy remains. Even more so is this true when isotropy takes over, for then the reaction time steadily gets larger compared with the evolution time. It thus appears that, if the anisotropy lasted longer than something of the order of 10 years, essentially no helium would have been produced in the big bang.

Table 1

Log β	$t(10^{10}$ °K)	$t(5\times10^8$ °K)	t_0
8	10^{-6}	8×10^{-3}	4×10^{11}
8·5	3×10^{-5}	$2{\cdot}5\times10^{-1}$	4×10^{9}
9	10^{-3}	8	4×10^{5}
9·5	3×10^{-2}	$2{\cdot}5\times10^{2}$	4×10^{2}
10	9×10^{-1}	4×10^{2}	4×10^{-1}
Isotropic	1	4×10^{2}	0

Table 2. Reaction Times for the Production of Deuterium at 10^{10} °K for a Present-day Radiation Temperature of 3° K and for Several Values of the Present Nucleon Density (n)

The actual values are obtained by taking $<\sigma v>=7\times10^{-20}$ and $n_p=3n_n$.

n(3° K)	$n(10^{10}$ °K)	t Reaction
10^{-5}	4×10^{23}	5×10^{-5}
10^{-6}	4×10^{22}	5×10^{-4}
10^{-7}	4×10^{21}	5×10^{-3}

If the anisotropy does not last long enough to ensure that little or no helium is produced, it might be thought that an amount less than the isotropic value would be produced. However, it seems that for some values of the anisotropy parameter the helium production exceeds that in the isotropic case. The reason for this is as follows. In the isotropic case the helium producing reactions which follow reaction (6) are rapid and (for values of the matter density which yield a present radiation temperature of 3° K) the amount of helium produced is essentially determined by the number of neutrons when reaction (6) and the subsequent reactions become important. If the universe is slightly anisotropic the early stages of the expansion are speeded up but perhaps not sufficiently to prevent complete burning of neutrons. However, any speeding up of the expansion makes it more difficult for the reactions changing neutrons into protons, and this means that, at a given temperature when deuterium and helium production start, the neutron density is higher than in the isotropic case. As the anisotropy parameter $(1/\beta)$ increases from zero there should thus be an initial rise in helium production while the greater neutron density is the dominant factor and the subsequent fall should occur when the anisotropy parameter is large enough for reaction times to exceed the evolution time.

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Homo habilis, *Homo erectus* AND THE AUSTRALOPITHECINES

By Dr. L. S. B. LEAKEY

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TOBIAS[1], in his reply to Robinson[2], has dealt very adequately with his questions about the validity of our new species of *Homo*, and I heartily agree with his arguments that *Homo habilis* stands completely distinct from any *Australopithecus*. I would like, however, to stress certain of the morphological aspects in which the mandible of the australopithecines differs from that of *Homo sapiens*, *Homo erectus* and *Homo habilis*, all three of which share *Homo* features.

These basic features can be clearly seen in Figs. 1 and 2. The "V"-shaped structure of the australopithecinae is illustrated by a specimen of the so-called 'gracile' species, *Australopithecus africanus*, and the mandible of *Australopithecus* (*Zinjanthropus*) *boisei* from Peninj. Contrasted with them we have in Fig. 1 the mandible of the paratype of *H. habilis* from Olduvai site *MNK* II and a specimen of *H. sapiens*. In Fig. 2 we have views of three species of *Homo*—*erectus*, *sapiens* and *habilis*—contrasted with *Australopithecus* (*Zinjanthropus*) *boisei*. Comparing Figs. 1 and 2 there can be no doubt that at least the paratype of *H. habilis* from *MNK* II belongs with the genus *Homo*, not with *Australopithecus* (not even the species *africanus*).

Similarly, if the views of the two species of *Australopithecus* in Fig. 1 are compared with Tobias's illustration of the type of *H. habilis*, the same conclusion must be drawn.

It must here be noted that it was this striking difference in mandibular structure which, among other factors,

rightly led Broom and Robinson to separate what used to be called *Telanthropus capensis* from the genus *Australopithecus*.

Examination of illustrations 1 and 2 seems to me to eliminate Robinson's view that *H. habilis* is nothing but a variant of *A. africanus*, or, as he has more recently suggested, that *A. africanus* is really *Homo*. *H. habilis*, however, in its mandibular structure, is unquestionably of *Homo* type. It shares features with both *H. sapiens* and *H. erectus* and is quite distinct from any australopithecine species so far described.

I wish next to make it clear that I do not share the opinions of my colleague Tobias, which he expressed in Moscow[3] and elsewhere (and some of which he reiterated in the closing stages of his reply to Robinson), when he comes to deal with the relationship of the australopithecines to the genus *Homo* or of *Homo habilis* to *Homo erectus*.

Tobias holds the view that the evolution of Man has been through a series of 'stages' represented, respectively, by *Australopithecus africanus* and *H. erectus*, leading on to *H. sapiens*. He goes further and expresses the opinion

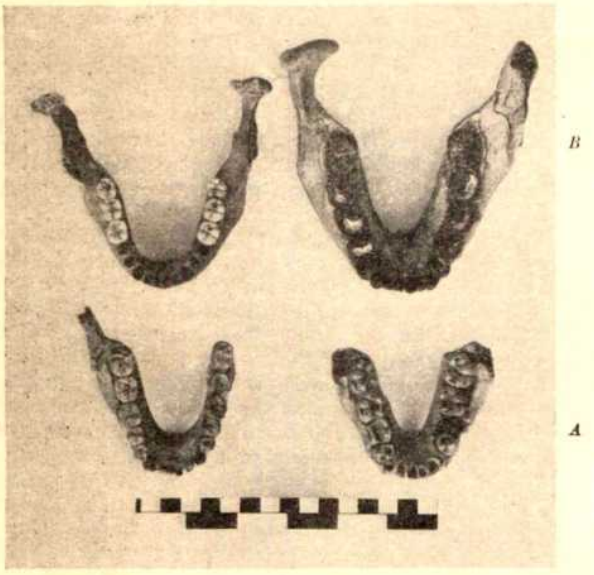


Fig. 1. Mandible of: *A, Australopithecus africanus; B, Australopithecus (Zinjanthropus) boisei; C, Homo sapiens; D, Homo habilis*

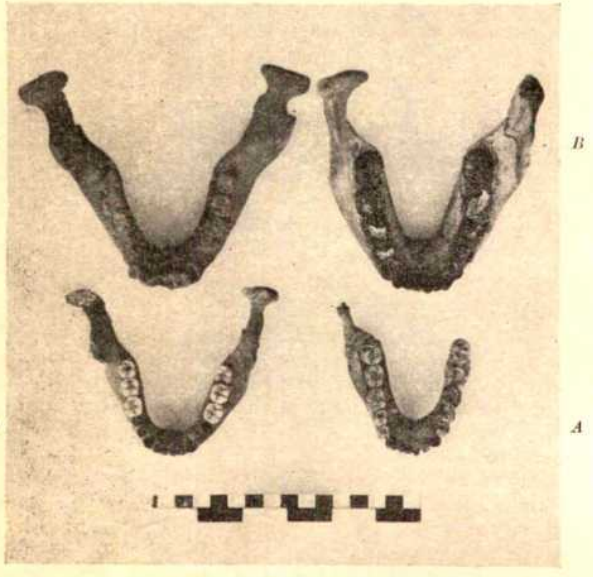


Fig. 2. Mandible of: *A, Homo habilis; B, Zinjanthropus; C, Homo erectus*/North Africa; *D, Homo sapiens*

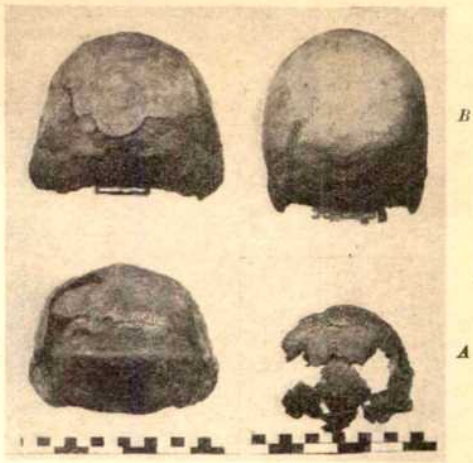


Fig. 3. Occipital views of: *A, Homo habilis; B, Homo sapiens; C, Homo erectus*/Olduvai *LLK; D, Homo erectus*/China

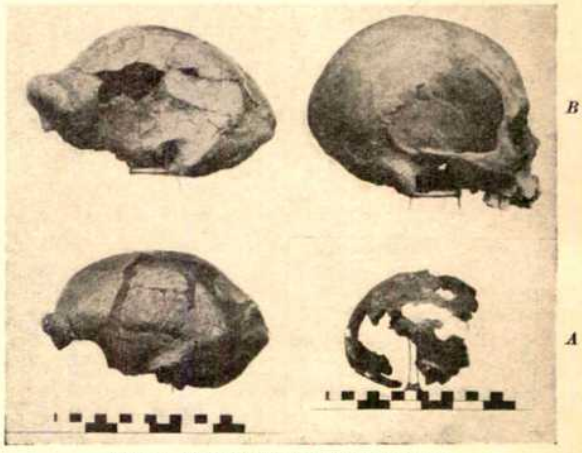


Fig. 4. Same as Fig. 3 but profile views

that "*Homo habilis* may represent a stage between *Australopithecus africanus* and *Homo erectus*".

I do not believe that the available evidence in any way supports such a conclusion. It seems to me more likely that *H. habilis* and *H. erectus*, as well as some of the australopithecines, were all evolving along their own distinct lines by Lower Pleistocene times. This would mean that their shared common ancestor must be sought in the more remote past and that when examples of the parent stock are found they will not much resemble any one of the three subsequent branches.

I submit that morphologically it is almost impossible to regard *H. habilis* as representing "a stage between *Australopithecus africanus* and *Homo erectus*". In both the two latter hominids, the maximum cranial width is to be found near the base of the skull on the temporal bones. *H. habilis*, on the other hand, shares with *H. sapiens* the characteristic of having the greatest width of the vault on the parietals (see Figs. 3–6). *H. habilis*, also, shares an occipital morphology with *H. sapiens*, which, moreover, is quite unlike either *H. erectus* or any known australopithecine. It therefore seems to me most unlikely that the *H. habilis* cranial structure—either parietal, occipital or basi-occipital—could represent a stage "intermediate between *Australopithecus* and *Homo erectus*".

Even though *H. erectus* and the australopithecines share a number of cranial characters in their occipital bones, and in having a maximum width at the base of the skull, I have never been able to accept the view that *Australopithecus* represented a direct ancestral stage leading to *H. erectus*, and I disagree even more strongly with the present suggestion of placing *H. habilis* between

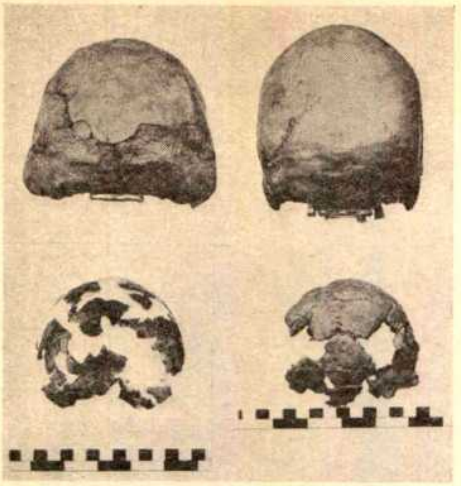


Fig. 5. Occipital views of: *A*, *Homo habilis; B, Homo sapiens; C, Homo erectus*/Olduvai *LLK* II; *D*, Protopithecanthropine

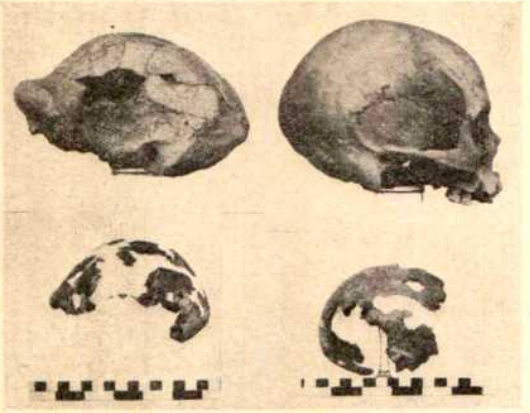


Fig. 6. Same as Fig. 5 but profile views

them. It places a hominid type which has a wholly distinct cranial morphology and one recalling that of *H. sapiens* type between these two. It is possible that *H. habilis* may prove to be the direct ancestor of *H. sapiens*, but this can be no more than a theory at present. If true it would mean that there had been two distinct species of the genus *Homo* at a very early stage—one leading to *H. erectus* and subsequent extinction and the other to *H. sapiens*.

We must also take into account the fact that at the very base of Olduvai Bed II, at *FLK* Maiko Gully, we have a skull, as well as the greater part of the associated dentition, of what seems to be an ancestral stage of *H. erectus* (see Fig. 6). This specimen was found stratigraphically between the earlier and the later representative of *H. habilis*, which also makes it unlikely that *H. habilis* evolved from a pithecanthropine.

On the other hand, it does suggest that there were two parallel species of the genus *Homo* evolving at Olduvai, one from the earlier to the more advanced *H. habilis* and the other from the protopithecanthropine to the very specialized representative of the African variety of *H. erectus*, which is represented by the skull from Olduvai *LLK* II.

The stratigraphical position of the different hominid finds from Olduvai, that are referred to in this article, is shown in Table 1. Table 1 includes the *Zinjanthropus* mandible from the nearby Peninj site, where the combined evidence based on geology, fauna and culture suggests a correlation with the Middle and Upper part of Bed II, Olduvai.

Table 1. Stratigraphical Relationships of Hominid Finds at Olduvai and Peninj

Type of hominid	*Homo habilis*	*Pithecanthropine sensu Lato*	*Australopithecus* (*Zinjanthropus*)
Main Bed II and its Peninj equivalents	+ *MNK* II	+ *LLK* II	+ *BK* II + Peninj
Lower Bed II	Major climatic break	+ *FLK* II Maiko Gully	
Bed I	+ *FLK NI* ? + *FLK* I + *FLK NN* I + *MNK* I		+ *FLK* I
	LAVA		

Table 1, taken in conjunction with the morphological factors briefly summarized here which are clearly seen in Figs. 1–6, should serve as a deterrent to the many who would like to draw up evolutionary sequences based on insufficient numbers of specimens.

All that need be said at present is that there was a time at Olduvai when *H. habilis*, *Australopithecus* (*Zinjanthropus*) *boisei* and what seems to be a primitive ancestor of *H. erectus* were broadly contemporary and developing along distinct and separate lines.

[1] Tobias, P. V., *Nature*, **209**, 593 (1966).
[2] Robinson, J. T., *Nature*, **205**, 121 (1965).
[3] Tobias, P. V., paper presented at the seventh Intern. Congr. Anthrop. and Ethnol. Sci., Moscow, 1964 (in the press).

CLOTHING INSULATION AND ACCIDENTAL HYPOTHERMIA IN YOUTH

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An investigation was undertaken with the view of improving our understanding of the environmental factors concerned in exposure accidents among hill-walkers and climbers in Britain. The effects of exercise, wind and wetting on the thermal insulation of a typical clothing assembly were observed. The clothing examined had been worn by one of the youths who died of exposure on the 'Four Inns Walk' in Derbyshire in March 1964 (ref. 1). It consisted of the following items: anorak with hood, woollen jersey, 'Vyella' type shirt, string vest, cotton drawers, jeans, one pair of socks, shoes and gloves. The experiments were conducted in a small climatic chamber in which a minimum temperature of 10° C (relative humidity 50 per cent) and a maximum wind velocity of 8·9 m.p.h. were obtainable.

The subject was a normal adult aged twenty-one. His height was 182 cm, weight 80·5 kg, surface area 2 m² and mean subcutaneous fat thickness 7·9 mm. He was examined at rest and during standard exercise on a bicycle ergometer at a work rate of 300 kg/min. The experiments were carried out in 4·5 h sessions beginning between 1200 h and 1300 h. The ambient temperature was 9·4° C dry bulb and relative humidity 50 per cent. The following experimental situations were investigated. (1) Rest in a

sitting posture: (*a*) at air movement 12 ft./min; (*b*) at air movement 220 ft./min (2·5 m.p.h.). (2) Exercise in still air. (3) Exercise in a wind of 790 ft./min (9 m.p.h.) velocity. (4) Exercise in a 9 m.p.h. wind after the clothing had been saturated with water by standing under a shower bath, the water temperature being either 10° C or 18° C. (5) As under (4), but wearing a plastic over-anorak and windproof over-trousers which kept the clothing dry.

The experiments were conducted in the following order: Day I, situations (2), (3) and (4). Day II, situations (1*a*) and (4). Day III, situations (1*b*) and (5). A final experiment was carried out in less well-controlled conditions in a laboratory at an ambient temperature of 16° C, relative humidity 60 per cent, and air movement 25 ft./min.

Measurements of rectal and body surface temperature were begun after 40 min in each situation and repeated at intervals of 20–30 min for 90 min at rest and 73 min during exercise. Rectal temperature was measured with an indwelling thermocouple at a depth of 15 cm. Skin temperatures were measured with a thermocouple probe inserted through small holes in the clothing or held in contact with the exposed skin. A Cambridge potentiometer was used for measuring rectal temperatures and an 'Ellab' thermocouple thermometer (Elecktrolaboratoriet, Copenhagen) for skin temperatures. When the subject's clothing was wet the skin temperatures of the lower limbs and extremities fell below 16° C and could not be measured with the probe. The experiment was repeated with thermocouples attached to the skin, and readings were taken on the Cambridge potentiometer.

The respiratory exchanges were measured by the bag method before each set of temperatures; expired gas was collected over 15 min in resting experiments and 4–6 min in work experiments. Gas samples were analysed on a Lloyd–Haldane apparatus.

The rate of heat loss in warming and saturating inspired air was calculated from the formulae given by Belding *et al.*[2]. Evaporative heat loss from the skin was assumed to be constant at 18 g/h[2].

Table 1

(*a*) Location of Thermocouples and Factors used in determining Mean Skin Temperature after Belding *et al.*[2]

Region	Multiply by
Forehead	0·07
Arm lateral	0·07
Arm medial	
Forearm medial	0·07
Hand	0·05
Foot	0·07
Pectoral region	0·35
Abdomen	
Lumbar region	
Scapular region	
Thigh medial	0·19
Thigh lateral	
Leg anterior	0·13
Leg posterior	
	1·00

(*b*) An Abbreviated Table of Standard Values of Air Insulation (I_A)[4]

I_A (Clo)	Air movements (ft./min)	(cm/sec)	(miles/h)
0·1	4,500	2,280	51
0·15	2,000	1,015	22·7
0·2	1,050	534	11·9
0·4	210	107	2·4
0·6	75	39	0·85
0·8	35	18	0·4
1·0	10	5	0·1

Mean skin temperatures were computed from 2–4 readings taken over each of thirteen regions of the body surface using the weighting factors shown in Table 1. Mean body temperature (T_B) was calculated as 2/3 rectal + 1/3 skin temperature. Skin temperature, for this purpose, was computed without the extremities, the weighting factors being adjusted accordingly[3,4]. Stored heat loss (S) was computed from the following equation:

$$S = \Delta T_B \times W \times 0{\cdot}83 \times \frac{60}{t} \qquad (1)$$

where ΔT_B is the change in mean body temperature in time (t) min, W is the body-weight in kg, and 0·83 the specific heat of tissues in kcal/kg.

The insulation value of the clothing was calculated as follows:

$$I_{Cl} + I_A = \frac{T_S - T_A}{0{\cdot}18\,H} \qquad (2)$$

$$H = \frac{M - W - E - S}{S.A.} \qquad (3)$$

where I_{Cl} is the insulation value of clothing (in Clo units); I_A the insulation value of air (in Clo units); T_S, the mean skin temperature (in °C); T_A, the ambient dry bulb temperature (in °C); H, the non-evaporative heat loss through clothing (in kcal/m² h); 0·18, the factor for converting c.g.s. units $\left(\frac{°C}{\text{kcal/m}^2\text{ h}}\right)$ to Clo units; M, metabolism (in kcal/h); W, mechanical work (in kcal/h—1 kcal = 427 kg/min); E, evaporative heat loss from lungs and skin plus heat loss in warming inspired air (in kcal/h); S, stored heat loss (in kcal/h); $S.A.$, surface area (in m²).

From the same data it was possible to calculate values for the insulation (I_T) and conductance (K) of the tissues, thus:

$$I_T = \frac{T_R - T_S}{H}, \text{ in } \frac{0°\text{ C}}{\text{kcal/m}^2\text{ h}} \qquad (4)$$

and

$$K = \frac{H}{T_R - T_S}, \text{ in } \frac{\text{kcal/m}^2\text{ h}}{°\text{C}} \qquad (5)$$

In the calculation of H in equations (4) and (5) evaporative heat loss from the skin was omitted.

Clothing and air insulation (I_{Cl} and I_A). The effect of exercise, wind and wetting on the insulation afforded by clothing and air is illustrated in Fig. 1, and compared with the values observed when the subject was at rest in 'still' air (that is, less than 25 ft./min of air movement). The numerical data from which these results were calculated are shown in Table 2. An estimate of the *S.D.* of a single measurement based on six pairs of observations was ± 0·10 Clo.

Rest. In 'still' air in the climatic chamber the total insulation afforded by the dry clothing and surrounding air ($I_{Cl} + I_A$) was 2·49 Clo (Table 2, No. 2). A com-

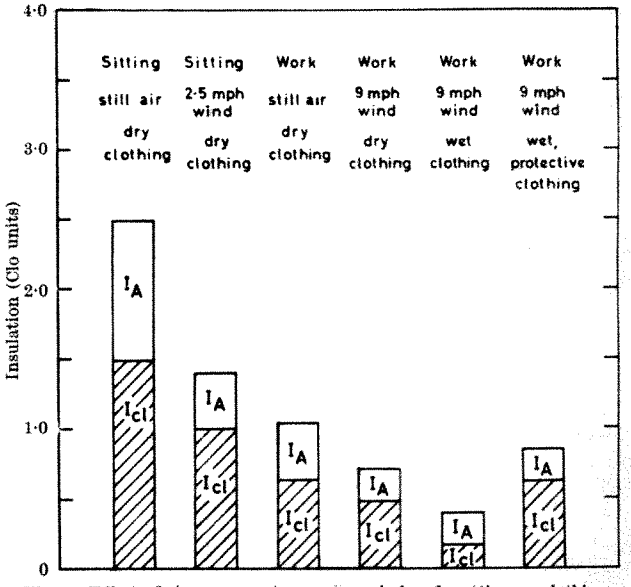


Fig. 1. Effect of air movement, exercise, wind and wetting on clothing and air insulation ($I_{Cl} + I_A$). With wet clothing in the presence of wind, overall insulation was comparable with that of air alone at low wind velocity

parable result was obtained under ordinary conditions in the laboratory (Table 2, No. 1). From Table 1 the value of I_A for still air is 1·0 Clo, therefore I_{Cl} was equal to 1·49 Clo. This result may be compared with the standard value of 1 Clo for ordinary business clothing[4], and 3 Clo for military polar clothing[5,6]. With modern down clothing, values up to 7 Clo have been obtained.

In a slight breeze (wind velocity 2·5 m.p.h.) $I_{Cl} + I_A$ fell to 1·40 Clo. The value of I_A at this wind velocity is 0·4 Clo; therefore, the value of I_{Cl} was 1·00. This 30 per cent drop in I_{Cl} was associated with a marked fall in skin temperature over the face and extremities and to a lesser extent over the limbs.

Exercise. During exercise in 'still' air $I_{Cl} + I_A$ fell to 1·04 Clo, a finding which could be attributed to the 'bellows' action of movement in displacing static air from the clothing. I_{Cl} could not be estimated for this situation, since the effect of exercise on I_A is not known. However, a likely value of 0·4 Clo has been assumed for the purpose of illustration (Fig. 1).

The combination of wind and exercise reduced $I_{Cl} + I_A$ to 0·71 Clo.

Wetting. When the subject worked on the ergometer wearing wet clothing and facing a 9 m.p.h. wind, $I_{Cl} + I_A$ fell to 0·4 Clo. Wetting therefore reduced total insulation by 0·7 − 0·4 = 0·3 Clo, or nearly 50 per cent, and if I_A is assumed equal to 0·23 Clo, which is the appropriate value for a wind velocity of 9 m.p.h., the value of I_{Cl} is only 0·17 Clo, which is only 15 per cent of the insulation value of this clothing, when dry and in the absence of wind or exercise.

When the subject wore waterproof over-garments which kept his clothing dry and at the same time protected him from the wind, $I_A + I_{Cl}$ rose to 0·85 Clo, which was close to the observed value for exercise in dry clothing in still air.

Change of stored heat (S). The values of S in most of these experiments were within the limits of experimental error (Table 2), indicating that a state of thermal equilibrium had been reached. This was achieved in 1–1·5 h in the resting experiments and in about 45 min during exercise. In two experiments a steady state was not achieved; in one of these (experiment 3) clothing insulation was inadequate for the conditions (ambient temperature, 10° C; air movement, 2·5 m.p.h.); in the other there was an excess of stored heat from a previous 15-min period of exercise at 600 kg/min before the experiment.

Skin temperature. The regional and mean skin temperatures observed at the end of each experimental period are shown in Table 3. The resting mean skin temperature at a comfortable ambient temperature of 16° C was 32·1° C, which was lower than expected since the values observed in clothing experiments in comfortable temperatures are usually in the range 33°–34° C. This finding

Table 2. Experimental Results

No.	Date	Conditions	T_a	T_S (° C)	T_R (° C)	M (kcal/h)	W (kcal/h)	E (kcal/h)	S (kcal/h)	H (kcal/m²h)	I_{a+Cl} (Clo)	I_a (Clo)	I_{Cl} (Clo)	I_T (Clo)	K $\frac{\text{kcal/m}^2\text{h}}{\text{°C}}$	Air movement
1	31/5	Rest dry	16	32·14	37·50	88	0	25	+3	33	2·68	0·88	1·80	0·767 (0·138)	7·26	25 ft./min
2	30/4	Rest dry	9·4	30·58	37·50	127	0	34	+2	48	2·49	1·0	1·49	0·72 (0·129)	7·76	12 ft./min
3	14/5	Rest dry	9·4	26·93	37·48	115	0	30	+51	69	1·40	0·4	1·00	0·79 (0·142)	7·06	230 ft./min (2·5 m.p.h.)
4	28/4	Work dry	9·4	31·24	38·20	300	41	46	+22	118	1 04			0·31 (0·056)	17·73	25 ft./min
5	28/4	Work dry	9·4	26·44	38·28	370	41	54	−8	134	0·71	0·23	0·48	0·47 (0·085)	11·82	9 m.p.h.
6	28/4	Work wet	9·4	21·10	38·06	450	43	71		168	0·40	0·23	0·17	0 54 (0·097)	10·27	9 m.p.h.
7	30/4	Work wet	9·4	21·50	37·76	458	54	77	0	171	0·40	0·23	0·17	0 51 (0 091)	10 87	9 m.p.h.
8	14/5	Work protective over-garments	9·4	28·17	37 63	329	10	44	0	123	0·85	0·23	0·62	0·41 (0·074)	13·65	9 m.p h.

T_S, Mean skin temperature; T_R, rectal temperature; M, metabolism; W, mechanical work; E, evaporative heat loss from lungs and skin plus heat loss warming inspired air; S, rate of change of stored heat, a plus sign denotes cooling, because S is then added to M in calculating the rate of heat loss; H, non-evaporative heat loss through clothing; I_{a+Cl}, clothing plus air insulation; I_a, air insulation; I_{Cl}, clothing insulation; I_T, tissue insulation; K, tissue conductance. The values are the means of the last two observations during each period. Values of I_T in $\frac{\text{°C}}{\text{kcal/m}^2\text{ h}}$ are shown in parentheses below the corresponding values in Clo units.

Table 3. Regional Skin Temperatures (°C)

No.	Conditions	Date	Trunk	Upper limb	Lower limb	Forehead	Hand	Foot
1	Rest							
	Still air	31/5/65	35·1	33·7	29·4	34·9	28·4	27·9
	Laboratory		35·2	33·4	28 6	34·9	34·7	25·9
2	Rest							
	Still air	30/4/65	35·1	32·9	26 6	34·3	26·3	27 8
		30/4/65	35·1	32·7	26·0	33·5	20 8	22 9
3	Rest							
	Wind	14/4/65	34·1	30 3	22·7	26·6	12·6	18·3
		14/4/65	33 1	29·0	22·4	27 7	12 6	16·3
4	Work							
	Still air	28/4/65	34·8	33·9	29 1	35 0	29 9	24·5
		28/4/65	34·5	30·9	27·8	38 0	28 3	29·2
		28/4/65	33·7	32·6	28·3	34·6	24·7	28·0
5	Work							
	Wind	28/4/65	30·8	30·9	22·9	26·8	< 15 0	20·1
			31·8	30·3	23·2	26·5	< 15·0	18·2
6	Work							
	Wet and wind	28/4/65	30·5	25·2	15·4	22 7	11·3	7·81
7	Work							
	Wet and wind	30/4/65	30 0	27 3	14 2	24 0	11 3	7 81
		30/4/65	30·3	25·7	14·2	24·9	11 3	7 81
8	Work							
	Wet and wind with protective over-garments	14/4/65	34·7	31·8	27·0	22·4	18·9	13·7

Note the low skin temperatures over the limbs associated with the wearing of tight jeans. The fall in trunk temperature in the presence of wind (5) shows that the anorak was inadequate. The low trunk and peripheral temperatures seen with wet clothing indicate severe cold stress.

was explained by the comparatively low temperatures over the lower limbs and the fact that the subject was rather fat[7]. In a group of middle-distance runners recently examined, mean subcutaneous fat thickness was 3·2 mm (range 3·8–2·9 mm)[8], compared with 7·9 mm in this subject.

Oxygen intake. During rest at an ambient temperature of 9·4° C, the subject felt cold, and although shivering was not perceptible, there was considerable elevation of metabolism. Mean oxygen intakes of 0·424 l./min and 0·385 l./min were observed on different days, compared with 0·300 l./min in a warmer environment in the laboratory, the latter being the predicted value for conditions of thermal comfort.

When the subject's clothes were dry and he was exercising in 'still' air or wore protective over-garments in the presence of a 9 m.p.h. wind, mean oxygen intakes of 0·99 l./min–1·10 l./min were observed (Table 4), these being typical values for the 300 kg/min work-rate. When his clothes were wet and he exercised facing a 9 m.p.h. wind, the subject experienced severe cold stress. The measured work-rate increased by 25 per cent due to faster pedalling, and the oxygen intake rose by 50 per cent to 1·50 l./min, which was the predicted value for twice the work-rate (that is, 600 kg/min). Since there was no perceptible shivering, the reason for this disproportionate increase in oxygen intake was not apparent. A similar effect was observed by Adolph and Molnar[9] in their experiments on nude subjects in the cold, and is important in that it affords a partial explanation of early exhaustion in exposure accidents.

Table 4. Oxygen Intake and Observed Work-rate during Exercise at a Nominal Intensity of 300 kg/min, Showing Influence of Cold Stress.

Conditions	Date	Observed work-rate (kg/min)	Oxygen intake (l./min S.T.P.)
Dry clothing, minimal air movement, no cold stress	28/4	294	1·009
	30/4	283	0·986
	14/5	291	1·096
	Mean		1·030
Wet clothing, wind, cold stress + +	28/4	309	1·501
	30/4	386	1 511
	30/4	337	1 575
	Mean		1 529

Tissue conductance and insulation. The resting values of tissue conductance (K) and its reciprocal tissue insulation (I_T) are shown in Table 2. The resting values compare well with published data on the minimum tissue conductance or maximum tissue insulation of resting subjects exposed in air[10,11] or in water[12,13], and suggest that cutaneous vaso-constriction was maximal in all the resting experiments[14]. The results observed during exercise in wet clothing agree with minimum values calculated from the data of Nielsen[15] and Adolph and Molnar[9] for subjects exercising in the cold air at low temperature. They show a 30 per cent increase in conductance over the resting values. This is less than the increase in K reported in swimmers in cold water[16].

The foregoing results show satisfactory agreement with published data on military clothing. Belding[5] reported a 50 per cent reduction in I_{cl} associated with exercise, but there is as yet no satisfactory estimate of the effects of exercise on I_A. Breckenbridge and Woodcock[6] measured the effect of wind on the insulation of military cold-weather uniforms and observed a 30–40 per cent reduction at a wind velocity of 24 m.p.h. These uniforms had windproof trousers and were superior in many respects to the 'Four Inns Walk' clothing. Larose[17] has shown that in some fabrics, such as pile, the insulation is but little affected by winds up to 10 m.p.h. but falls steeply at higher velocities as the wind begins to penetrate the fabric. The best windproof fabrics now available show less than 1 per cent penetration at 100 m.p.h. wind velocity and yet are permeable to water vapour. Hall and Polte[18] and Hall *et al.*[19] reported a 50 per cent reduction of insulation associated with wetting. But no previous measurements have been reported on the combined effect of wind and wetting on clothing insulation.

The conditions of our experiments differed in many ways from outdoor conditions. The effects of wind and exercise are likely to be greater in hill walkers than in a subject working on an ergometer at constant wind velocity, and greater still in bad weather. On the other hand, there is some small gain outdoors from short-wave radiation even with overcast skies. The wetting effect was perhaps magnified in these experiments because of the rather low relative humidity. In natural conditions when rain is falling, evaporative cooling is less; but considerable amounts of water fall on the clothing and have to be warmed. For example, the volume of water falling on a surface 1 sq. m in area amounts to 10 l. for each centimetre of precipitation. In spite of these differences from natural conditions, the conclusion can safely be drawn that the overall insulation of a man's clothing, plus air, when he is wet through and exposed to a strong wind, will be extremely small—and probably of the order of 0·3 Clo—which is the insulation value of air alone at a lower wind velocity (for example, of 5 m.p.h.). He is therefore in the same situation *vis-à-vis* total insulation as if he were nude at the same ambient temperature but at somewhat lower wind velocity. (The distribution of skin temperatures in various body regions would, however, be different, the trunk being warmer and the limbs cooler than in the nude subject.)

The principal defect of the 'Four Inns Walk' clothing was the lack of insulation and protection from wind and wetting over the lower limbs. Even at rest in still air, skin temperatures over the legs and thighs were unduly low and accounted for the comparatively low mean skin temperatures. With wind and wetting, the skin temperatures over the lower limbs fell to 14° C, values at which rather severe muscle cooling would be expected at least in thin subjects[20,21]. This finding afforded a possible explanation for muscular weakness as an early symptom in exposure[16,22]. Further investigations of this aspect are being undertaken.

The evaluation of heat exchange. There are several uncertainties connected with the evaluation of heat exchanges by indirect calorimetry; among them are the estimation of evaporative heat loss and the evaluation of energy expenditure as mechanical work.

Evaporative heat loss. In clothing experiments a rather wide discrepancy may be observed between the change of nude weight and clothed weight over a given period of time, and this was the case in the present experiments. There are reasons for doubting whether either of these measurements gives an accurate estimate of evaporative heat loss from the skin in cool conditions. We have therefore used Belding's estimate of 6 kcal/m² h for evaporative heat loss from the skin in clothed subjects[3], and calculated respiratory heat loss from the measured volume of ventilation rather than attempting to calculate evaporative heat loss from weight changes. The second uncertainty in the evaluation of heat exchange is the estimation of energy expenditure as mechanical work. In bicycle ergometry we multiply the work performed by 1/427 to obtain its thermal equivalent and deduct this value from metabolism in calculating metabolic heat production. A similar procedure may be used to evaluate heat production in persons walking uphill, in which case one deducts from metabolism the thermal equivalent of the work done in lifting the body. On the other hand, in persons walking on the level, energy expenditure as external work is negligible[23]; and in going downhill there may well be heat gain from dissipation of potential energy. No direct measurements of the latter have yet been made,

although the energy cost of so-called 'negative' work has been a subject of interest to physiologists for many years.

In hill walkers, variation in mechanical work associated with changing terrain appears to be more or less balanced by variation in the rate of progress and therefore of oxygen intake. Consequently, metabolic heat production may remain reasonably uniform in spite of variation in oxygen intake. In recent observations on hill walkers, the oxygen intake of men walking at their natural pace was 2·5 l./min on ascents, 2·0 l./min on horizontal ground and 1·5 l./min on descents. If one deducts or adds 20 per cent for mechanical work during ascent and descent, respectively, the heat production for uphill, downhill and level sections seems to be much the same, that is, about $2 \times 5 \times 60 = 600$ kcal/h.

Fatigue and exposure. Although these experiments were conducted primarily for the purpose of determining Clo values, the results are also of interest in connexion with the physiology of hypothermia. The striking elevation of metabolism during standard exercise under cold stress affords a possible explanation of the early onset of fatigue in exposure casualties. A similar finding was reported by Adolph and Molnar[8]. Results such as these suggest that within certain limits metabolism rises to a level necessary to maintain thermal balance and that this may be achieved (*a*) by increasing the work-rate, (*b*) by shivering, or (*c*) by other unexplained means. In severe cold stress the metabolic demand may be so high that only very fit persons can meet it over a prolonged period.

A quantitative approach to this subject has been attempted in Fig. 2 by treating the tissues, the clothing and the air as thermal resistors in series along the pathway of heat flow from the central body mass to the surroundings[4], thus:

$$\frac{T_A - T_R}{H} = I_A + I_{Cl} + I_T = \Sigma I \qquad (6)$$

A difficulty arises from the fact that evaporative heat loss from the skin is deducted from metabolism in calculating I_{Cl}, but not in the calculation of I_T. However, for cold conditions evaporative heat loss from the skin is small and H can be taken as being equal to metabolism minus respiratory heat loss so that $H = 0{\cdot}9$ M. Fig. 2 shows the relation of $R_R - T_A$ to metabolism for various values of ΣI.

The chart can be used to predict the final value of rectal temperature for any given combination of ambient temperature, wind velocity, clothing insulation, tissue insulation and metabolism.

The following examples will serve to illustrate the method of calculation: From the results in Table 2 and Table 1*b*, I_{Cl} for wet clothing = 0·17 Clo, I_T during exercise = 0·51 Clo and I_A for gale force wind = 0·10 Clo, so that $\Sigma I = 0{\cdot}78$ Clo. At an ambient temperature of 0° C and oxygen intake of 1·5 l./min, the equilibrium value of T_R is 28·5° C and hypothermia would be inevitable. On the other hand, if mean oxygen intake is 2·0 l./min, the T_R at thermal equilibrium will be 37·9° C.

For a person in wet clothing who is sheltering from the wind at 0° C, $I_{Cl} = 0{\cdot}5$ Clo, $I_T = 0{\cdot}8$ Clo (in thin subjects I_T may be as low as 0·55 Clo at rest and 0·35 Clo during exercise), and $I_A = 0{\cdot}4$ Clo, so that $\Sigma I = 1{\cdot}7$ Clo. In order to prevent a rectal temperature falling below 37° C, oxygen intake must be 0·9 l./min or 3·6 times basal. Whether or not a person can manage this over a long period will depend to a large extent on whether or not he was exhausted on taking shelter. On the other hand, in dry clothing with waterproof over-garments, I_{Cl} will be 1·5 Clo and ΣI will be 2·7 Clo and oxygen intake need be only 0·56 l./min or twice basal.

The chart also brings out the fact that the effect of clothing and tissue insulation on rectal temperature is larger at high than at low metabolism; this means that relatively small variations in I_{Cl} or I_T become important at high rates of metabolism. For the same reason a person with average subcutaneous fat may be able to maintain a normal rectal temperature at a work-rate which will cause an unusually thin person's rectal temperature to fall dangerously low. Thus for the subject of these experiments ΣI for wet clothing and gale-force wind would be 0·78 Clo during exercise, and with oxygen intake of 2 l./min and ambient temperature of 0° C, he would have a T_R of 37·8° C. For a thinner person with ΣI of 0·68 Clo, the corresponding T_R would be 33·0° C.

Now in order to maintain his rectal temperature at 37° C the thin subject would need to raise his oxygen intake to 2·24 l./min, which is an increase of 12 per cent. Such an increase is small compared with the sixteen-fold possible range between basal and maximal oxygen intake. On the other hand, the associated 12 per cent increase in $T_R - T_A$, when $T_A = 0°$ C, amounts to $37 - 4 = 33°$ C, which is the difference between normality and incipient hypothermia. Thus apparently trivial differences in metabolism or total insulation can have disastrous consequences in the wet cold situation, in which exposure casualties commonly occur.

Thus in summary:

(1) The thermal insulation value of clothing worn in the 'Four Inns Walk 1964' (ref. 1) was 1·5 Clo units, which may be compared with 1·0 Clo for business clothing[4] and 3·0 Clo for polar clothing assemblies[5,6].

(2) The effects of exercise, wind and wetting on clothing insulation were studied. When the subject exercised in wet clothing in a 9-m.p.h. wind, clothing insulation fell to 0·17 Clo, which was 15 per cent of the control value for dry clothing in still air with the subject at rest.

(3) The thermal insulation of the body tissues was also determined and it was shown how apparently small changes in oxygen intake and insulation can have disas-

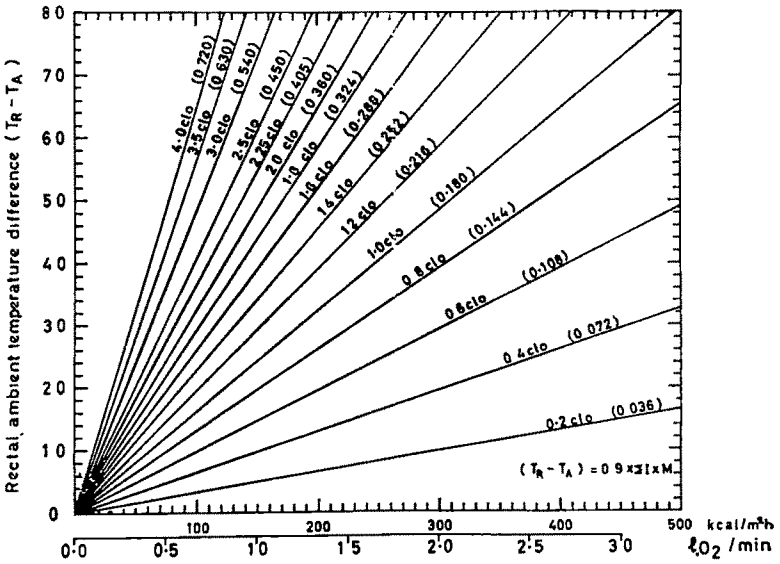


Fig. 2. Chart for predicting final body temperature (R) under given conditions of ambient temperature, total thermal insulation and metabolism. Ordinate shows difference between rectal and ambient air temperature ($T_R - T_A$) in ° C, abscissa shows metabolism in kcal/m² surface area/h and oxygen consumption for an average man of 75 kg weight and 2 m² surface area. The values of total insulation (ΣI) are given in Clo units with c.g.s. units in parentheses. $\Sigma I = I_A + I_{Cl} + I_T$. The equation of the graphs is $(T_R - T_A) = 0{\cdot}9 \times M \times \Sigma I$, ΣI being expressed in c.g.s. units. To convert Clo to c.g.s. units, multiply by 0·18

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OBITUARIES

Prof. Saul Adler, O.B.E., F.R.S.

Saul Aaron Adler died in Jerusalem on January 25, 1966, at the age of 70. He was the leading Jewish parasitologist of this century, yet so modest that he was always astonished when honours came his way, or at the crowds which gathered to welcome him at airports on his frequent journeys throughout the world.

Adler was born in the town of Karelitz in Russia in 1895, and five years later was taken to England where the family settled in Leeds. He went to school there and qualified in medicine during the First World War at the University of Leeds, at this time showing no clear signs of the brilliant future which lay ahead. Soon after taking his degree, he joined the Royal Army Medical Corps and served in the Middle East, which gave him his first taste of tropical medicine. It was natural, therefore, that on demobilization he should take the Diploma in Tropical Medicine of the University of Liverpool and join the staff of the Liverpool School. He went out to the Sir Alfred Lewis Jones Research Laboratory at Freetown in West Africa, and became immersed in the study of the parasites in the local inhabitants and animals. He was under the guidance of Profs. Blacklock and Gordon, and he realized the debt he owed them until the end of his life. In 1923, he married Sophie Husden of similar Russian Jewish extraction to his own, and they had two sons and a daughter, all of whom survive him. In 1924, he was called to Jerusalem by Chaim Weizman, at that time president of the Jewish Agency for Palestine, in order to develop the new Institute of Microbiology. He was soon appointed professor and was associated with the Hebrew University for the remainder of his life.

Adler's researches led him, however, far afield; his main subject of interest was leishmaniasis, in all its forms, and at every stage of its life-history. He investigated the disease around the Mediterranean basin and far into the Middle East; and, in later life, in East Africa, China, Brazil and Panama. In every phase of his investigations, he tried to start with the basic elements. Thus, a good experimental host for the parasite was lacking, so he sought a suitable animal, and found one in the golden hamster in the desert near Aleppo. He colonized it in his institute and, with typical generosity, distributed the progeny to laboratories everywhere. The invertebrate host of *Leishmania* was unknown when Adler began his research, although species of *Phlebotomus* were suspected. With O. Theodor, he made a profound study of these insects, beginning with the anatomy and physiology of their mouth parts and alimentary tract, continuing with observations on the behaviour of cultures of the parasites in the gut and their exit through the proboscis in the act of biting, and, finally, demonstrating transmission of *L. tropica* to volunteers by experimentally infected sandflies. From the experimental host, and the invertebrate vector, Adler passed to work on the parasite itself, and he became convinced, after a lifetime of observations, of the multiplicity of species which exist in the genus *Leishmania*. Biological and clinical features of the disease had at first led him to this opinion, which, in the final years of his life, he was able to confirm by a serological reaction, the technique of which he demonstrated to students in Israel, Britain, Italy, Brazil and elsewhere, realizing that he had not much time left for establishing the unique usefulness of this test.

A leishmanial stage in the course of the life-cycle of *Trypanosoma cruzi*, the cause of Chagas's disease, naturally aroused the interest of Adler and, on visits to Brazil, he became well acquainted with this infection. The disease, however, had another attraction for him, because his second great interest was Darwin and evolution; he had studied Darwin's life and works in the greatest of detail (translating his *Origin of Species* into Hebrew), and, in 1959, he put forward the hypothesis that Darwin suffered and died from Chagas's disease. He showed that Darwin must almost inevitably have become infected with *T. cruzi* on landing in a highly endemic area of South America in 1835 and that the chronic invalidism, including angina, which followed was characteristic of the cardiac form of Chagas's disease. Adler applied the subject of evolution to protozoa, and showed the operation of its laws not only in the species complex of *Leishmania*, but also in *Trichomonas*, insect crithidia, and in rodent malaria parasites.

Malaria was another subject on which Adler's original mind cast light. His first researches in West Africa were devoted to species of *Plasmodium* present in the local chimpanzees and lizards, and it was during his sojourn in Sierra Leone that he first used himself as a human volunteer, attempting without success to pass the parasites of the chimpanzee to man. He never ceased to carry out such experiments, which were more successful in leishmaniasis and relapsing fever; in fact, an infection of the latter nearly killed him. From work on the parasites of apes, Adler changed in the later years of his life to a study of the plasmodia of rodents, and produced interesting adaptations of *P. vinckei* in hamsters. He was convinced that this field in general offered possibilities of great promise to the geneticist.

Adler was the recipient of many honours; he most prized his election as a Fellow of the Royal Society in 1957; he appreciated the award of O.B.E. from his 'second' country, Great Britain, in 1946, and of the Israel Prize for Medical Science on his deathbed in Jerusalem. Other honours included the Laveran Medal, the Gaspar Vianna Medal, the Chalmers Medal of the Royal Society of Tropical Medicine and Hygiene, the Order of the Cross of the Phoenix of Greece, and the honorary degree of doctor of science conferred on him by the University of Leeds in 1965. Adler's life was too short to enable him to carry out everything he would have liked to do. He had still much research before him in parasitology; like his father, he was a Hebrew scholar and wished to work on the Dead Sea

Scrolls; he could have made his name in pure mathematics, chess or literature. Adler created a school of ardent followers in Israel; his influence was scarcely less in many other parts of the world, where his many friends and students deplore his passing. P. C. C. GARNHAM

Dr. Maurice N. Hill, F.R.S.

THE sudden death at Cambridge of Maurice Hill, on January 11, 1966, at the age of 46, has made the lives of many people the poorer. It is difficult to think where he will most be missed, so wide were his interests: whether it was as research scientist, supervisor of research students, devoted college man, tireless member of committees, or simply as a warm, lovable family man that he made most impact, is a question that fortunately does not need answering. However, it is certain that it was his warmth that was the key to everything.

He was born on May 29, 1919, son of Prof. A. V. Hill, and, after schooling at Highgate, set out on an undergraduate career at King's College, Cambridge, in 1938, which was interrupted by the Second World War. He left Cambridge in 1939 and was assigned to the Anti-Submarine Establishment at Portland; later he moved to the Establishment at Edinburgh that was developing methods of sweeping magnetic and acoustic mines. Here he made many friends, including Ben Browne, Edward Bullard, Leslie Flavill and Tom Gaskell, with whom much of his later work was done.

When the War ended, Hill had definite plans to carry out research into marine geophysics at Cambridge, but he had first to complete his undergraduate training. He had married Philippa Pass in 1944 and, having now a son, he took his degree as soon as possible, to move into the less restrictive world of academic research. He was to stay with the Department of Geophysics at Cambridge for the rest of his life. His Ph.D. was on the subject of marine seismic prospecting. It was a notable dissertation—he had developed a new technique of geophysical exploration and it was soon to become the most valuable tool available to marine geophysicists.

In 1949 he became an assistant in research and from that time he was able to devote himself fully to elucidating the nature of the Earth's crust and upper mantle beneath oceans; it was soon realized that the relative thinness of the oceanic crust in comparison with the continental crust enabled us to learn much about the forces and processes at work in the mantle, and Hill ensured that British contributions to knowledge about ocean basins and what lay beneath were of high standard. The *Challenger* Expedition of 1950–53, which he helped to organize, was a spectacular example of this, but there were many other expeditions to specific oceanic features by means of which Hill, with his colleagues, gradually got the 'feel' of sub-oceanic geology.

Instruments for this purpose steadily developed—the precision echo-sounder, the towed magnetometer, the sea gravimeter, heat flow apparatus, improved corers, long-range seismic apparatus, ocean bottom seismometers, a reflexion seismic profiler and a deep diving magnetometer. Hill was responsible for the evolution of many of these, and he and his growing number of research students put them to intensive use.

It could well be said that 1963 was an *annus mirabilis* for Hill. Apart from being chief scientist on R.R.S. *Discovery* during the highly successful geophysical ventures in the Indian Ocean, he also saw published, under his editorship, *The Sea*—three very comprehensive volumes on physical and chemical oceanography and the underlying rocks.

Apart from his continued assault on the Atlantic Ocean, in recent years he was much concerned to improve sea-going facilities for university research departments—he was largely instrumental in the conversion of a ship for university use; this will come into service later this year.

He became a Fellow of King's College in 1949 and during 1961–65 was director of studies in natural sciences there. In 1954 he was made assistant director of research in the Department of Geophysics and in 1965 became reader in marine geophysics. He was elected a Fellow of the Royal Society in 1962, and, in 1963, was awarded the Charles Chree Medal and Award of the Physical Society.

And yet, effective scientist that Hill was, it was his humanity that left an indelible impression. The practical side of sea-going places greater demands on a scientist than mere intellectual distinction and cleverness. An apparently paradoxical combination of the rugged and gentle is necessary, as is more than average common sense and a deep understanding of people. All these he possessed in great measure. It goes without saying that he was an admirable supervisor of research students to whose problems, scientific and otherwise, he was always responsive. Not for him the occasional formal interview with his students—they used to occupy him full time, and he would not have had it otherwise. He was aware that the strength of a science lies in a steady inflow of young research workers, and their well-being and happiness are of fundamental importance. To Maurice, the human element of science—the encouragement, sympathy, support, and even just the attentive ear—was uppermost.

One aspect of his life that was characteristic and which extended beyond the bounds of science was his generosity. He and his wife were deeply concerned with the problems of anyone in need. It is impossible to guess how much charitable work he did—most of it was never revealed—but his giving was both munificent and imaginative.

He was a man of many aspects, all of them genial. He will be remembered with affection far beyond the scientific community. D. DAVIES

NEWS and VIEWS

Experimental Pharmacology in the University of Strathclyde: Prof. W. C. Bowman

DR. W. C. BOWMAN, at present reader in pharmacology in the School of Pharmacy, University of London, has been appointed to a professorship in experimental pharmacology in the University of Strathclyde. Dr. Bowman was a student, research student and lecturer at the School of Pharmacy. After qualification he worked with Dr. (now Prof.) E. J. Zaimis on neuromuscular transmission. He has been interested in the mechanism of action of neuromuscular blocking agents. Working with Dr. M. J. Rand, he found that triethylcholine produced a temporary effect in the cat and rabbit which closely resembled that seen in man in the case of the syndrome myasthenia gravis. He found that triethylcholine had a prejunctional site of action and suggested that the compound might be of use in the treatment of neurogenic spastic states. More recently he has been interested in the effect of adrenaline in potentiating the contraction of skeletal muscle and has investigated the effect of sympathomimetic amines on both innervated and chronically denervated skeletal muscles. Bowman has contributed a great deal towards the success of pharmacology at the School of Pharmacy, both as a teacher of undergraduates and of postgraduates. He has proved a most lucid and

stimulating teacher and has enabled many students to achieve success in this subject. He has published many papers and has given communications and demonstrations to societies both in Britain and in the United States. He has become an international authority on this work. Dr. Bowman will take up his post at the University of Strathclyde on September 1.

American Association for the Advancement of Science

Dean Don K. Price, jun., assumed office as president-elect of the American Association for the Advancement of Science on January 15. He is one of the few social scientists to hold this office, the most recent being the economist, W. C. Mitchell, in 1938. Since 1958 Don Price has been Dean of the Graduate School of Public Administration of Harvard University, and has the broad background necessary to guide the association, the wide objects of which are "to further the work of scientists, to facilitate co-operation among them, to improve the effectiveness of science in the promotion of human welfare . . .". Don Price was a Rhodes scholar at Oxford from 1932 to 1935.

Aid to India

In reply to a question in the House of Commons on February 17, the Prime Minister, Mr. H. Wilson, said that the Governments of the United Kingdom, Canada, Australia and New Zealand, and of some non-Commonwealth countries, were already in close touch with each other and with the Government of India regarding the threatened famine in India. Britain had made an immediate and interim contribution in the form of an interest-free loan to India of £7·5 million. The Australian Government on February 17 announced a very large shipment of food to India, and the problem was, above all, one of port capacity and internal distribution; some very massive shipments of food were being organized. Replying for the Prime Minister on February 24, Mr. G. Brown added that the Government was in touch with the Indian Government about supplies of food which could be made available if they were needed. Discussions about further aid were still in progress.

Anglo-French Co-operation in Oceanography

In reply to a question in the House of Commons on March 3, the Minister of State for the Department of Education and Science, Mr. R. E. Prentice, said that details of the French Government's intentions for undersea research had not been made known to the British Government, but there had been unofficial exchanges between scientific organizations in the two countries, and the Secretary of State was prepared to consider any proposals that might be made. British oceanographers and geographers at research establishments of the Natural Environment Research Council and at universities had co-operated with French scientists in such research and would continue to do so. The Natural Environment Research Council was at present reviewing the future orientation of oceanographic research in Britain. Both Britain and France, along with other countries, were represented on the Inter-Governmental Oceanographic Commission.

Research in Mental Health

The debate in the House of Commons on February 18 on Mr. W. Hamling's motion to transfer responsibility for the education and training of all mentally handicapped children from the Ministry of Health to the Department of Education and Science was noteworthy for the repeated reference to the need for more research in this field. Sir David Renton, commenting on the unevenness of scientific and social advance, pointed out that the great progress in antibiotic therapy had intensified the problem of dealing with the mentally handicapped. Progress in detecting, preventing, and curing mental defects did not match the advances in antibiotics and obstetrics and there was immense scope for further research. Dr. E. Summerskill also referred to the growing magnitude of the problem and advocated the establishment of an Educational Research Council. At present, only about twelve people in Britain were investigating the learning processes of severely sub-normal children, though Dame Joan Vickers paid tribute to the quality of some of this work. She herself suggested that the Ministries of Education, Health and Labour should set up an interdepartmental committee to review the scale of the problem. Mr. J. Snow was concerned at the disparity between the scale of mental health research and the administrative action of the Ministry of Health in this field. He urged that more attention be paid to the problem of the autistic child. Lord Balniel strongly urged more support of research into the learning processes of backward children and referred to the work now in progress at the University of Manchester. Mr. L. Pavitt fully supported those who urged more research into the problems of the mentally handicapped, as did Mr. A. Mackenzie and Sir Edward Boyle, who added that the limiting factor to-day was the supply of trained workers for such research. On the proposed transfer of responsibility from the Ministry of Health to the Department of Education and Science, Sir Edward, like numerous other speakers, had reservations, while welcoming the motion generally. The Parliamentary Secretary to the Ministry of Health, Mr. C. Loughlin, replying on the debate, warmly welcomed the pleas for more research into mental health generally and promised to see that research was as far as possible extended, but he had reservations on the proposed transfer of responsibility, and the resolution was, by leave, withdrawn.

Durban Museum and Art Gallery

The director of the Durban Museum and Art Gallery reports that during 1964–65 the Department of Local History was moved from the Old House to the refurnished Old Court House (*Annual Report of the Durban Museum and Art Gallery for the Municipal Year 1964–1965*. Pp. 18. Durban: Durban Museum and Art Gallery, 1965). The Old House was later altered and redecorated and will shortly be opened as a period house. Work on the displays in the Museum was devoted to the installation of the new geological exhibits and much time was spent in planning the rearrangement of the Hall of South African Birds. Collecting trips included one to Northern Natal and another to the eastern area of Cape Province.

Oil and Gas Pipelines in Europe

The network of large pipeline systems established in Europe for the transportation of crude petroleum, refined products, and gas, has now reached an advanced stage in development, which has so far taken only about 12 years to realize. During this comparatively short period, some 1,700 miles of large diameter crude oil pipelines have been built in various systems, delivering more than 70 million tons of crude oil per annum to European refineries. Such are the projects now planned, some well under way, that within the next 2 years more than 500 miles of pipeline, capable of delivering a further 50 million tons of crude oil to Central Europe each year, will, it is anticipated, be completed. Implicit in this vast network are a number of 'products pipelines' designed for the speedy transference of a full range of refined petroleum products, including fuel oil, from strategically placed refineries to the major centres of consumption, and many more such lines are already being planned. In addition, the gas industry has already completed many hundreds of miles of high-pressure gas distribution pipelines; one result of this is to make obsolete the many small individual gas-producing plants that previously existed.

Among the most ambitious of these European oil pipelines is undoubtedly the Trans-Alpine Pipeline (commonly known as TAL), from Trieste, at the head of the Adriatic, to Ingolstadt in South Germany, the centre of a large refinery complex; some details of this venture have already been recorded in these columns (*Nature*, **208**, 435; 1965). The largest pipeline at present operating in Europe, completed in 1962, runs 782 km from L'Avera on the Mediterranean, northwards to feed refineries in Strasbourg, Karlsruhe and Mannheim. This pipeline is sponsored by the Société Du Pipe-Line Sud Européen (SPLSE), a consortium of major oil companies operating in France and Western Germany (including Compagnie Française des Pétroles—a French Government concern); by means of another system (Rhine Donau Oelleitung, RDO), supplies are fed to other refineries in Bavaria. Other notable lines are from Ludwigshafen to Wilhelmshaven (Nord West Oelleitung, NWO); also the Central European (CEL) system serving Italy, Switzerland and Southern Germany. An informative article, with a good explanatory map of locations of existing and projected pipelines and refineries, by R. E. Watkins, is included in what is, most regrettably, the last issue of *BP Magazine* (British Petroleum Company, London. No. 18; winter 1965). This magazine first appeared in 1960 and it has consistently set a remarkably high standard in design, illustration and presentation of subject-matter.

The San Mateo–Hayward Bridge, California

A NEW bridge of unusual design is under construction which will eventually carry four to six lanes of traffic across the southern part of San Francisco Bay, California, when it is completed in 1967. This bridge will incorporate nearly a mile of orthotropic spans in its 7-mile length. In this particular orthotropic design, the deck carries most of its own weight and this eliminates the need for the overhead steel supports and cables common to conventional steel bridges. Use is made of new high-strength steels, pressed into corrugated form; the resulting ribs possess enough strength to enable the deck to be self-supporting over spans of up to 750 ft, the length of the central span in this new bridge. It is claimed that the orthotropic design for bridges offers advantages both in economy and speed of construction. A similar bridge has already been completed from Montreal to an island in the St. Lawrence River, the site of the 1967 World Exhibition. According to an article entitled "A Big Order" (*The Lamp*, Standard Oil Company (New Jersey), New York, **47**, 1965), all supporting towers of the San Mateo Bridge will be aluminium in colour, the body of the bridge being in gold. A remarkable ancillary feature of this construction is the very large quantity of the protective coatings required for coating the steel of the structure. The Humble Oil and Refining Company (a subsidiary of Standard Oil Company (New Jersey)) is scheduled to supply 25,000 gallons of undercoat ('Rust-Ban 191') and 125,000 gallons of vinyl-based top-coat to protect all the steel. These protective coatings are being applied to the prefabricated steel sections in a specially constructed building 15 miles away from the site, to which the sections are afterwards transported by barge.

Syntex Corporation of America

THE Syntex Corporation is internationally known for its pioneer research activities and manufactured products in the pharmaceutical industry. It operates through various marketing subsidiaries, of which, in the United States, Syntex Laboratories, Inc., with administrative and research centres at Palo Alto, California, is the principal. Other subsidiaries are located in Mexico (Syntex, S.A.), in Canada (Syntex, Ltd.), while in England the group has now established its own marketing organization to cover Great Britain and most Commonwealth countries. To accelerate the dissemination of medical and marketing information to distributors of Syntex products, new regional offices have been opened in London, Madrid and Milan, to augment the work of the centre previously established in Zurich. Among the distributors of Syntex products are such well-known firms as Imperial Chemical Industries, Ltd. (United Kingdom); Laboratories Cassenne (France); Chemie Gruenenthal GmbH (West Germany, Austria and Switzerland); Instituto Farmacológico Latino, S.A. (Spain); Recordati Laboratorio Farmacologico S.p.A. (Italy); and Tanabe Seiyaku Co., Ltd. (Japan). According to the *Annual Report of the Syntex Corporation for the Fiscal Year ending July 31, 1965* (Pp. 24. Panama: Syntex Corporation, 1965), substantially increased sales resulted in net earnings of more than 10 million dollars, the highest in the history of the Corporation. Sales for the United States market constituted 46 per cent of total sales in the fiscal year 1965, Europe accounted for 29 per cent, Latin America for 14 per cent, and other countries 11 per cent. Of the drugs and other products with which Syntex is concerned may be mentioned steroids for treatment of inflammatory diseases, including the 'Synalar' group comprising compounds for treatment of skin conditions; drugs used in the area of reproductive physiology, consisting primarily of several oral contraceptives; steroid intermediates, and finished sex hormones. Animal health products feature strongly in another category of Syntex manufacture. During the year under review, a new form of 'Synalar' (fluocinolone acetonide) was introduced to veterinarians in the United States designed for animal therapy. This was followed by the drug 'Flucort' (flumethasone), a new anti-inflammatory corticoid used in treating various rheumatic, allergic and metabolic disease conditions in large and small animals.

The California Bearing Ratio Test on Loess

THE California bearing ratio test, one of the mainstays of soil mechanics and conventionally popular with civil engineers, especially when concerned with highway and aerodrome runway construction, has undergone some critical modifications both in technique and interpretation, since O. J. Porter first described his original methods in two papers, "The Preparation of Subgrades" and "Foundations for Flexible Pavements" (*Proc. U.S. Highways Res. Board*, **18**, Part 2, 1938; and **22**, 1942, respectively). Porter's original text was essentially a soil classification test, as we know it to-day. With the advent of the Second World War, the U.S. Corps of Engineers had to build many aerodromes and access roads under many different subgrade conditions, often in a very short space of time. From various flexible pavement design methods the California bearing ratio test was selected, and after a thorough examination of its merits and applications, the U.S. Corps of Engineers made some important changes in Porter's original procedures. These were, briefly, the use of dynamic compaction instead of static compaction; the compaction of soil to dry density and the use of the moisture content expected during compaction in the field (thus the test became a measure of soil shear-strength, not just a classification test); a correction was also introduced for California bearing ratio load-penetration curves (initially concave-upwards). After 1945, the test was universally adopted in highway construction work. In Britain, however, further amendments were made, such as dispensing with soaking procedure, and reversion to static compaction. It is clear from a timely paper by J. P. Blakeley entitled *The California Bearing Ratio Test on Loess* (National Roads Board: Road Research Unit. Bulletin No. 2. Pp. 18. Wellington, New Zealand, 1965) that further modifications of this test are both desirable and imminent. As he points out, the trend towards the use of bound base-courses and stiffer surfacings is causing difficulty in the application of the California bearing ratio test, and this method of pavement design will have to be modified to make rational allowance

for the all-important load-spreading properties of the pavement. The author divides his paper into two parts. The first part consists of a review of the California bearing ratio test, and the second deals with relationships between soil dry density, compaction moisture content and soaked/unsoaked bearing ratio. The first part is an objective survey of the California bearing ratio test past and present. The second gives the results of considerable experimentation in terms of its title, conducted on one soil only, Heathcote Loess; but he finds that trends in the variation of bearing ratio values with moisture content and density should be true of a variety of soils.

A Virus Disease in Crustacea

Prof. C. Vago, of the Laboratoire de Cytopathologie, Saint-Christol-les-Alès, has written to the Editor reporting the incidence of a virus disease in a crustacean. The virus diseases so far described in invertebrates have been either from insects or from Acaria. The present virus was observed in *Macropipus depurator* L. (*Portunus depurator*). Diseased animals collected on the French 'Languedoc' coast of the Mediterranean showed a slow development of paralysis; sometimes a slight darkening was observed at later phases of the disease. Inoculation of blood from diseased animals into healthy ones provoked these symptoms. An infectious fraction was obtained from the suspension of homogenized muscle, heart and gland tissue by filtering them through 450-mμ 'Millipore' membranes and by alternate centrifugation at a maximum of 30,000 r.p.m. An electron microscopic study of this fraction, after negative staining with phosphotungstic acid, revealed numerous paraspherical virus bodies, 50–60 mμ in diameter (Fig. 1). The same viruses were also obtained from the tissue suspension by purification with trichlorotrifluorethane. Histological investigations of infected tissues and further analysis of the virus are in progress.

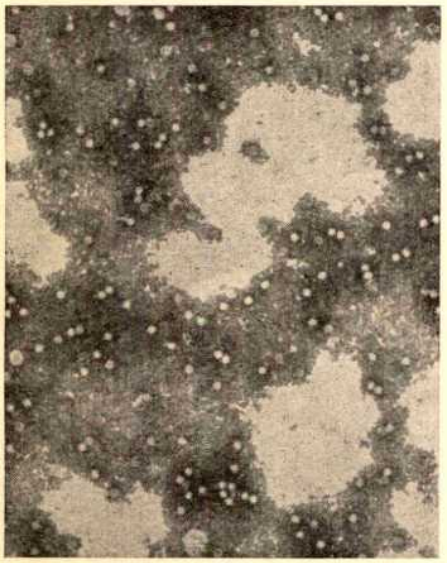


Fig. 1. Virus bodies isolated from paralysed *Portunus depurator*. Negative staining with phosphotungstic acid (×30,000)

Hot Springs and Fumaroles at Jebel Marra, Sudan

D. Hammerton, of the Hydrobiological Research Unit, Faculty of Science, University of Khartoum, has written to the Editor as follows: "The volcano of Jebel Marra (3,024 m. latitude 24° 16′ E., longitude 12° 58′ N.) in Darfur Province, Sudan, has hitherto been regarded as extinct. It consists of a well-developed caldera 3 miles in diameter with a central cone containing a deep crater lake. A larger, shallow lake occupies part of the eastern floor of the caldera. While engaged on a biological investigation of the two lakes in January, 1966, I made two discoveries which suggest that the volcano must be regarded as dormant. First, a series of hot springs, ranging in temperature from 65°–85° C, were found along the north-west margin of the inner crater lake. The volume of flow is probably greater than can be accounted for by precipitation and it is suggested that the springs consist either of juvenile water or of water which leaks through the floor of the lake and is subsequently heated and forced to the surface. Secondly, exploration of the outer north-west slopes of the inner crater revealed the presence of a valley in which there are numerous fumaroles emitting hot gases under pressure. The whole valley was filled with a strong smell of sulphur dioxide, and numerous dead birds and insects were observed in the vicinity of each fumarole. Three adjacent valleys, though not explored in detail, appeared to contain similar fumaroles. This record of fumarolic and hot spring activity is of considerable vulcanological interest in Africa because of the northerly position of the volcano. Detailed chemical analyses have been made of the waters of the lakes and of the hot springs in conjunction with biological studies, and the results will be published elsewhere."

Research Facilities at Naples, Plymouth and Windermere

How many workers in the biological sciences to-day are aware of the research facilities available at the Biological Stations at Plymouth and Windermere and the Naples Zoological Station? The British Association for the Advancement of Science was actively concerned in the foundation of all three of these Stations, and its continuous financial and moral support has entitled it to table space and the use of research equipment and other material at the Stations. The opportunity to use these facilities—in some respects unique—is open to teachers, senior students and research workers in universities, university colleges and other educational or research establishments who apply to the British Association. There is no limit to the time available to applicants at the Marine Biological Station, Plymouth, and the Freshwater Biological Station, Windermere; but at the Zoological Station, Naples, the Association is entitled to only one month's table space during any twelve months running from July 1 to the following June 30, and for administrative reasons applications should be submitted as long in advance as possible. Successful applicants in all cases will be offered a place, free of charge, in the laboratory in question, and the use of the equipment and material necessary for carrying out the programme of work involved. Applications should be addressed to the Secretary, British Association for the Advancement of Science, 3 Sanctuary Buildings, 20 Great Smith Street, London, S.W.1, and should include a statement of qualifications, the work in which it is desired to engage, and the dates for which the use of table space is required. Applications will be submitted to a committee of the Association (and in the case of the Naples Station to a Committee of the Royal Society) for approval.

Scientific Research in France

An article in *Le Progrès Scientifique* (No. 91, December 1965) analyses expenditure on research and development in France in 1963. Public expenditure was 4,508 million francs, of which 110 million francs was for space research and 1,453 million francs for military research. A further 2,073 million francs was expended by private enterprise on industrial research, about 18·7 million francs by non-profit institutions and about 82·9 million francs was provided by international organizations, making a total of 6,249 million francs. Of this total, 2,267·5 million francs was spent in the State sector, 844·1 million francs in the educational sector, 3,103 million francs in the industrial sector, and 34·2 million francs by non-profit institutions;

3,347 million francs was spent on research and 2,901 million francs on development.

Scientists in Mexico

PART I (*A–G*) of the volume on Mexico in the series *Scientific Institutions and Scientists in Latin America*, issued by the Centre for Scientific Co-operation in Latin America of the United Nations Educational, Scientific and Cultural Organization and the Organization of American States, contains the information regarding scientists in Mexico which was contained in the earlier three volumes issued in 1951, and in 1955 brought up to date and with many new references (Pp. 372. Montevideo, 1965).

Royal Geographical Society: Awards

THE Royal Geographical Society has announced the following medals and awards for 1966: *Patron's Medal*, Prof. E. J. H. Corner, leader, Royal Society Expeditions to North Borneo and the Solomon Islands; *Founder's Medal*, G. Hattersley-Smith, for glaciological investigations in the Canadian Arctic; *Victoria Medal*, G. R. Crone, librarian and map curator, Royal Geographical Society; *Murchison Grant*, Prof. S. G. Davis, University of Hong Kong; *Back Grant*, Dr. S. Evans, Scott Polar Research Institute, Cambridge; *Cuthbert Peek Grant*, Lieut. Commander M. K. Burley, leader, Combined Services Expedition to South Georgia; *Gill Memorial*, P. Haggett, University of Cambridge; *Mrs. Patrick Ness Award*, E. C. Evans and J. P. M. Long, Welfare Branch, Northern Territory Administration, Government of Australia.

University News: Belfast

DR. K. J. IVIN, at present reader in physical chemistry in the University of Leeds, has been appointed to the chair of physical chemistry.

Bradford Institute of Technology

DR. A. BOTTOMLEY, at present Ford Foundation adviser to the Institute of Public Administration and Development of the Lebanese Government, has been appointed professor of economics. Dr. W. R. A. D. Moore, reader in high polymer chemistry at the Institute, has been appointed professor of polymer science.

London

PROF. A. C. OFFORD, at present professor of mathematics at Birkbeck College, has been appointed to the chair of mathematics at the London School of Economics and Political Science. Dr. G. R. Davis, resident mining consultant in Northern Rhodesia to the British South Africa Co., has been appointed to the chair of mining geology tenable at the Imperial College of Science and Technology. Dr. P. J. Grant, at present reader in engineering science at the Imperial College of Science and Technology, has been appointed to the chair of nuclear power tenable at that College.

Announcements

DR. N. P. ALLEN and Dr. O. Simpson have been appointed deputy directors at the National Physical Laboratory as from April 1. Dr. Allen, who joined the National Physical Laboratory as superintendent of the Metallurgy Division in 1944, will become deputy director (A), and Dr. Simpson, who joined the Laboratory in 1963 as superintendent of the Basic Physics Division, will become deputy director (B).

THE sixteenth annual meeting of the Société de Chimie Physique will be held in Paris during May 31–June 3. Further information can be obtained from Prof. G. Emschwiller, Société de Chimie Physique, 10 rue Vauquelin, Paris 5.

A COURSE on "Life in the Past", arranged by the Joint Biology Committee, will be held in the University of London on May 21. Further information can be obtained from the Treasurer, Joint Biology Committee, 11*a* Lonsdale Gardens, Tunbridge Wells, Kent.

AN international symposium on "Electron Microscopy and Cytochemistry" will be held in Leiden during May 30–June 4. Further information can be obtained from the Secretariat of the Symposium, Laboratory for Electron Microscopy, 62 Wassenaarseweg, Leiden.

A REVIEW symposium on "Modern Applications of Thermoelectricity" will be held at the Welsh College of Advanced Technology during April 20–22. Further information can be obtained from the Organizer of Short Courses, Welsh College of Advanced Technology, Cathays Park, Cardiff.

A SYMPOSIUM on "Traffic Safety—a National Problem" will be held in Washington on April 28, during the second annual meeting of the National Academy of Engineering. Further information can be obtained from the Secretary, National Academy of Engineering, 2101 Constitution Avenue, Washington, D.C.

A CONVENTION on "Thermodynamics and Fluid Mechanics", arranged by the Thermodynamics and Fluid Mechanics Group of the Institution of Mechanical Engineers, will be held in Liverpool during April 13–15. Further information can be obtained from the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

THE spring meeting of the Western States Section of the Combustion Institute will be held at the Denver Research Institute during April 25–26. The subjects under discussion will be: fire research; pyrolysis of solid fuels and oxidizers; high-pressure, condensed-phase reactions; metal combustion. Further information can be obtained from the Secretary, Western States Section, the Combustion Institute, 16902 Bollinger Drive, Pacific Palisades, California.

AN international symposium on "Generalized Networks", arranged by the Microwave Research Institute of the Polytechnic Institute of Brooklyn, in co-operation with the Institute of Electrical and Electronics Engineers, the Optical Society of America and the Society for Industrial and Applied Mathematics, will be held in New York City during April 12–14. Further information can be obtained from the Symposium Committee, Polytechnic Institute of Brooklyn, 333 Jay Street, Brooklyn, N.Y.

A SYMPOSIUM on "Advances in Polymer Characterization", organized under the auspices of the Manchester and District Advisory Council for Further Education, will be held in the University of Manchester during April 20–21. The programme will include sessions on: solution properties and the determination of molecular weight distributions; characterization of tacticity; properties of amorphous polymers; crystallinity and crystallization. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Manchester.

THE fifth general meeting of the International Mineralogical Association will be held at Cambridge during August 30–September 3. The programme will include symposia on crystal growth with special reference to minerals or bonding forces in mineral structures and on pyroxenes and/or amphiboles, and an evening session on problems of the quantitative measurement of reflectivity in ore minerals. Further information can be obtained from the Local Secretary, International Mineralogical Association Meeting, Department of Mineralogy and Petrology, Downing Place, Cambridge.

EXTENDING THE USEFULNESS OF MATERIALS

THE Materials Science Club held the third of its 1965 series of technical meetings on December 14 and 15 at the Atomic Energy Research Establishment, Harwell (see *Nature*, 206, 335; 1965). The meeting was divided into three sessions: the first was devoted principally to recent advances and future prospects in diverse fields such as ferrous and non-ferrous metals, ceramics, concrete, glass, paper, plastics and graphite. The second session covered general trends of development in materials science with particular emphasis on the inter-relationship between different fields. After a brief introduction covering the economics of new materials development, the final session was devoted almost entirely to a general discussion of the topics already covered during the meeting.

Recent advances in particular fields. The first material, graphite, was surveyed by Prof. A. J. Kennedy (the College of Aeronautics, Cranfield). Conventional polycrystalline graphites form a range of very varied materials the properties of which depend strongly on the origin of the cokes used as starting materials. The methods of preparation and purification of graphite can also profoundly affect the properties, as can be seen from the increase in density. That of conventional graphite lies in the range 1·5–1·9 g/ml.^{3}, recrystallized material is raised to 2·1, and the highly oriented pyrolytic form (deposited from the vapour phase) has a value of 2·2. The highest figure, that for a single crystal of graphite, is 2·26.

In principle, graphite is an outstanding material for high-temperature applications (with strength increasing to a maximum at about 2,700° C), and much effort has been devoted to developing new forms which might pay dividends in this respect. Graphites are characterized by a relatively high porosity—20–30 per cent is typical—and it is possible to achieve an improvement in mechanical and thermal properties by such techniques in impregnation and hot working.

The pyrolytic graphites are highly anisotropic, a feature which has both limitations and advantages. The fact that they possess high strength parallel to the crystal planes at 2,700° C, and that heat is rapidly and evenly distributed along the surface, makes their use in rocket engines and nose cones of considerable importance. The utilization of graphite in nuclear engineering and particularly in advanced gas-cooled reactors raises another singular feature of its behaviour, namely, its ability to reduce the internal stresses developed by irradiation through the mechanism of irradiation creep. The properties of graphite fibres, although they have only about half the mechanical strength of carbon fibres, are interesting, particularly at elevated temperatures.

In the discussion, attention was centred on the nature of the voids in graphites which apparently are normally small intercrystalline spaces (5–10μ). These, in addition to being able to be filled by impregnation, can also provide accommodation for creep. Delamination of graphite was found to occur because of the anisotropy of shrinkage resulting from the cone or circular type of growth exhibited in pyrolytic graphites. The high resistance to corrosion of graphitic materials was of value when used in the electrolysis of brine, and it was suggested that the particular orientation of graphite provided a system most capable of accommodating and resisting corrosion stresses.

The second paper, on "Steel Plant Refractories for High Temperature Processes", was given by Dr. J. N. Chesters (United Steels Co., Ltd., Rotherham). He began by summarizing the development of steel-making techniques from the original crucible process to the present-day electric arc furnaces. More than half the refractories produced in the world are consumed in steel-making furnaces. During the past decade there has been a radical change due in the main to the introduction of tonnage oxygen in the Bessemer-type of furnace. This has resulted in operating temperatures at least 100° C higher than normally used and concentrations of iron oxide many times those previously experienced. The use of high-current arc furnaces has also contributed to higher temperatures. Although material developments have arisen from work on atomic energy and rocketry, these have proved largely inapplicable to steel-making due to their instability under oxidizing conditions and their inadequate resistance to iron oxide. It seems that the development of suitable materials for furnace linings is very likely to come from the use of modified conventional materials; for example, in the use of denser materials of higher purity fired at unusually high temperatures. As a replacement for the conventional fire-clay and silica bricks, increased use has been made of basic refractories, particularly dolomite and magnesite. Many of these are used in the unfired state—frequently tar bonded—and it is increasingly certain that the carbon resulting from the tar plays a vital part in resisting slag attack.

Even the densest of these dolomite refractories are, however, proving inadequate for the worst conditions, and the latest swing is towards increasingly pure magnesia either in the fired or unfired state. Bricks made from relatively pure magnesia, fired at high temperatures, are also possessed of unusually high thermal shock resistance. A further decrease in porosity would be likely to improve this performance still more. Another major development has been the use of high alumina (85–98 per cent) bricks.

In the discussion which followed, Dr. Chesters instanced many cases of anomalous behaviour in refractory furnace linings, and pointed out that very little was really known about the atmosphere inside the furnace and the precise mechanism of attack. In one case, for example, a layer of special high-resistance material set in dolomite was found to have distorted considerably more than expected. The question of whether or not a better performance was obtained with fired or unfired materials probably depended on the relative conductivities and the penetration of the slag melt into the refractory. Graphite had been used as a furnace-lining material in the United States, but it was known that graphite burns rapidly in the presence of oxygen, and therefore some experiments had been done to reduce the oxygen concentration in the furnace atmosphere by replacement with nitrogen or steam. There was a suggestion that the considerable increase in temperature in certain areas of the lining leading to rapid breakdown was due to the influence of plasma jets which, of course, were capable of generating extremely high temperatures.

Mr. J. P. Proctor (Pilkington Brothers, Ormskirk) next gave a talk on "Glassy Materials". Three types of compound are found in normal glasses: (*a*) network-forming compounds which are oxides of such elements as silicon, phosphorus, boron and germanium; (*b*) network modifiers which are essentially alkali metals such as lithium, magnesium, caesium and barium; (*c*) intermediates such as the oxides of beryllium, aluminium, zinc and lead.

It was also possible to produce glasses containing fluorides, sulphides, tellurides, etc. In the laboratory at least, the properties of glass can be tailored to cover a wide range of specifications and the important factors in obtaining the desired result are: (1) chemical composition, and (2) the thermal history—important aspects of which are the transformation temperature, the strain release temperature and the annealing temperature.

Methods of improving the strength of glass include chemical toughening as exemplified by the replacement of small by large ions using diffusion processes. Strengths in the range 50,000–80,000 lb./in.^{2} can be obtained in this

way. All glass-like substances are prone to the process of devitrification but, by the use of controlled nucleation with elements like gold, silver or copper, the crystallinity can be controlled, and this technique, together with chemical toughening, can provide glasses with strengths in the range 100,000–250,000 lb./in.^{2}.

Mr. Proctor showed how the use of silver halides in a glass of the sodium–boron–silicate type gave a photochromic substance which darkened on exposure to light.

Questions on the properties of different glasses revealed that it was possible to produce complex structures made up of layers of different glasses.

Some discussion followed on the use of slag/ceram compounds as building materials, and Mr. Proctor pointed out that it is necessary to control the crystallization in order to preserve strength. For this reason it was impossible to roll β-eucryptite glass without destroying the crystallites since any subsequent treatment always tended to alter the crystalline nature. It was apparently not possible to modify the surface properties of glass in order to reduce the amount of condensation which occurred in Britain's humid climate!

Mr. R. W. Nichols (Reactor Materials Laboratory, Culcheth), in his talk on "Recent Developments and Future Prospects for Ferrous Materials", said that a growing understanding of the mechanisms available for strengthening metals, coupled with new production processes which permit greater freedom of composition, is enabling the development of structural steels with higher strengths than the mild steel which they are beginning to replace. One method of strengthening steel is the introduction of additives such as carbon or other alloying elements which act as obstacles to fracture propagation. The proportions of these additives must, however, be kept to a minimum in order to avoid affecting the toughness and welding characteristics of the steel. A striking example of alloying developments is the series of maraging steels giving weld strengths of 90 tons/in.^{2} upwards with excellent weldability.

The important requirement of steels for high-temperature usage has involved work to determine the parameters controlling high-temperature strength and high-temperature oxidation resistance. In addition to employing alloying techniques, considerable gains can be made by the more efficient use of existing materials, as a consequence of a better understanding of the limiting failure processes. Control of the grain size can, for example, be achieved by the use of aluminium (as AlN) and niobium (as NbC). Further improvements can be made by increasing the purity of steel—most important is the reduction of sulphur—and also by the use of hot rolling techniques to break up the carbides into smaller particles.

After this paper it was decided to proceed without discussion to the joint contribution by Dr. A. R. Harding (Aluminium Laboratories, Ltd., Banbury) and Mr. R. W. George (Royal Aircraft Establishment, Farnborough) entitled "Aluminium and its Alloys". This paper, incidentally, provided an encouraging example of a joint approach by supplier and user. Dr. Harding described the present stage of development of aluminium alloys, showing how their properties and application are already well exploited, and pointing out the importance of the economic factors in deciding what materials should be developed and how they should be produced. He then showed how the present development of conventional alloys is governed by the need to satisfy conflicting requirements. Mr. George then discussed the improvement of fatigue properties in aluminium and its alloys and showed how increases in tensile strength obtained in the development of aluminium alloys have not been accompanied by proportionate increases in fatigue strength. From various metallographic studies it became increasingly clear that the plastic deformation resulting from cyclical loading can break down or destroy those micro-structural features which confer tensile strength. In attempting to develop an improved aluminium alloy, a material containing 5 per cent magnesium was selected, since this alloy, although of low tensile strength, has a relatively high fatigue strength. Improvements in tensile strength were attempted by further alloying without interfering with the intrinsic fatigue-strengthening mechanism of the aluminium–magnesium matrix. Various aluminium–magnesium–copper and aluminium–magnesium–zinc compositions have been made and tested and an aluminium–zinc composition was chosen as a working basis for further development.

Discussion on improvements in ferrous metals showed that again developments centred on the principle of limiting fracture by the use of additives, and by careful attention to control of grain size. The use of carbon, for example, could be tolerated provided the carbides formed were small and uniformly distributed; weaknesses were often encountered due to the lath-like shape of certain carbides. It was also observed that inter-metallics can occur as small brittle particles in the matrix, and this leads to fracture. Pearlite colonies can also contribute to fracture as these show weakness across the lamellae.

There was a suggestion that in view of the endless series of papers describing work on aluminium containing 4 per cent copper, very little progress has been made in commercial aluminium alloys over the past 10 years or so. Dr. Harding said that this surely indicated that aluminium–copper alloys cannot be extended into the more sophisticated requirements range and he felt that we had reached the maximum degree of improvement which can be achieved by purely empirical methods. It was time for science to have a look-in. Improvements in materials can be obtained by the trick of keeping some of the additives in solution so that they would be available for precipitation when the appropriate conditions were encountered.

The first day's discussions ended with a dinner attended by about fifty delegates at the Shillingford Bridge Hotel. After the meal the meeting was addressed by Dr. P. Murray (assistant director of the Atomic Energy Research Establishment).

Dr. Murray summarized the advances made in reactor technology at Harwell since its formation. The increasingly severe operating conditions demanded by these advances had made necessary the creation of a whole range of new materials with highly specific properties. These developments had been shared by a number of the divisions at Harwell and had, in fact, represented a truly 'materials science' approach to the solution of problems.

A vigorous discussion followed Dr. Murray's talk in which the economics of development on such a scale was often mentioned even to the extent of one member suggesting that the country and the tax-payer would be better served by closing down Harwell altogether!

The theme of "Recent Advances in Particular Fields" was continued at the start of the second day by a lecture from Dr. K. Newman (Imperial College of Science and Technology) on "Concrete". He remarked that the most useful properties of concrete are its ability to be moulded easily into any shape in the fresh state, and its high compressive strength and durability in the hardened state. Its main disadvantages are its low tensile strength, which has led to the use of mild and high tensile steel as reinforcement, and the considerable shrinkage it undergoes on drying out. Despite its widespread use little was known until recently about the structure of concrete and how this affects its physical and mechanical properties. The internal structures of hardened cement pastes, mortars and concretes were briefly described and their influence on stiffness, strength and the mechanisms of shrinkage and creep were explained. It was then apparent that considerable advantages can be gained in the use of concrete as a structural material by providing even a moderate, reliable tensile strength and a significant reduction in shrinkage. Several methods of improving tensile

strength were proposed, including the use of aggregate of similar stiffness as hardened paste to reduce stress concentrations, the formation of chemical bonds at the aggregate–paste interface, and the introduction of small pieces of steel wire, glass or other fibres to arrest crack propagation. Most of the adverse effects of shrinkage can be overcome by more sensible construction techniques. However, drying shrinkage can be reduced considerably by sealing the concrete in impermeable surface finishes, by the use of expanding cements, or by steam curing at temperatures more than 180° C. A range of materials with properties between those of concretes and ceramics could be produced by autoclaving low water content, pressure compacted, concrete products.

In considering methods of reinforcing concrete, Dr. Newman during the discussion indicated that a large number of different additives had been put into concrete, including organic reinforcement; the only criterion to emerge from these trials was that for satisfactory performance the reinforcing material should be stiffer than the material into which it is placed. The presence of a large number of small cracks also served to increase the strength of concrete by acting as fracture terminators.

After this, Dr. H. Corte (Wiggins Teape, Research and Development, Beaconsfield) gave a talk on the development of paper and board. The early use of paper was confined almost entirely to writing and printing and it was only the application of mechanization, leading to increased production of cheap paper, which opened up new fields of application. These come into four main categories: (*a*) Packaging paper (such as disposable milk containers and multi-wall corrugated containers). (*b*) Sanitary papers (such as disposable incontinence pads). (*c*) Communication papers (such as '*NCR*' paper and electrographic copying paper). (*d*) Special technical papers (such as the eighteen different functional papers which are used in and for the motor-car).

In attempting to improve the performance of paper, three essential properties of the material must be borne in mind. These are: porosity, sensitivity to water, and strength.

Paper has a random porosity with a considerable variety of pore sizes. This scatter can be a disadvantage in such applications as oil filters, etc. Control of porosity can be done by various techniques including coating, which is often done for other reasons and also by the closure of pores as in the preparation of vegetable parchment by sulphuric acid or chloride ions. The mechanism of this latter technique is not yet fully understood but is being investigated. It is also possible to reduce porosity by means of resin impregnation. Multiple spark techniques can also be used to impose a controlled porosity, and, finally, the porosity can be further reduced or eliminated by lamination with plastics or aluminium foil. The sensitivity to water depends mainly on the presence of hydrogen bonds, and two techniques are available for increasing wet strength. Either additives which form bridges can be used, or alternatively, the incorporation of plasticizers (as in the wrappings of sweets) can be effective. Paper tears essentially by delamination, and improvements to tear strength are achievable by coating with latices or by impregnation with epoxy resins. A more advantageous fibre distribution (felting) can also be used but the technique is difficult.

Dr. J. P. Berry (Rubber and Plastics Research Association) next gave a talk on plastics. He began by indicating the close relationship between some of the materials called plastics impregnated papers by Dr. Corte, and visualized as paper reinforced plastics by himself! Plastics have many attractive properties but their complex mechanical behaviour poses problems in design. Inadequate understanding of these problems has tended to restrict the use of plastics to non-critical applications or has resulted in over-conservative and hence uneconomic design. In selecting a plastic for a particular use it is necessary to establish the factors which will render it unserviceable. After this the mechanical properties will determine the dimensions of the fabricated component. By achieving an understanding of the properties and limitations of plastics, and in particular the effect of time and temperature, more efficient use can be made of existing materials in present applications, and extensions can be made to new applications. There also will exist the possibility of developing materials with special improved properties.

In reply to a question on the use of paper fabrics, Dr. Corte pointed out that the principal difficulty was to achieve a satisfactory drape, and also indicated that the paper fabric-like non-woven materials were mostly composed of non-cellulose materials and were therefore expensive. Cheapness in 'paper making' depended on long runs, and the type of run used in textile production would be quite uneconomic. There was, however, a development in the United States of such disposable items made from paper as laboratory coats and hospital sheets.

Several members raised the question of ageing, odour absorption and unattractive appearance associated with plastic domestic utensils. Dr. Davies pointed out that articles which were cheap were often 'nasty' and both he and Dr. Berry emphasized that improvements were continually being made to plastics, and that it was also necessary to educate consumers in the choice of suitable materials. There was some discussion on the similarity of paper and wood, particularly in the case of some resin-reinforced products which Dr. Corte showed. The difference between wood and paper depended on whether or not the lignin was left in or removed from the product.

General trends of development in materials science. This session began by a survey of 'Fracture' by Mr. S. F. Pugh (Metallurgy Division, Harwell). Mr. Pugh plunged straight into classical materials science by observing that Galileo was wrong in assuming that the strength of materials was independent of dimensions, and remarked that Leonardo da Vinci found after experiments with iron wires of different lengths that tensile strength was dependent on the length of the specimens. It was suggested that a longer specimen contained more dislocations. A knowledge of fracture mechanisms may help both in the development of materials with improved fracture resistance and in improving engineering design techniques. Similarities and differences between the fracture characteristics of materials with as wide a variety of structures as mild steel and magnesium oxide were discussed. In developing a coherent science of fracture of materials, the subject can be divided in different ways according to the criterion adopted. So far there is no way of predicting which types of fracture a new material will suffer, because the critical factors are not known. Further investigations of fracture mechanisms are required, perhaps with more attention being paid to comparison of a given type of fracture in materials of widely different structure and physical properties. A complementary problem is in finding the relevance of the various fracture models to each particular type of fracture in each type of material. Mr. Pugh gave examples of the phenomenon of ductile fracture in glassy polymers subjected to prolonged stresses and said that a typical instance was to be found in stainless steel. He also dwelt on the use of reinforcing agents and, like Dr. Newman, indicated that it was often effective to set a crack to stop a crack, and instanced the poor bond between the reinforcing material and the resin in fibreglass as a means of providing a strong composite.

In the discussion it was observed that as materials were produced which had greater strength, the problem of brittleness became more acute and much of the work on improvement of materials lay in the reduction of brittleness. A technique was the provision of a multitude of crack-inhibiting centres. These can be either voids (as in the case of graphites) or particles and voids as produced in refractories resistant to thermal shocks by the incor-

poration of ground-up bricks in the clay matrix. Soft reinforcements in materials like concrete would also achieve the same end.

Dr. C. Edeleanu (Imperial Chemical Industries Materials Group, Billingham) then spoke on the development of engineering materials. The factors which have to be considered when making the critical decision of what material to use in an engineering application are: (1) What materials are available. (2) Costs—not of the material itself, but of the whole job, including processing and fabricating. (3) The time limit—whether or not the material can be developed and processed in the time available. (4) Human factors which include whether or not the material is pleasing in the particular application.

All these factors, but particularly the last, entail close co-operation between the designer, the fabricator and the user. It is also essential to understand the real and not the alleged requirement of a product, since often the customer does not really know what he wants. It was pointed out also that in many cases important developments occurred not in the material but in the development and use of economical ways of fabrication and production. Examples of this type of improvement emerged in the discussion particularly with reference to the manufacture of aluminium and the continuous process for steel fabrication.

Mr. J. M. Hutcheon (Reactor Materials Laboratory, Culcheth), in his talk entitled "A Plea for the Humdrum", said that although the development of new materials to the full scale in a very short time is possible, it is very expensive, and can only be undertaken under very exceptional circumstances. In most countries, for most of the time, the requirement is that the investment in research and development be kept to a reasonable proportion of the gross national product. There is, however, still ample scope for the materials technologist to use his influence with the engineer to ensure that materials specifications are rationally related to the proposed use; to investigate also the causes of variability within what is nominally the same material, and to seek means of improving existing materials by relatively small changes in the manufacturing route. This shifts the balance of research and development responsibilities away from the user laboratories into those of the manufacturer. Rapid test procedures are required, together with the detailed examination of every stage of the manufacturing process, and it is possible that some central organization such as the Ministry of Technology should finance developments.

The speaker also pleaded that university graduates should work in operating plants. He also pointed out that the statements made in his talk underlined the necessity for co-operation between user and manufacturer at the earliest possible stage of a project.

The final paper in this session was given by Mr. J. E. Gordon (Explosives Research and Development Establishment, Waltham Abbey). The speaker wondered whether the practice of cheapening a material in order to extend its market was making the most economic use of Britain's scarce research resources. Bessemer was said to have reduced the cost of steel by a factor of 5, but it is unlikely that the cost of mild steel could be reduced in the future by so much as 20 per cent. If this did happen, the value to the motor-car user, and perhaps the economy as a whole, would be no greater than a reduction of 1 per cent on petrol consumption. Reductions in the region of 25 per cent in petrol consumption could clearly be achieved by cutting the weight of the car. It is therefore arguable that steel is too cheap and if it were more expensive manufacturers would produce more efficient designs. Cheap materials may be as doubtful a blessing as cheap labour. Improved materials development could produce two benefits: (*a*) a more efficient product by weight saving, or (*b*) cheaper fabrication, since the cost of fabrication is nearly always much greater than that of the material.

The former point is receiving much attention, but the latter needs further consideration. Mr. Gordon believes that we may be nearing the end of the road with regard to cheapening existing metal-working processes, and we must begin to think more about moulding and casting processes. Some attention should also be paid to two-component systems, and here one can either increase the strength while leaving the density constant, or leave the strength unchanged and reduce the density. This could be achieved with steels, but not with some of the intrinsically lighter materials. An example would be the reinforcement of soft steel with hard ceramics; also, the quantity of wood consumed per year is about 10^9 tons compared with 4×10^8 tons of steel. Wood is cheap and has a similar modulus to steel, it has low density but does have other disadvantages. Could not more use be made of it? Plastics have too low a modulus to be used much as engineering materials, but might be modified. The use of very small quantities of reinforcement—less than 2 per cent—was found to give startling increases in mechanical properties. The discussion included remarks on the cost of training graduates, the type of training normally given to Ph.D. students and a suggestion that universities are far from being bands of learned men training intelligent machines. The only reason for research is to keep the teachers' minds alert in their task of machine training. It was also suggested that different fabricating techniques should be combined to provide new components based on design requirements and it was also contended that the policy of planned obsolescence, so prevalent in motor-cars, should be extended to things like houses so that the owner of a plot of land can replace his house easily and cheaply every 10 years or so.

Economics of new materials development. It was intended that most of this session should be devoted to a general discussion of topics raised in the Conference as a whole, and by way of a curtain raiser Mr. F. Roberts (Chemical Engineering Division, Harwell) gave a short talk on the economics of new materials development. He emphasized the importance of correctly allocating costs in any manufacturing operation and suggested that the important factors were: (1) raw materials, (2) power consumption, (3) capital costs, (4) reject rate and recycling costs, (5) degree of quality control (inspection), (6) scale of production (that is, (*a*) bench scale; (*b*) experimental or pilot scale; (*c*) large scale), (7) research and development charges.

In considering raw material costs it is necessary to consider the economic advantages of either starting with pure materials and ensuring they are kept clean during processing, or by starting with cheaper and less pure materials, and incorporating a refinement stage during the process. Turning to the question of continuous or batch production, Mr. Roberts emphasized that if continuous production were used it must be really continuous, since stoppages would inevitably affect the whole production system.

Finally, he emphasized the advantages of costing as early as possible in the development of the process.

Dr. Chesters began the discussion by pointing out that although labour costs were often the target for economy they were in many cases a small fraction of the total bill. For example, in the production of a ton of steel the total cost was £25, of which the main single item was £14 for scrap and only 7*s*. for labour costs. It is, therefore, better to improve techniques or to improve the quality and lasting power of such things as electrodes than to attempt to economize on labour costs. It was suggested that the number of different types of steels could profitably be reduced, but against this there was the position that the customer insisted on a variety to meet his own needs, and if this were not supplied would simply go to someone who was prepared to make it for him. There was agreement, however, that often the customer did not know what he wanted, and that there was a need for a closer

investigation of requirements. Also, such matters as methods of test could easily be undertaken by joint user co-operation. If this co-operation were further extended and the users could be persuaded to do more research on requirements, it would then be possible to bully suppliers more successfully, and oblige them to provide new materials with properties which were really needed.

Some discussion ensued on the differences between the development of a new material and a new product. In the former case the development was usually for a specific purpose, whereas in the latter, having made the development, it was often necessary to search for a market. There were dangers attending on the change of manufacturing processes from, say, batch to continuous operation, since the product often possessed different properties, and it had been found in the past necessary in some cases to start from different raw materials.

Some surprise was expressed at the lack of satisfactory composite materials, and this was attributed to the fact that scientific method had only recently been devoted to the development of composite materials. These were very often very expensive in the early stages and also the techniques were often time consuming. However, it was felt that considerable advances in composite materials would occur over the next few years.

It is impossible to sum up in a few words a conference which covered such a wide field of materials and ideas, but a point which emerged very strongly—and which was commented on by a number of speakers—was the very keen interest displayed by lecturers and contributors to the discussion alike in economic matters. Even during a discussion on what the organizers felt would be purely technical matters, the conference was continually being reminded of the need to consider economics.

W. A. Holmes-Walker

IDENTIFICATION OF ELUTED COMPONENTS IN GAS CHROMATOGRAPHY

An informal symposium with "Identification of Eluted Components" as the main theme was held by the Gas Chromatography Discussion Group of the Institute of Petroleum on September 23, 1965, at the Bodington Hall residence of the University of Leeds. The members were officially welcomed to the University by Prof. H. M. N. H. Irving.

The first paper, presented by Dr. R. P. W. Scott (Unilever Research Laboratories, Colworth House), summarized recent work on the development of a chromatograph that would operate intermittently, allowing a 15-min time interval to elapse after the elution of each peak.

Experience in the separation of complex mixtures had shown that simultaneous low-resolution mass and infra-red spectra were needed to assign identities unequivocally to peaks. This being so, a single chromatographic run extending to as long as 24 h with each eluted component being given the attention it deserved could result ultimately in a net gain in analysis time, particularly if compared with duplicate runs, one for high-speed low-resolution mass spectrometry and the other for trapping and regeneration of samples for infra-red examination. Tailoring the gas chromatographic and infra-red equipment to meet the preferred approach was the subject of the presentation.

Because of the time needed to make a normal infra-red scan, it was essential to be able to stop the chromatographic run for a 15-min period without adversely affecting the chromatographic resolution of components still in the column. This was found to be possible using a set sequence of stopping the carrier gas flow at the exit of the column, directly after trapping out a peak and releasing the column pressure at the inlet. Elution was re-started when required by opening the inlet and outlet valves and re-applying the column pressure evenly over a period of about 30 sec. This procedure has found extended applicability for the analysis of flavours using a 60-ft. packed column having a performance of 30,000 theoretical plates.

To obtain the desired mass of component in the infra-red cell a concentration step was required; this has been achieved by condensing each fraction on to a 10-cm column packed with 40 per cent 'Carbowax 200 *M*' at ambient temperature, from which it is automatically eluted rapidly at high temperature into the infra-red cell. A sixty-fold concentration is possible by this means. Simultaneously with the infra-red examination, a mass spectrum would also be obtained using a low-resolution mass spectrometer, such as '*M.S.* 10', which would give similar resolution to the normal fast-scan mass spectrometers but at less than one-fifth of the cost. For infra-red spectra, a normal '*SP.*200' infra-red spectrophotometer with a 15-min scan has been used for which 25 μg of sample was adequate, provided the cell was designed so that the sample was situated wholly in the light path. A suitable heated cell, plated internally to minimize adsorption of sample and with a total volume of 25 ml., was described and the determination of the spectrum of eugenol which has a vapour pressure of 30 mm at the cell temperature of 150° C was cited as an example of its use.

The whole sequence of events has been automated to such an extent that injection of the sample in the first instance is the only manual operation required.

The next paper, presented by Mr. L. Davies ('Shell' Research, Ltd., Woodstock Agricultural Research Centre, Sittingbourne), dealt with the problems of identification encountered in the determination of pesticide residues in crops, soils, etc., where the solvent extract can often contain very much less than 1 p.p.m. of pesticide together with large amounts of co-extracted material.

The electron capture ionization detector, which possessed selective response to certain halogenated compounds at levels below 10^{-9} g, had been used successfully with both planar and radial arrangements of anode to cathode using a d.c. mode of operation with oxygen-free nitrogen as carrier; undesirable ionization effects had been observed when using argon. The halogen sensitive leak detector in its several forms (Associated Electrical Industries, Ltd.) was also useful as a gas chromatographic detector at the nanogram level although to achieve this sensitivity with acceptable base-line stability the electrode potential required optimizing and the cell (types *HA* and *J* had been used) should be operated in a stream of nitrogen. The electron capture and leak detectors were used to complement each other, in so far as when tied in series they exhibited differing relative responses to certain halogenated compounds. This enabled some degree of certainty to be attached to identification and considerably reduced the possibility of mistaking a product of low electron affinity present in gross amounts for a trace of chlorinated compounds, as was possible when electron capture was used alone. Chromatograms illustrating this type of application were shown. While the linearity of response of the leak detector cell was about three times that of electron capture and the elements are inexpensive, a degree of electrical instability and the need for conditioning are features that have to be contended with. Mr. Davies pointed out, however, that the cells were being used for an application for which they were not designed.

For non-halogenated organo-phosphorus pesticides a sodium thermionic detector, adapted from a flame ionization detector by fusing sodium sulphate to a platinum wire collector electrode, had been the subject of investiga-

tion. A response to nanogram quantities of organophosphate pesticide had been obtained by increasing the electrode temperature but this had resulted in rapid loss of selectivity and base-line stability; similar characteristics were found when other alkali metal salts were used and when the salt was introduced into the flame at various positions. Some reduction in the rapid loss of selectivity at high-sensitivity settings had been obtained by using a tube of thin platinum 1 mm in diameter filled with sodium sulphate as the 'pick-up' electrode. There was considerable scope for development of this detector and the leak detector for use as selective detectors in gas chromatography.

Using the chromatograms obtained for a mixture of *n*-alkane, iso-alkane and alicyclic hydrocarbons, Dr. D. R. Deans (Imperial Chemical Industries, Ltd., Heavy Organic Chemicals Division, Billingham), in the third paper presented, showed that superior separation of certain components difficult to separate could be obtained by using two columns of different type in series and backflushing part of the sample through the first column at some critical stage during the elution. The degree of separation which could be achieved was better than could be obtained by straightforward elution through either of the columns used alone or in series. In addition to this novel application of backflushing there was also its more conventional use in conjunction with pre-columns for isolating heavy components. Despite these applications, the technique was not widely used and Dr. Deans thought this was due to the mechanical difficulties of operating taps enclosed in ovens and the attendent contamination of sample with grease and increase in dead volume causing band spreading. These objections had been overcome by using a system in which a capillary *T*-junction was used to connect the two series columns with one limb of the junction extending outside the oven to the control system. Similarly the controls for injection and backflush vent were outside the oven (for details see *J. Chromatog.*, 18, 477; 1965).

The combinations of columns (two packed, two capillary or packed and capillary in series), of liquid-phase type on the columns, of cutting and of treating the backflush (to vent or back to the column system) were innumerable. Fractions or compounds monitored out of the stream at some intermediate stage could be subjected to in-line reaction using micro-reactors or pyrolysis units. A fascinating example of this type of application was described in which the identification of the components of a complex mixture of heptenes, for which pure compounds for use as references were not available, was achieved by hydrogenating monitored peaks and re-chromatographing them against the more readily available saturated hydrocarbon reference compounds. Operation of the various systems described was considered to be very simple in practice and a switching unit had been constructed for connexion to any existing chromatograph allowing all possible schemes of operation to be used.

Mr. M. B. Evans (Natural Rubber Producers' Research Association) described his work concerned with the role of the solid support in retention measurement. One aspect which had been investigated was the apparent increase in polarity of non-polar liquid phases when used under conditions where oxygen is not rigorously excluded from the column. The retention characteristics of a wide range of solute types on freshly prepared squalane columns to which known weights of high molecular weight alcohols, aldehydes and ketones had been added were compared with retentions on a squalane column which had been deliberately oxidized at 100° C; for the columns with added oxygenated compounds there was evidence to show that retention indices are linearly dependent on oxygen content, whereas for oxidized columns there was an initial drop in retention for polar solutes (also a pronounced reduction in peak tailing) followed by an increase. It was suggested that the initial drop observed was due to the first formed strongly polar compounds deactivating the surface and the values at the minimum of the curve corresponded to the retention index due to true partitioning in the squalane.

Evaluation of a number of glycols as support deactivators led to the conclusion that diglycerol was most efficient and its use had enabled improvements in peak symmetry and reduced solute isomerization and decomposition. Detailed investigation of the effect of diglycerol-treated support on the retention of weakly polar solutes by dinonyl phthalate and squalane phases showed retention to be almost independent of the ratio of liquid phase to the diglycerol deactivator; the retention of polar solutes such as alcohols was found to display marked dependence similar to that observed when squalane was oxidized.

It was considered feasible that the extent of the initial high retention of polar solutes when low liquid phase loadings were used could be a measure of the support activity. The evaluation of a wide range of support material using squalane as the liquid phase and a wide range of solutes at constant sample size provided convincing evidence that support activity could be measured in this manner and indicated that a commercially available silane-treated product was the least adsorptive of those investigated. It was also considered that an evaluation of the support could be made by the reversed procedure of assessing the extent of the deactivation of polar groups of the liquid phase by the support at constant proportion of liquid phase, thus the highest retention index for a polar solute should be given by the least active support. This was confirmed experimentally using polyethylene glycol liquid phase on the same range of supports as were used for the other investigation.

The final paper of the symposium was presented by Dr. J. F. K. Huber (University of Amsterdam) under the title "Two-Dimensional Gas Chromatography". He discussed factors relevant to the identification of components using two columns having different liquid phases as coatings. After listing the various forms of retention by which a solute could be characterized, Dr. Huber examined in some detail the factors affecting the accuracy of such data, among which were effects due to interfaces, pressure, flow changes and non-linearity of the partition isotherm. The results obtained by sample injection into two different types of column could be correlated by combining relative peak area values and known retention values although one source of error was the presence of masked peaks. With two columns combined so that fractions of the effluent from the first column were injected into the second column more definite results could be obtained as well as enrichment of a trace relative to a main component.

C. G. Scott

URBAN TRAFFIC PROBLEMS

A MEMORANDUM issued by the Ministry of Transport/Scottish Development Department deals with the principles of applying traffic engineering techniques to urban road traffic problems*. It is designed to enable suitable short- to medium-term solutions to be found for urgent traffic problems, while providing a useful guide to the wide range of traffic engineering methods and techniques, many of which must be used in formulating the long-term traffic solutions which will be incorporated in comprehensive land use/transport plans. It does not attempt to give guidance on the surveys needed for

* Ministry of Transport/Scottish Development Department. *Advisory Memorandum on Urban Traffic Engineering Techniques.* Pp. xi + 92. (London: H.M.S.O., 1965.) 7*s.* 6*d.* net.

preparing these plans. The shorter-term traffic solutions should not be looked at in or by themselves, however, but should be viewed so far as possible in relation to parking policies, to the needs of environmental as well as traffic management and to the likely ultimate pattern of town roads. Together they constitute the broad approach to the problem of traffic in towns; in particular, any short-term proposals which would determine road widths or alignments for a long time ahead should be carefully considered in this wider context.

The memorandum includes a report on a preliminary reconnaissance to identify the nature of traffic problems, the information available on traffic data, accidents and land-uses, and the extent to which further information is needed. This is followed by a section on methods of taking traffic censuses, origin and destination surveys, surveys of public transport, measurements of speed, parking surveys and of collecting accident data. In the third section, the processing and presentation of survey information ready for some formulating scheme are considered, and in the fourth, the design of short-, medium- or long-term schemes to meet the needs of both traffic and road safety. The fifth section considers methods for comparing the advantages and disadvantages of alternative proposals, including the economic assessment of road improvements, and, in the sixth section, investigations needed to verify the adequacy of the measures taken and to reveal any need for further measures are discussed. Finally, the value of quantitative assessment of traffic problems and improvement schemes, the need to study and train engineers in the methods of meeting traffic requirements and to take advantage of new, or little-used, techniques are considered.

New methods of meeting traffic problems, such as new types of signal-controlled pedestrian crossings, "give way to traffic from the right" signs at roundabouts, the use of vehicle presence detectors, tidal traffic working and area traffic control, are likely to come increasingly into use as their possibilities are explored, with also increasing application of pedestrian segregation, channelization, urban motorways, and grade-separated systems. Short-term measures may offer much relief of congestion at little cost, and waiting restrictions, banning of right turns, or one-way systems often increase road capacity by 20 per cent or more. Improvements of 20 to 50 per cent can often be effected at signal-controlled junctions, by rephasing and simplifying the traffic pattern, by restricting certain movements, or by small carriageway widenings at the signal approaches, as well as through increasing use of the more flexible modern traffic signal controllers to meet peak-hour demands.

With medium-term measures, the emphasis should be on junction improvements, provision of roundabouts, new signal-controlled junctions and widening roads, particularly at junctions or bottlenecks. In narrow streets lay-bys for buses or delivery vehicles can be of great value in promoting a freer flow. Traffic engineering techniques can help longer-term measures which require programming in order of priority. In determining priorities and in designing various schemes for new or improved roads and junctions and general road layout, to meet the future requirements of a town, it is emphasized that in establishing environmental areas and freeing them of unnecessary traffic, the traffic on the primary road network will be increased, and the network must therefore be designed to take heavier flows.

SOCIAL INFLUENCES ON MEASURED INTELLIGENCE

THE November issue of *Educational Research* (8, No. 1) contains five separate papers on various aspects of the influence of social factors on the measured intelligence quotient (I.Q.). Taken together, they are of outstanding public interest. One paper is entitled "Further Evidence on the Effect of Date of Birth on Subsequent School Performance", by R. Freyman, and is based on a study of: (*a*) 3,419 children born during 1952–55 from eight streamed junior schools in different parts of Middlesbrough; (*b*) 4,120 children born during 1953–55 from seven streamed secondary modern schools, four grammar schools and two other selective secondary schools; (*c*) 364 junior school children attending remedial reading groups; (*d*) 312 children referred to the child guidance service; and (*e*) 156 children attending the day special school for educationally subnormal (E.S.N.) children. Among the children in streamed junior schools, of those born during the period September to December, 39·7 per cent were in *A* classes, 25·8 per cent in *B*, and 22·4 per cent in *C*, while of those born in the period May to August, 28·2 per cent were in *A*, 36·6 per cent in *B*, and 43·7 per cent in *C* classes. These differences were statistically highly significant, along with the finding that the percentage in the *A* stream increased with length of schooling.

Similar results were obtained from all the children attending secondary schools. Of those born during September to December, 36 per cent were in selective schools and 28·4 per cent in secondary modern schools, whereas, of the ones born during May to August, 32·3 per cent were in selective schools and 36·1 in secondary modern schools. Again, a larger proportion of younger than of older children obtained low and average marks.

Of the children in remedial reading groups, 46·7 were born during May to August, and 27·8 per cent during January and April periods, a discrepancy which was wider still among those referred to the educational psychologist because of backwardness in reading. The children attending the local E.S.N. school produced less clearly cut differences.

From his survey, Freyman concludes that the date of birth exerts a significant influence on a child's school grading, and that at junior level, streaming in schools tends to perpetuate differences due to date of birth. The disadvantages of the children born during May to August were greatest in those referred because of difficulties in reading; and "secondary psychological symptoms such as lack of confidence and emotional maladjustment were often displayed".

It might be thought that these discrepancies arise because children born in September to December are almost 8 years old when transferred from infant to junior schools as compared with the 7 or 7 and 4 months age of those born in the summer. But in itself, that factor, although real, can be allowed for; it does not account for the whole of the difference, which seems to be determined by some specific condition, not simply delayed mental development; and Freyman calls for a new educational deal for children born during the summer, which might include longer time in the infant school and more flexible forms of educational organization.

In his paper on "Date of Birth and Scholastic Performance", D. A. Pidgeon is concerned with the same kind of theme while carrying the discussion somewhat further. He makes the point that children who have had more schooling will have learned more and in examinations are likely to give better results; but in addition, children born in summer usually remain the youngest in their group throughout the junior school, a situation which imposes additional stress. Supporting evidence comes from Sweden.

It is a moot point whether, as a whole, the innate intelligence varies as between children born at different

times in the year. This question can be approached only through tests, which at school age give results similar to those already indicated. To some extent it may be clarified through appropriate tests carried out on children of pre-school age. But at present, many children are handicapped for life through being born during summer months and having to fit into the teaching year.

Pidgeon thinks that the abolition of streaming would remove one obvious and distasteful effect, but "while classes consist of younger and older children, the younger may continue to suffer in other ways". To him, the only true solution would be the individualizing of learning, along with a change of approach from present class teaching methods.

In the next two papers, interest shifts to educational performance between different kinds of children and in different types of secondary schools. W. H. King writes on "Differences in Mathematical Achievement Related to Types of Secondary Schools and their Geographical Locations". This is part of a much larger study based on comparisons between children attending schools in South-East England and others in the twin cities St. Paul–Minneapolis, Minnesota. A special sampling technique was used on 58 secondary schools representing all the different English types, and in each the unit of testing was a complete form.

In addition to questionnaires completed by teachers and students, three standardized tests were used: Mathematics Test 1 by MacFarlane Smith; Co-operative Sequential Tests—Mathematics *STEP* Test; and Raven's Progressive Matrices, 1956 edition. 3,134 children had scores on all three tests. Taking grammar, technical, secondary modern, and comprehensive schools in order, on Raven's test the mean scores were 49·81, 49·72, 44·18 and 45·76; on the *STEP* test, 37·13, 33·44, 24·22 and 26·62; and on the Maths 1 test, 83·83, 80·28, 55·69 and 57·96. Contrasting pairs of schools were also tested. On Raven's test the differences were not great but they were very marked with the other two, particularly the Maths 1 test.

In all these tests, the grammar school scores were the least variable, the differences widening progressively in secondary modern, technical, and comprehensive schools, and especially because of wide differences in mean scores, with considerable overlap between schools. It is clear that comparisons between mean scores in different types of secondary schools are of little value. Nevertheless, it was estimated that two-thirds of the high ability group came from grammar schools, while three-quarters of children of low ability were to be found in secondary modern schools. Still, "no one type of school has a monopoly of students in the high or low ability category".

In his conclusions, King directs attention to the very wide gap in scores between children in grammar and technical schools on one hand and secondary modern ones on the other, suggesting that the causes, motivations and levels of aspiration are not linked solely with differences of ability. These points urgently need attention; and the research has uncovered a problem of raising the achievements of the lower ability group in secondary modern and comprehensive schools without lowering it in grammar and technical schools. Particularly in the two mathematical tests, the findings were disappointing. Clearly, in relation to mathematics, the minds of many children are not being fully stretched. That conclusion may also apply to the United States public schools with which the project is linked, where the students' scores again are relatively low.

King does not go on to discuss the social influences which might account for these differences. Instead, in a paper on "Meritocratic and Social Class Selection at Age Eleven", D. F. Swift approaches intelligence tests on school children from another angle. Drawing on the work of Floud, Banks and Halsey, he states that "in developing industrial societies, the educational system is replacing all others as an avenue of social mobility". His own research was carried out in the North of England, covering 132 families, and was designed to compare the attitudinal environment of the home with the ability of the children, making use of the 11+ allocation procedure. The group under test represented a 10 per cent sample of all families in two Local Education Authority Divisions. Taking parental occupational groups, under the State School Sampling Frame, I to VI, the 278 children who passed at age eleven plus were distributed in the order 108, 78, 62, 13, 13 and 4, while by contrast, of the 922 children who failed, the corresponding figures were 98, 120, 290, 44, 157 and 213. Grouped by social class, 60 per cent of the middle class children passed, and 40 per cent failed, as against 11 per cent of working class who passed while 89 per cent failed. Again, middle class children had a mean score of 117 in verbal reasoning, 122 in English, and 117 in arithmetic, as against working class children, whose scores in these respective subjects were 100, 103 and 101.

On these results, ostensibly, the British ideal of equality of opportunity is being met, but present psychological knowledge recognizes the importance of motivation in test performance. Had borderline cases been taken into consideration, one-half of the working class children but only one-tenth of those of middle class would have been rejected for grammar schools.

Swift then goes on to consider what should be the basis for the 'meritocratic' system of selection. It seems to him that because of similarities between value-orientations of middle class and school life, "the use of middle class children as a base group is justified"; but any scheme for the organization of talent-producing institutions would need to be based on research covering large numbers in many different areas. And it is important to bear in mind that perspectives must be concerned with the contribution of education to allocations in society, for which the first step would be to establish how far education was important in the total process of meritocratic or social class selection.

The longest and most far-reaching paper is by O. Ferron, on "The Test Performance of 'Coloured' Children". He is working at Najala University College, Sierra Leone. He draws on the findings of many surveys of which the following may be taken as representative. Many early I.Q. tests in the United States tended to show that white children were superior to Negro and Red Indian ones and that children of North European stock were superior to those coming from South Europe. In 1926, Hirsch measured the I.Q. of immigrant children, and found that the mean score of British Canadians was 93·8, French 85·3, Negroes 84·6 and Portuguese 82·7. But in the same year, Helmer devised tests which contained subject-matter relating closely to the life of Red Indians, in accordance with which when compared with them white children were definitely inferior. On the other hand, Rohrer attributed the low scores of Red Indians in other tests to such factors as differences in social and economic status, differences in cultural environment, language differences, differences in test motivation, and inaccuracies in estimating chronological age.

Another worker in the field was Pyle, who found that Chinese children attending public schools were superior to American ones in rote learning, but inferior in tests involving logical relationships and in speed of learning generally. Then, in 1930, the University of Hawaii published the results of testing 4,500 children in the United States and Hawaii—where differences in social status between Chinese, Japanese, Portuguese, Puerto Ricans and Filipinos have been much reduced—which indicated that when differences in environmental opportunities are not too great, differences in intelligence performance could be expected to be correspondingly small.

Early work on African children produced results showing that they were markedly inferior to the Whites. For example, Fick, in 1939, concluded that Zulu children

aged 10–14 were from four to five years behind children of European descent on American Army Beta tests, and Oliver, on work initiated in 1932, concluded that in East Africa only 14 per cent of Africans reached or exceeded the European mean. But results like these stimulated interest in environmental factors, such as illiteracy among parents, and extremely low standards among teachers in primary schools, together with the very poor equipment of native African homes.

Ferron goes on to point out that in Africa the gap between white and non-white children can be reduced by the use of non-language tests, but the cultural ways of life and patterns of child rearing are significantly different, as also are cultural modes and traditional attitudes; but he thinks that "it would be far more constructive to continue with the use of Western type tests, since African aspirations are in the direction of a western technological civilization". Indeed, comparisons between British and African test performance can be justified in that they show where emphasis should be placed in the matter of environmental deficiencies. As a result of an investigation which he began in Freetown in 1960 and completed in Zaria in 1964, he concluded that the test quotient of an African group is to a large extent dependent on the degree of assimilation of western culture. He emphasizes that for African children, "the difficulty of passing from one world to another as the child grows older, of living in two worlds simultaneously, and of acquiring the newly imposed and rather unrealistic symbols of success and status, represents a struggle for survival with which the biological struggle of the survival of the fittest can bear no comparison, for even those who survive the former appear to be emotionally, psychologically and intellectually maimed for life". But he does not despair of the future, although until a simultaneous attack is made on many fronts, progress will continue to be slow; and the effort should be spread over the whole educational field, from birth onwards. In this campaign, the chief impediments at the present time appear to him to be found. (*a*) in the school, because of a mechanical approach to teaching in the early years particularly, in the teacher's relative lack of facility in the use of the English language, through discouraging the child from asking questions and exploring his environment, severe and capricious corporal punishment, along with mental rigidity, and an attitude of dogmatic authoritarianism; (*b*) in the home, because of birth injuries and incidence of subsequent malnutrition and disease, certain early child-rearing practices that are in the nature of traumatic experiences, the nature of the emotional relationships between adults and children, illiteracy, mainly of the mother, and lack of appropriate stimulation in the home and neighbourhood environment, and lack of definite teaching on the part of older children and adults; and (*c*) in government action through lack of proper physical surroundings in home and school, and relative lack of stimulation from mass media of instruction.

Because colour prejudice and discrimination against coloured people are not so severe in Britain as in the United States, Ferron thinks that a useful link and exchange of ideas might be established between research workers in Britain and in other parts of the world.

R. Weatherall

EMISSION BAND SYSTEMS OF Ag_2 PRODUCED IN DISCHARGE

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In recent years, use has been made of King's furnace by several workers in their studies of the band spectra of silver and other high boiling-point metals. As a result, five band systems of the diatomic silver molecule have now been found. They are: (1) the system λλ4100–5200 first studied by Ruamps[1] in emission, and later by Kleman and Lindkvist[2], both in emission and in absorption; (2) the system λλ2750–2850, by Ruamps[3] and by Joshi[4] in absorption; (3) the system λλ2640–2700, by Ruamps and by Maheshwari[5] in absorption; (4) the system λλ2560–2620, by Ruamps in absorption; (5) the system λλ2460–2560, by Ruamps and by Maheshwari also in absorption. According to the vibrational analyses of these workers, these five band systems have a common lower electronic state of vibrational frequency 192·4 cm^{-1}. It is generally believed that this lower state is the ground-state of Ag_2. More recently, two of us[6], making use of quartz absorption tubes in their investigations of the atomic and molecular spectra of silver, were able to detect the visible band system at as low a temperature as 1,100° C.

Following our work on the absorption spectra of silver, attempts have been made to obtain emission band systems of the metal in discharge. This discharge tube was made of fused quartz. It was composed of a central stem of length about 20 cm and bore of 8 mm, two shorter and larger end stems with flat windows, and two side-tubes enclosing cylindrical aluminium electrodes of rather great heat capacity. After having introduced a few decigrams of spectrally pure fine grains of silver through a detachable window of the stem and having distributed them evenly along the central stem, the discharge tube was set along the collimator axis of a Hilger medium-size quartz spectrograph. The central stem was then wound about with a nichrome heating coil, and wrapped in turn with asbestos cloth, so that its temperature could be raised electrically for vaporizing the silver therein. A platinum–platinum rhodium thermocouple, previously attached to the side of the central stem, served to indicate the temperature during heating. In performing an experiment, the discharge tube was connected to a pumping and feeding device for introducing pure neon at a pressure of about 1 mm of mercury which served for carrying an electric discharge. Preliminary tests showed that: (1) the temperature of the central stem should be raised to 1,100° C and preferably to 1,200° C to get sufficient amounts of silver vapour for the discharge; (2) the side tubes, in which electrodes were enclosed, should be surrounded by solid carbon dioxide in order to get rid of the troublesome OH bands in the emission spectrum; (3) the average life of the heating coil was about 6 h at 1,110° C, but it rarely exceeded 2 h at 1,200° C. A high-tension transformer furnishing a power of 150 W was used to run the discharge tube. Exposures ranging from 5 to 20 min were found sufficient to record the band spectra on Ilford '*HP*3 Hypersensitive Panchromatic' plates. Iron arc served as the comparison spectrum.

Under our experimental conditions, six emission band systems attributable to the diatomic molecules of silver have been found. Five out of the six systems are previously known in absorption, and one of them also in thermal emission. The new system is situated in the region λλ3150–3630. Vibrational analysis of the old and new systems reveals some interesting and important facts.

Visible system λλ4100–4700. About thirty bands have been observed in the region λλ4100–4700, and they are all degraded to the red. Most of the bands are found to group into well-marked sequences. With the dispersion used, which was about 50 Å per millimetre in this

region, no rotational structure of the bands was observable. All the bands, except those belonging to the last three sequences on the long wave-length side of the region, can be represented fairly well ($\pm$ 4 cm^{-1}) by the formula:

$$\nu = 23{,}005 + \left[152\left(v' + \frac{1}{2}\right) - 0{\cdot}3\left(v' + \frac{1}{2}\right)^2\right] - \left[193\left(v'' + \frac{1}{2}\right) - 0{\cdot}6\left(v'' + \frac{1}{2}\right)^2\right]$$

In this formula, ν represents the wave-number per centimetre of the band-heads, v' and v'' the vibrational quanta in the upper and lower electronic states of the molecule. It results from the foregoing formula that the fundamental frequencies of vibration at the upper and lower states are $\omega'_e = 152$ and $\omega''_e = 193$ cm^{-1}, respectively, and the origin of the system is $\nu_e = 23{,}005$ cm^{-1} ($\nu_{00} = 22{,}984$ cm^{-1}). Within the experimental error, these molecular constants are in good agreement with those found by Kleman and Lindkvist for the system λλ4100–5200. Hence the system produced in absorption and in thermal emission and our system produced by discharge are one and the same system. According to these workers, the lower electronic state of the system was the ground-state of Ag_2, and so they designated this system as system $(A - X)$. We shall see that whether the lower state of this system is the ground-state is still open to question.

In our emission system, the v'' progression corresponding to $v' = 0$ comprises eight bands. Now, in following this progression it is found that the vibrational quanta $\Delta G''$ decrease at first very slowly, but drastic changes occur at $\Delta G''_{9/2}$, $\Delta G''_{11/2}$ and $\Delta G''_{13/2}$. Indeed, the values of the first and fourth vibrational quanta are $\Delta G''_{1/2} = 191{\cdot}8$ and $\Delta G''_{7/2} = 190$ cm^{-1}, while those of $\Delta G''_{9/2}$, $\Delta G''_{11/2}$ and $\Delta G''_{13/2}$, as inferred from our observed data, are about 236, 239 and 217 cm^{-1} respectively; in other words, there are deviations of whole bands from the regular course of a band progression. Moreover, band-heads, well visible in the other sequences of the system, become vague in the sequence $\Delta v = -4$ and practically disappear in $\Delta v = -5$, $\Delta v = -6$ and $\Delta v = -7$. Beyond $\Delta v = -7$, a continuum sets in, which continues to as far as 4800 Å. No doubt, we have here an example of vibrational perturbation and predissociation. A similar example was observed by Choong[7] in the case of a band system of the molecule of TeO.

Although the banded region of the system stops in the vicinity of 4700 Å, we have found eleven weak and complicated bands in a region succeeding the continuum mentioned in the foregoing paragraph. The wave-lengths of these bands are:

4807·0, 4811·4, 4861·4, 4884·4, 4915·1, 4917·1, 4957·6, 4972·2, 4994·4, 5016·3, 5024·4 Å.

These bands cannot fit into the foregoing formula. They may be due to polyatomic molecule of silver or impurities.

Ultra-violet system λλ3150–3630. The remarkable feature of this new system is that it consists of two strong wing-like bands, and patches of weak continua and flutings which link with the wing tips of the bands. The wings are found to originate from the lines of wave-lengths 3280·7 and 3382·9 Å, that is, the first doublet lines of the principal series of silver, and are almost symmetrical with respect to the corresponding lines. Under the specified conditions of experimentation, the span of the λ3280·7 band and that of the λ3382·9 band are about 140 Å and 80 Å respectively.

The production of a continuous spectrum that joins on to the long- or the short-wave-length side of an emission line has been known for a number of cases, and the phenomenon was first investigated in detail by Oldenberg[8] in the fluorescence spectrum of mercury vapour to which inert gases at high pressure had been added. In our case, it seems fairly certain that the wings are arising from a transition between an unstable upper state and an unstable lower state of Ag_2 resulting from $Ag(^2P_{1/2,3/2}) + Ag(^2S_{1/2})$. In regard to the patches of weak continua and flutings, they might be due to weakly bound molecules of Ag_2 or AgNe.

It may be of interest to point out that the doublet lines in question, produced in absorption[6] under neon at a pressure of 30 cm of mercury and at a temperature of about 1,200° C, were found to have a broadness of the order of 3 Å only. On the other hand, the same doublet lines, produced by a silver arc in an atmosphere of air, were found to be strong and to take the form of wing-like bands similar to the bands obtained in discharge. In the light of the present investigation, it may be said that unstable Ag_2 resulting from $Ag(^2P_{1/2,3/2}) + Ag(^2S_{1/2})$ must exist in prominent abundance in a silver arc.

Ultra-violet system λλ2750–2880. Twenty-one bands have been observed in the region λλ2750–2880. They are all degraded to the red and their heads can be represented in good approximation ($\pm$ 4 cm^{-1}) by the formula:

$$\nu = 35{,}646 + \left[180\left(v' + \frac{1}{2}\right) - 3{\cdot}8\left(v' + \frac{1}{2}\right)^2\right] - \left[207\left(v'' + \frac{1}{2}\right) - 0{\cdot}1\left(v'' + \frac{1}{2}\right)^2\right]$$

From this formula it may be seen that the values of ω'_e and ω''_e are 180 and 207 cm^{-1} respectively, and the value of ν_e is 35,646 cm^{-1} ($\nu_{00} = 35{,}631$). It should be pointed out that, according to this result, none of the upper and lower electronic states are in common with those of the known systems of Ag_2. The wave-lengths of the observed band heads, their estimated intensities, and the corresponding vibrational quantum numbers are listed in Table 1.

Table 1

λ	I	v',v''	λ	I	v',v''
2756·8	1	4 0	2825·4	8	1·2
2761·4	2	5 1	2828·6	5	2 3
2768·4	4	3·0	2839·8	4	0 2
2771·7	3	4 1	2842·7	5	1·3
2780·1	8	2·0	2845·6	4	2·4
2792·6	10	1·0	2849·3	2	3·5
2796·0	5	2 1	2856·4	1	0·3
2806·5	8	0 0	2861·9	2	2 5
2809·6	7	1·1	2866·7	2	3·6
2812·5	5	2 2	2873·3	1	0 4
2822·5	8	0 1			

Ruamps[3] designated the absorption system λλ2750–2850 as system $(B–X)$ of Ag_2, and suggested the values of its main molecular constants as: $\nu_e = 35{,}827{\cdot}3$ cm^{-1} ($\nu_{00} = 35{,}806{\cdot}1$), $\omega'_e = 151{\cdot}3$ and $\omega''_e = 192{\cdot}4$ cm^{-1}. Now, out of the nineteen bands observed by him, there are, within 0·5 Å, thirteen stronger ones having wave-lengths the same as ours. Hence his system and ours are undoubtedly one and the same system. The discrepancy in molecular constants appears to be due to the choice of the value of ν_{00}; that is, the (0,0) band. We find that with our choice the irregularity in the values of the vibrational quanta $\Delta G''$ is much less. From our results we believe that the fundamental frequency of vibration of the ground-state of Ag_2 is $\omega''_e = 207$ cm^{-1}, and not 192·4 cm^{-1} as is generally believed.

Three far ultra-violet systems. The three far ultra-violet absorption systems of Ag_2 observed in the regions λλ2640–2700, λλ2560–2620 and λλ2460–2560 and designated by Ruamps[3] as systems $(C–X)$, $(D–X)$ and $(E–X)$ are also found in discharge but with much fewer bands. Our emission bands attributable to the so-called system $(C–X)$ are:

2645·4(1,0), 2656·5(0,0), 2658·0(1,1), 2660·3(2,2), 2661·7(3,3), 2663·2(4,4), 2665·2(5,5), 2666·8(6,6), 2668·7(7,7), 2674·9(10,10).

Our emission bands attributable to the system $(D–X)$ are:

2562·4(0,0), 2564·2(1,1), 2566·0(2,2), 2568·0(3,3), 2570·0(4,4).

Our emission bands attributable to the system $(E–X)$ are:

2481·9(1,0), 2485·2(2,1), 2488·1(3,2), 2490·6(0,0).

Besides the aforementioned bands of Ag_2, eight persistent bands of SiO have been detected as very weak bands in the region λλ2290–2590. Of course, lines of silver and neon were also present in the working ranges of the different systems. Fortunately they were not present in great number.

In conclusion, our work on the emission band systems of Ag_2 reveals: (1) the existence of one new system in the region λλ3150–3630; (2) all the known absorption systems have their counterparts in emission; (3) the fundamental vibrational frequency of Ag_2 is 207 cm^{-1} and not 192·4 cm^{-1} as is generally believed; (4) the existence of vibrational perturbation and predissociation in the emission system λλ4100–4700.

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MONTE CARLO MODEL OF A FRACTURE PROCESS

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During the course of an investigation of the role of energy in comminution[1], a model of an elementary random fracture process was investigated with the view of illustrating some of the probability aspects of size reduction. Particles are subjected to events in which stresses are applied and released successively. The magnitude of the stresses and the specific energy (energy per unit volume of particle) which they generate are statistically distributed. In the simplest case, the particles are identical and they fracture if the specific energy, E, equals or exceeds a certain threshold level, E_f. After fracture, the fragments leave the stress field under consideration for a subsequent operation and the stresses are brought to bear on a fresh particle. If fracture does not occur during an event (that is, $E < E_f$) the strain energy is assumed to be dissipated and the particle remains in the stress field until fracture occurs. In reality, E_f is also distributed statistically, but for the present it will be assumed constant. Also it would be more realistic to postulate that some breakage occurs with values of E just below E_f, but is insufficient to reduce the size of the particle significantly; catastrophic failure occurs when $E \geqslant E_f$. If E_f is near to the maximum value of E, the curve frequency of fracture versus energy requirement will be severely skewed in a positive direction, indicating much wasted energy; the form of this relationship was required.

It is assumed that the specific energy imparted by the events is distributed rectangularly and takes integral values from 1 to 10 arbitrary units, each integer occurring in a random sequence with equal probability. Tables of random numbers (counting zero as 10) provide values of E. The energy consumed until a particle fractures is given by the sum of N digits along rows or columns between two values of $E \geqslant E_f$ and including the second value. Tabulation of results showed number of events necessary to cause breakage, N; E_f and $\Sigma^N E$. An extract from tables is: 9 0 3 7 4 8 6 3 1 1 5 5 8, which for $E_f = 8$ tabulates as:

N	E_f	$\Sigma^N E$
1	9	9
1	10	10
4	8	22
7	8	29

2,240 consecutive digits[2] were treated in this manner, producing 650 fractures; the total number of eights was 227; of nines, 216; of zeros, 207. Because the probability of occurrences of any digit is 0·1, each is expected to appear 224 times in a sequence of 2,240; thus, the particular extract of the random number tables sampled in this work was somewhat deficient in 9's and 0's. The entire assembly of data analysed into frequency, according to number of events and energy input to cause fracture, is shown in Fig. 1. Also given are theoretical and experimental averages; in all cases the agreement is good.

By evaluating the various permutations of digits 1 to 9 and 10 in summing to give integers 8, 9, 10 . . ., the probability of the entries in Fig. 1 can be calculated. In particular, the sudden decrease in frequency of occurrence when particles require eleven energy units for fracture can be confirmed; this is because at least two events are now necessary to cause fracture. The expectations of 10, 11, 12 and 13 are, respectively, 0·121 × 672 = 81·4, 0·0331 × 672 = 22·2, 0·03641 × 672 = 24·5, and 0·040051 × 672 = 26·9. These are near to the values obtained by the Monte Carlo procedure; improved expectation estimates can be obtained by using the experimental value 650 instead of 672. In the same manner, the other expectations can be obtained, but the calculations become unwieldy as energy requirements increase; however, it is clear that the probabilities for the higher values of N are vanishingly small, being of the order of 10^{-N}. The two-stepped lines give the limits for the formation of integers from the sum of N digits. The upper line is given by $10 + 7\,(N-1)$, while the lower line is $8 + (N-1)$; the average, $9 + 4\,(N-1)$, provides a good fit to the experimental averages of N.

The analysis in Fig. 1 illustrates the reason for the uneven nature of the frequency versus energy distribution; it is composed of the sum of a series of simple distributions, each for a particular value of N. If E was graded into smaller intervals, the curve would be expected to smooth out. The analysis has been extended by dividing the data into three sets depending on whether the *coup de grâce* was delivered by an 8, 9 or 10; these are generally similar with only local random differences. Such a detailed analysis would be of importance in relating energy input to fragment size, because the strength of the dispatching blow will determine the resulting size distribution.

It is to be emphasized that this aspect of the comminution process is not primarily concerned with the production of fine material, but rather with coarse size-reduction. Here, for maximum effect, the objective should not be to bring to bear as much stress as possible, but rather to limit the stress to that only just necessary to cause fracture; general considerations show that, as expected, more effective energy utilization would result if the spectrum of E were reduced below and above E_f. It is of interest that in slow compression, which is a relatively efficient comminution process, E is steadily increased to E_f as a maximum value.

As already pointed out, the results of the present model can be calculated from elementary probability theory. A number of refinements can be suggested which, while providing a more realistic model, would, however, make direct calculation too involved. (*a*) Increase the

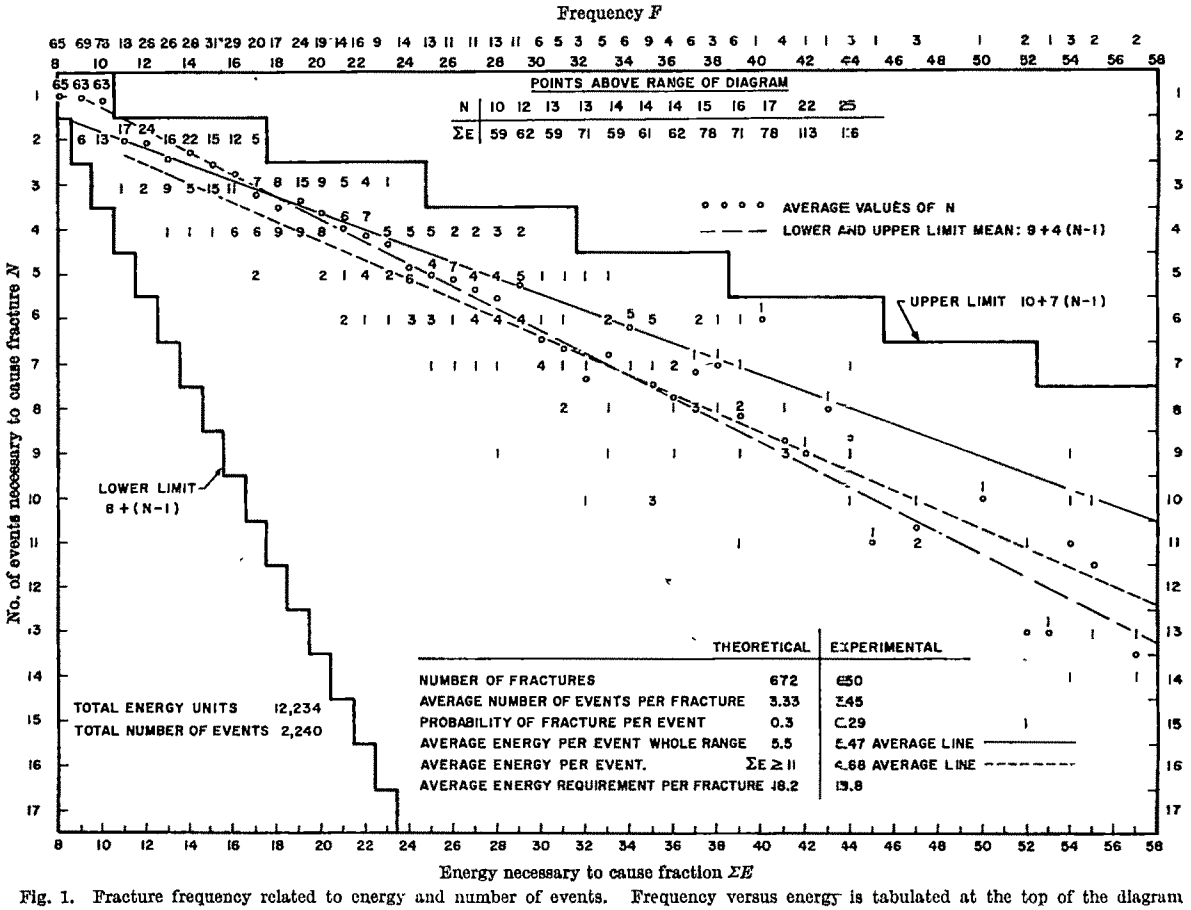


Fig. 1. Fracture frequency related to energy and number of events. Frequency versus energy is tabulated at the top of the diagram

intervals by which E progresses. An obvious procedure would be to cover the range 1–100 arbitrary units; this should smooth the frequency-energy distribution curve. (*b*) Incorporate fatigue into the particle characteristics; for example, they might fracture after, say, the tenth event irrespective of the magnitude of the blow sustained. Or, alternatively, E_f may diminish with each unfruitful event. (*c*) The strength of the particles may be distributed randomly; this can be obtained by allotting values of E_f from a second table of random numbers. (*d*) The distributions may be other than rectangular. (*e*) If fracture does not occur during an event, only a portion of the strain energy may be dissipated. In all these cases effective solution would require a digital computer to carry out essentially the same procedure as that described here.

Finally, although this model was devised in order to investigate an elementary comminution process, it appears appropriate to systems, inanimate and animate, where the fundamental unit responds only when the magnitude of the externally applied stimulus reaches a threshold value, both of these values being distributed statistically.

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PALAEOGEOGRAPHY OF THE TORRIDONIAN APPLECROSS GROUP

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THE Precambrian Torridonian sediments overlie the Lewisian Gneiss along the north-west coast of Scotland from the Inner Hebrides to Cape Wrath (Fig. 1). Strata are low-dipping, often nearly horizontal.

The sub-Torridonian gneiss surface. Detailed work in the Cape Wrath area reveals that, with rare exceptions, the sub-Torridonian surface is thoroughly decomposed to depths of 3 m and closely parallels the major bedding planes of the overlying sediments. Local etching and exfoliation of the surface suggest that the weathering occurred in Torridonian times. From Quinag southwards, however, the unconformity is highly irregular and the gneiss little weathered. At Quinag, arkoses overlap a hill at least 300 m high, and at Loch Maree the pre-Torridonian relief reached about 600 m (ref. 1).

Torridonian stratigraphy. The Torridonian has been divided into three groups[1] (Table 1).

Table 1. MAJOR DIVISIONS OF THE TORRIDONIAN

Group	Approximate thickness in m	Lithology
(3) Aultbea Group	900 to 1,400 in Coigach	Sandstones, flags and grey shales passing down into red sandstones and grey flags with shaly partings.
(2) Applecross Group	450 at Cape Wrath; 1,800 to 2,400 in the Gairloch-Torridon area.	Red arkoses and conglomerates with numerous pebbles of quartz, quartzite, jasper, chert, felspar porphyry, etc. Occasional red shales. Local basal breccias
(1) Diabaig Group	15 at Quinag; 150 at Gairloch; 3,000 in Skye:	Sandstones and grey mudstones with rippled sandy lenticles. Basal breccias and boulder beds of local origin. In Skye, dark grey sandy shales and buff grits passing down into epidotic grits with occasional purple and green shales

Except in Skye, the Diabaig Group generally occurs in the low-lying areas between pre-Torridonian hills and mountains. The breccias probably represent locally derived screes[1]. The mudstones at Diabaig have been compared with ordinary shallow-water marine sediments, including intertidal deposits[2]. The group gradually thickens from 150 m at Gairloch to 3,000 m in Skye[1,3]. No Diabaig sediments occur north of Quinag.

The Applecross Group contains sub-rounded to rounded pebbles, tabular conglomerates, large-scale trough and planar cross-stratification, channel-forms, occasional small-scale ripplemarks and rare desiccation-cracked red shales. The sediments are comparable with those of modern braided streams[4] and alluvial fans[5]. The group thickens from 450 m at Cape Wrath to 1,800–2,400 m in the Gairloch–Torridon area. Mean grain size gradually decreases southwards. Concomitantly, maximum diameter of the pebbles decreases from about 180 mm at Cape Wrath to 50 mm at Applecross[1].

The upper Aultbea Group at Cailleach Head, comprising grey, occasionally desiccation-cracked shales and interbedded rippled, cross-stratified and laminated sandstones, suggests a shallow (intertidal ?) environment not unlike that postulated[2] for the Diabaig mudstones. The lower Aultbea sandstones may represent the transition from braided stream to shallow marine environment.

It is generally accepted that the three groups form a conformable sequence. However, at Stoer (grid ref. 042330) thick-bedded Applecross conglomerates and arkoses overlie atypical Diabaig red sandstones and shales with slight angular unconformity, the lower beds having been tilted slightly to the north-west prior to Applecross deposition. The contact is marked by a line of sandstone boulders derived from the underlying rocks. Hence the Diabaig Group includes strata much older than the Applecross sediments.

The age of the red breccio-conglomerate at Stornoway is uncertain[7]. However, the poorly consolidated nature of the deposits and boulders of Torridonian-like pebbly arkose in the basal beds at Swordale suggests a post-Torridonian age.

The Applecross Group. Detailed investigation of the unconformity and sediments northwards from Quinag suggests that this area holds the key to the regional palaeogeography of the Applecross.

At Cape Wrath the sediments are best divided stratigraphically into five facies (Table 2). The progressive upward fining and concomitant change of bedding character suggest a change in the depositing streams from conditions of predominantly

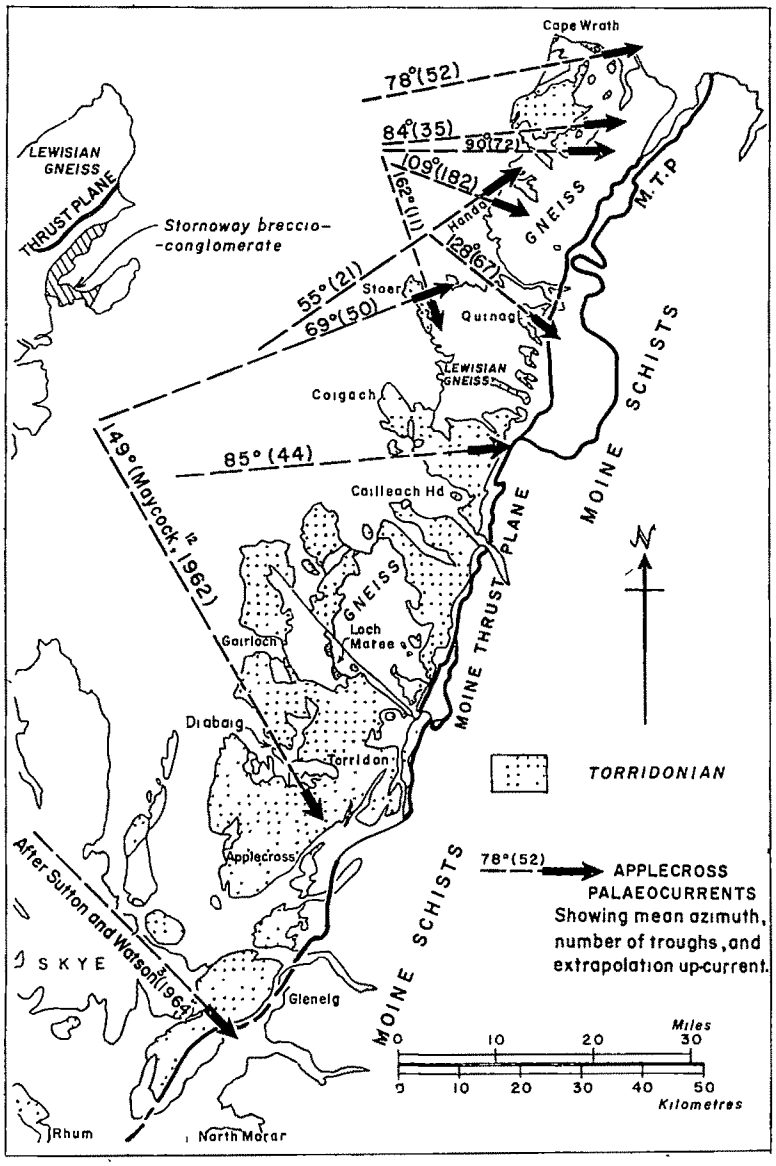


Fig. 1. Locality map, showing general geology and palaeocurrent directions determined from the Applecross Group

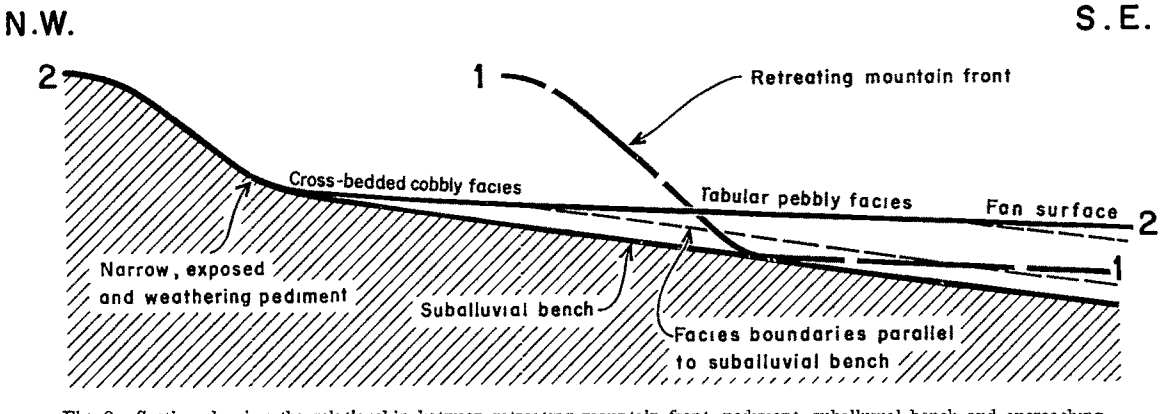


Fig. 2. Section showing the relationship between retreating mountain front, pediment, suballuvial bench and encroaching alluvial fan

rapid or shooting flow of the upper flow régime to those of predominantly tranquil flow of the lower flow régime[6]. Facies 5 is similar to the type Applecross sediments south of Loch Torridon. The vertical variations in the Cape Wrath sequence thus seem to reflect the lateral variations within the group as a whole.

Palaeocurrents. Palaeocurrent data from the Applecross Group are strongly unimodal. Each current direction was taken as the arithmetic mean of the azimuths of numerous trough axes exposed on nearly horizontal bedding surfaces. Regionally, the current pattern (Fig. 1) is more complex than has hitherto been assumed[7]. Although mean derivation from the north-west or west-north-west is now confirmed, the radiating arrangement of the currents suggests two large, coalescing fans. Coalescence appears to occur in the Coigach-Handa area, where the lower beds have been derived from the north-west and upper beds from the south-west.

Palaeogeography. Lithology, sedimentary structures and palaeocurrents suggest a piedmont or alluvial fan environment for the Applecross Group.

The flat, weathered gneiss surface beneath the sediments north of Quinag is interpreted as a buried pediment or suballuvial bench. This was formed at the foot of a north-westerly retreating elevated source area and progressively buried by the encroaching alluvial fan (Fig. 2).

All facies boundaries are diachronous and parallel to the suballuvial bench surface, dipping gently away from the base of the mountain front. The resulting upward fining of the sediments above the bench reflects the radial variation on the fan surface. As long ago as 1915, Lawson[8] postulated this relationship between an elevated source area, its flanking pediment and alluvial fan apron, and the planar rock surfaces commonly observed beneath dissected alluvial fans in south-western United States.

Relative uplift of the gneiss immediately north-west of Quinag probably initiated pediment formation and alluviation. It is noteworthy that north-east–south-west normal faulting is common in this area.

As the suballuvial bench must be intimately associated with the source area of the sediments, its weathered profile has been extensively studied. In the weathered gneiss, from 50 to 100 per cent of the plagioclase felspar has been altered to sericite and illite, haematite has partly replaced biotite, and hornblende has been entirely altered to sericite and chlorite. Microcline is only slightly weathered. It is clear that such a weathering of the Lewisian Gneiss could have supplied the microcline-rich arkoses and haematitic, illite-rich siltstones and shales constituting most of the Applecross Group. It also accounts for the almost total absence of locally derived basal breccias north of Quinag. The deep weathering of the bench and the abundant evidence for fluvial deposition of the sediments above suggest a warm, humid climate.

It seems likely that frequent sheetfloods on the upper portions of the fan deposited the tabular conglomerates of facies 2. The interbedded arkoses of this facies suggest a channel environment, occasional point-bar deposits indicating that stream migration was mainly away from the fan median. Progressively finer sediments accumulated downslope, probably as the channel and overbank deposits of braided streams.

Preliminary calculations based on slight vertical variations in palaeocurrent direction suggest: (1) the apex of the fan migrated approximately 40 km west-north-westwards during deposition of the Cape Wrath sequence; (2) a mean angle of slightly less than 1° between suballuvial bench and fan surface. Transport distances for each facies can also be calculated by this method.

Fig. 3 shows the relationship between the sediments astride the suballuvial bench and the remainder of the Applecross. This accounts satisfactorily for the lateral and vertical variations within the group, the palaeocurrents, the sudden change in structure of the sub-Torridonian surface, and the absence of Diabaig sediments north of Quinag.

Disturbed bedding of the Applecross Group. Disturbed bedding is abundant in facies 5 at Cape Wrath and the Applecross sediments south of Quinag. Structures include: (1) undulose and hazy bedding planes; (2) convolute bedding; (3) overturned and deformed cross-strata; (4) large-scale cuspate and diapiric structures. These structures probably formed *in situ* by the shifting and rearrangement of quicksands[9].

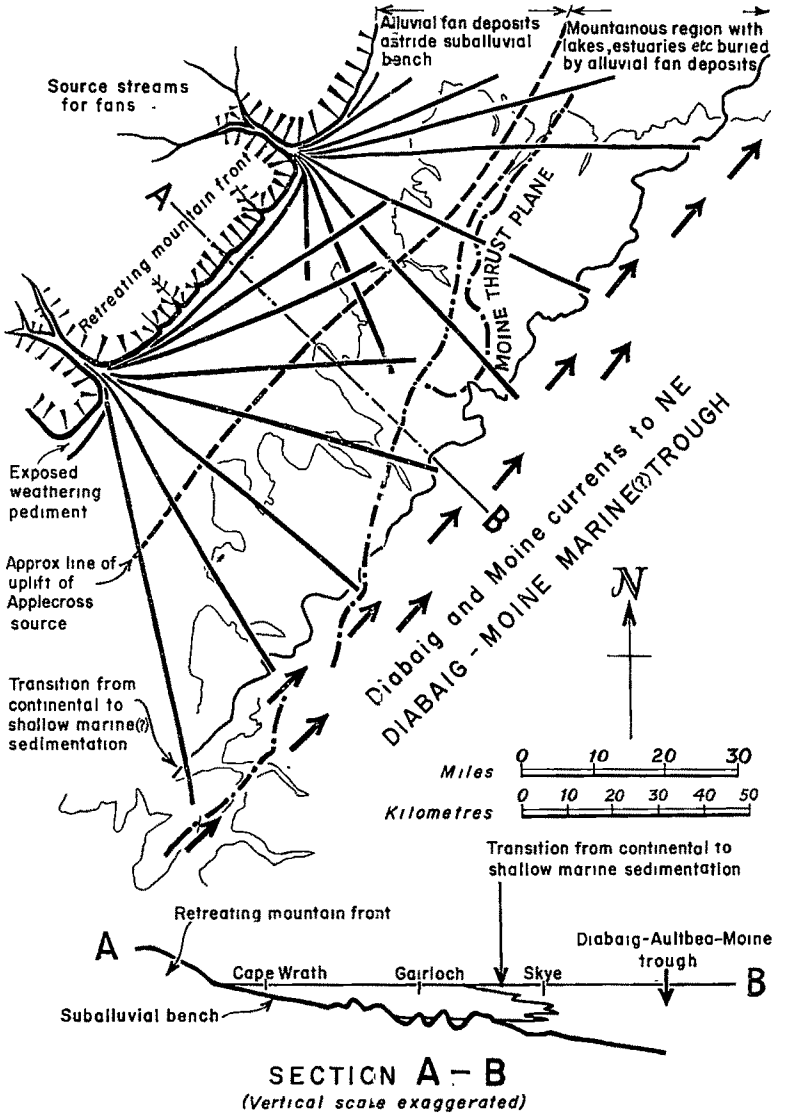


Fig. 3. Suggested palaeogeography of north-west Scotland in late Torridonian times

Table 2. Subdivisions of the Applecross Group at Cape Wrath

Geological survey divisions[1]	Facies	Main characters	Approximate thickness in m
t'''	(5) Contorted sandy* facies	Cross-stratified and tabular pink and grey arkoses; subordinate red fine sandstones and silty shales, rare gravelly bands with pebbles up to 50 mm in diameter. Channel-forms common. Ubiquitous disturbed bedding. Occasional small-scale ripplemarks.	90+
	(4) Cross-bedded sandy facies	Cross-stratified and tabular pink and grey arkoses, with occasional shale and conglomerate bands. Channel-forms. Disturbed bedding common near top of division Occasional small-scale ripplemarks.	90–120
t''	(3) Cross-bedded gravelly facies	Dominantly cross-stratified red felspathic gravels and arkoses; interbedded tabular gravels and conglomerates Channel-forms Disturbed bedding and ripplemarks rare.	90
	(2) Tabular pebbly facies	Flat-bedded red conglomerates up to 4 × 200+ m in section, grading down into or alternating with cross-stratified red felspathic gravels and arkoses. Channel-forms.	100–150
t'	(1) Cross-bedded cobbly facies	Cross-stratified and tabular red conglomerates, felspathic gravels and arkoses. Cobbles up to 180 mm in diameter. Channel-forms common.	0–30

* Wentworth grade scale terms used, except gravel (2–4 mm).

Thus quicksands probably were abundant on the outer portions of the Applecross alluvial fans. Water infiltrating into the coarser materials at the heads of many modern alluvial fans moves down the fans under hydrostatic head, often rising as springs at the fan margins[10,11]. By analogy, it is suggested that the bedding disturbances of the Applecross Group occurred in quicksands downslope from the water table-fan surface junction. The large diapiric and cuspate structures may have been caused by rising springs.

Bands and patches bleached light grey are common in the upper 120 m of the Cape Wrath sequence and have been noted in the Applecross to the south. Evidence suggests that the bleaching occurred shortly after deposition of the sediments. A moderately reducing environment seems likely, possibly promoted by a high, fluctuating water table.

The connexion between the Torridonian groups and the Moine Schists shown in Fig. 3 is based on Sutton and Watson[3]. The proposed lines of facies variation run obliquely to the present elongated outcrop of the Torridonian. Correction for post-Torridonian faults may alter these regional trends slightly.

Further work is necessary before the palaeogeographic picture is complete. However, it is hoped that this preliminary report has demonstrated the important connexion between geomorphic processes and contemporaneous sedimentation.

I thank my colleagues for helpful discussion and advice.

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DEPOSITIONAL ENVIRONMENT OF BRITISH LIASSIC IRONSTONES CONSIDERED IN THE CONTEXT OF THEIR FACIES RELATIONSHIPS

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ALTHOUGH the petrology of the economically exploitable oolitic ironstones in the British Lias has been intensively investigated and their conditions of formation repeatedly discussed[1–5], a number of outstanding problems still remain. It is believed that some of these can be resolved by studying the ironstones in the context of their facies relationships. It will be shown in addition how this approach facilitates the interpretation of early Jurassic palaeogeography.

The ironstones are typical minette ores and consist characteristically of chamosite ooliths, sometimes altered to limonite, in a sideritic and chamositic matrix, although numerous minor variants occur. For full petrographic details Taylor's monograph[6] on the lithologically similar Northampton Sand ironstone (Middle Jurassic) remains indispensable.

As indicated in Table 1, ironstones are widely distributed in the British Lias, being missing only from the Hettangian and Carixian stages. All are well known except the Ardnish Ironstone near Broadford, Skye, a 2 ft. band which was only recently discovered[7]. The most important ironstones in western Europe are the minette ores of Lorraine, ranging in age from Upper Toarcian to Bajocian (that is, 'Aalenian'); others occur in the Lower Sinemurian and Carixian of north-west Germany and the Carixian of Bornholm. Thin beds containing chamosite or limonite ooliths occur widely and include two in the Hettangian of Baden-Württemberg, the *Psilonotenbank* and *Oolithenbank*. It is evident, therefore, that in north-west Europe as a whole conditions suitable for minette formation occurred locally throughout the Lias.

Table 1

Stage	Scotland	Yorkshire	Midlands
Toarcian	Raasay	Rosedale	
Domerian or Upper Pliensbachian		Cleveland	Marlstone
Carixian or Lower Pliensbachian			
Sinemurian	Ardnish		Frodingham
Hettangian			

Facies Relationships

The extent to which the Liassic ironstones represent condensed deposits has not always been fully appreciated in discussions of their origin. As indicated in Fig. 1 the lateral equivalents of the ironstones in sandstones or shale are substantially thicker. Even this does not give an adequate picture of the extent of condensation, since

allowance must be made for post-depositional compaction of the shales. Furthermore, although the Yorkshire coast Liassic succession is one of the thickest in Britain, it still only signifies a moderate rate of sedimentation. Thus the Toarcian shales of the Nordoldenburg Trough in north-west Germany are some 400 m thick[8], about the same as the whole Liassic succession in Yorkshire.

Stratigraphical control for the correlations given in Fig. 1 is good and there is no likelihood of major non-sequences except possibly in the case of the Raasay Ironstone. (This last case has been allowed for so far as possible by correlating the ironstone only with the *commune* sub-zone of the *bifrons* Zone, though it may well span a much longer period of time[9].) On the other hand, it is likely that brief periods of erosion alternated frequently with periods of slow deposition.

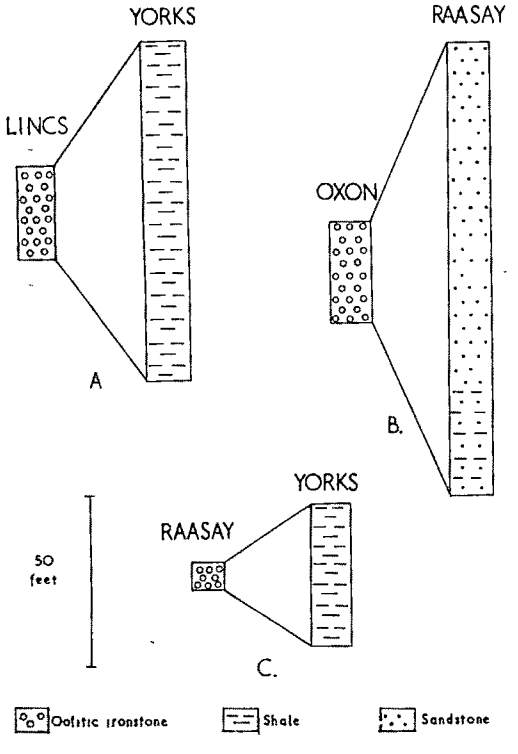


Fig. 1. Diagram to illustrate the condensed nature of oolitic ironstones compared with their lateral equivalents in shale and sandstone: *A*, Frodingham; *B*, Marlstone; *C*, Raasay

Brockamp[10] has observed that the German ironstones often occur at the boundary between sandstones and shales; a comparable association is characteristic of British Liassic ores. Both the Frodingham and Cleveland ironstones pass laterally into silty and sandy shales and the Rosedale and Marlstone ironstones pass laterally into fine sandstones. The lateral equivalents of the more poorly exposed Raasay and Ardnish ironstones are not known, but both beds occur in a sequence of silty and sandy shales.

In considering the general problem of iron enrichment in the sea-water or bottom sediment it is misleading to restrict attention, however, to the chamosite/limonite oolites. This is made evident by examining the facies relationships of the Cleveland Ironstone on the Yorkshire coast. Abundant ammonites allow a detailed correlation from the ironstone in a fairly normal, partly oolitic development near Staithes to its lateral equivalent a few miles to the south-east of a thicker sequence of silty/sandy shales with thin nodule bands of non-oolitic siderite mudstone (Fig. 2). It cannot be seriously supposed that the chemical constitution of the sea-water differed appreciably over such a short distance. Therefore it follows that the concentration of iron in the beds to the north-west, sufficient to make an exploitable ore, is the result of local physical conditions (a higher terrigenous sedimentation rate to the south-east in quieter and probably slightly deeper water). Although, considering the shales in bulk, the iron content is considerably diminished, it has been concentrated in the nodules owing to diagenetic segregation.

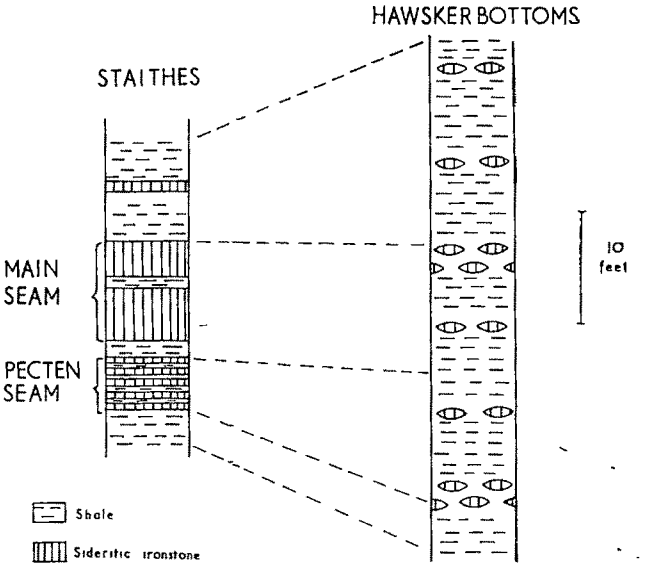


Fig. 2. Correlation of the *spinatum* Zone on the Yorkshire coast, based on Howarth (ref. 21). The Cleveland Ironstone at Staithes comprises the Main and Pecten seams

Obvious as the foregoing deduction may appear, it is nevertheless significant since the facies of silty/sandy shales with sideritic nodules is much more widespread in the Lias than the chamosite oolites. For example, in Yorkshire this facies begins just below the base of the Carixian and continues up to the top of the Domerian. There is a return to normal non-silty calcareous facies in the lower part of the Toarcian but the silty/sandy ferruginous facies returns in the Upper Toarcian.

In the Midlands and South-west England, in contrast, the Lower Lias is largely free of ferruginous beds, the most notable exception being the Lower Sinemurian Frodingham Ironstone in north Lincolnshire. However, the Lower Domerian, that is, the zone preceding that containing the Marlstone and Cleveland ironstones, is marked by a great spread of silty shales with sideritic nodules over most of this region. Apart from the local occurrence of diachronous sandy beds in parts of the south-west, the Toarcian marks a return to normal calcareous clay deposits.

British Liassic rocks can in fact be divided into two distinctive associations which may be termed the 'calcareous' facies and 'ferruginous' facies. The former is the more widespread and consists of argillaceous deposits and limestone. Smooth-textured shales, clays or marls with strongly sub-ordinate detrital quartz of silt grade and subsidiary layers and nodule bands of fine-grained argillaceous limestone were probably deposited in slightly deeper and quieter off-shore waters. Shallower near-shore deposits, as seen in Glamorgan, the Mendips and the Inner Hebrides, contain a higher proportion of limestone, which includes oolitic and bioclastic varieties.

The argillaceous rocks of the ferruginous facies are typically rich in detrital quartz of silt to fine sand grade (on the Wentworth Scale). This gives them a distinctive rough texture, while the abundant muscovite flakes produce a characteristic glint. The associated sideritic nodules correspond to the calcitic nodules in the calcareous clays just as the chamosite oolites may be thought of as the equivalents of the calcareous oolites. Calcareous nodules may also occur, however, though not in intimate association with the sideritic.

Sandstones are subordinate in both facies, although commoner in the ferruginous; they are almost invariably fine grained. Whereas those in the calcareous facies are well sorted, in a calcitic cement (for example, in the Lower Broadford Beds of the Skye area) the sandstones in the ferruginous facies are of several types. One type, well exemplified by the sandstones at the Carixian–Domerian boundary in Yorkshire, is well sorted and clean-washed, with a calcitic cement as in the calcareous facies. Other sandstones, though possessing a calcitic cement, contain limonite associated with the quartz grains (for example, the Upper Broadford Beds sandstones of the Skye area). A third type, strictly confined to the ferruginous facies, consists of intimate mixtures of clay and fine sand. The lithological contrast allows the recognition of mottling exhibiting several varieties of trace fossil including *Chondrites*, *Diplocraterion*, *Rhizocorallium* and *Thalassinoides*. Good examples include the topmost Sinemurian and the topmost Toarcian beds on the Yorkshire coast, and much of the Pliensbachian in the Inner Hebrides.

Bearing of Facies Relationships on Hypotheses of Ironstone Formation

The abundance and diversity of benthonic fossils in dominantly argillaceous sediments signifies a widespread sedimentary environment of quiet but shallow-water marine conditions in the British area during Liassic times, and implies that the associated landmasses were of very subdued relief. In the context of this environmental picture the occurrence of mixed sandy, silty and argillaceous rocks is likely to signify the proximity of rivers. Tidal and longshore currents are of course capable of transporting sand by traction considerable distances from river mouths but the deposits that result from such transportation are normally well sorted—mud and fine silt having been winnowed out. The mixed sand, silt and clay deposits that occur in the ferruginous facies have evidently been buried before any major winnowing action could occur. Such mixed deposits, lacking any trace of lamination because of the activity of burrowing organisms, are characteristic of the distal parts of pro-delta régimes of the present time[11,12]. In support of this interpretation, fossil driftwood appears to be more abundant in the ferruginous than in the calcareous facies of the Lias, due allowance being made for variations in rates of sedimentation. We must now consider the bearing of this interpretation on the various hypotheses of ironstone formation.

The orthodox views of minette ore formation all assume derivation of iron by continental weathering, although opinions differ on the mode of transportation and concentration on the sea bed. A radically different hypothesis has been proposed recently by Borchert[13,14], who believes that the conventional interpretation has several insuperable difficulties and argues that certain deposits on the sea floor, possibly far from land, themselves acted as sources of iron. Though Borchert's ideas were received with some scepticism in the discussion following the reading of his paper in London[15], they were treated sympathetically by Dunham[5] as being possibly valid in the case of certain Liassic ironstones.

It will be convenient to consider Borchert's hypothesis first. He envisages three depth zones in the sea with distinctive chemical characteristics and bottom deposits. The deepest is the hydrogen sulphide zone, characterized by anaerobic bottom waters and sediments consisting of pyritic bituminous mud. The shallowest is the oxygen zone, with a rich bottom fauna, where limonitic ooliths form in agitated water. Between these is the carbonic acid zone, rich in carbon dioxide, where the bottom deposits consist of sideritic muds; iron is supposed to be mobilized from the deposits of this zone. Part of the iron is carried by marine currents into shallower water where it contributes to the formation of limonite. Part is carried towards the hydrogen sulphide zone, where it encounters silica dissolved in alkaline water liberated from the bituminous mud; as a result chamosite forms.

A major difficulty of Borchert's interpretation is that the depth zones and chemical reactions are hypothetical so far as marine conditions are concerned. There is no evidence, for example, of a widespread carbon dioxide zone in seas of the present day where the deeper water is anaerobic. Apart from this, however, these are ample grounds for rejecting his hypothesis for the Liassic ironstones.

The necessary association of ironstones and bituminous shales at the same stratigraphic horizon exists nowhere in western Europe. On the contrary, there is a clear negative correlation, since laminated bituminous shales, such as occur in parts of the Hettangian and Sinemurian of South-west England and the Lower Toarcian of a much more extensive area, invariably belong to the calcareous facies. Borchert actually argues for deep-sea stagnation and bituminous facies on the grounds that, in the absence of polar ice caps during periods of ironstone formation, deep oceanic circulation would be far more sluggish than to-day. Liassic deposits occur in the Mediterranean region (Ammonitico Rosso facies) which were probably laid down in deeper water than anything in north-west Europe; they are, however, strikingly deficient in organic matter and are not especially rich in iron.

The evidence from facies associations lends support in fact to the conventional view of iron derivation, with ferruginous deposits forming close to river deltas. We must now examine briefly certain ideas on the site of ironstone deposition and the transportation and concentration of the iron, in so far as they are pertinent to the Liassic ores.

It has been suggested that ironstones formed close to old shorelines[10,16]. This is not necessarily so. The unequivocal shoreline deposits of Glamorgan and the Mendips are in calcareous facies and include normal oolites and skeletal limestone, as do the near-shore deposits of the Lower Broadford Beds in Skye. In contrast, none of the oolitic ironstones are obviously associated with an old shoreline, though because of inadequate exposure this cannot be decisively ruled out. It has been pointed out, moreover, that certain Asiatic rivers have been observed to carry iron-bearing sediments far into the open sea[17].

Another common belief is that the ironstones did not form in the open sea but in some sort of near-shore lagoon. To be meaningful, the term lagoon should be restricted to partly isolated regions of abnormal salinity, or at least regions protected from strong wave- or tide-induced water disturbance. The Liassic ironstones contain abundant benthonic fossils including many stenohaline types, with no suggestion of abnormal salinity. There is in addition abundant evidence from the rocks themselves of strong water agitation.

Two different solutions have been proposed to account for the rarity of detrital quartz grains in what are evidently shallow-water deposits that have been subjected to agitation sufficient to transport such grains. Hemingway[8], influenced by an approximation in the Yorkshire Lias on three occasions to a cyclic sequence of shale–sandstone–ironstone, has related the sedimentation to erosional cycles on the land. As the latter became peneplaned, mechanical erosion diminished so that finally only chemical

derivatives including iron were transported and deposited in shallow restricted arms of the sea. This hypothesis fails to account for the frequent lateral passage of ironstones into clastic rocks and is clearly incompatible with the interpretation adopted in this article. Some sort of 'clastic trap'[18] seems a more plausible explanation. That is, some iron was transported slightly beyond the limit of deposition of the bulk of the terrigenous sediments. We turn now to how this might have been achieved.

The problem of the transportation and concentration of iron to form exploitable ores is singularly acute because of the lack of modern analogies. As is well known, chamosite is apparently forming nowhere in recent marine sediments and siderite is rare, while the concentration of dissolved iron in sea-water is negligible. The most commonly held belief is that the iron was transported in rivers in the ferrous state protected by organic colloids and later deposited on entering the alkaline environment of the sea. Carroll[19] has recently put forward a different suggestion, pointing out that ferric oxide can travel to the sea as particles coating the surface of clay micelles and can be removed by a lowering of the redox potential to negative values, which converts the ferric iron into the more soluble ferrous form. She argues that chamosite may be formed in such a reducing environment by the interaction of iron released in this way with kaolinite.

It seems quite probable that siderite could form as a result of the process Carroll describes, iron released from clay minerals within silty pro-delta muds reacting with carbonate ions in the interstitial water and afterwards segregating to form nodules. In support of this, there is a clear association of siderite with clay in the Lias. Those sandstones in the ferruginous facies which have been winnowed free of clay particles, such as the Marlstone 'sandrock' in Leicestershire and the *davoei-margaritatus* Zone sandstones of Yorkshire, are cemented by calcite not siderite, suggesting that iron has been removed with the clay.

Unfortunately Carroll's interpretation does not solve the problem of chamosite concentrations. All the normal Liassic clays studied by me consist dominantly of illite, kaolinite being markedly subordinate. Yet chamosite is the most abundant clay mineral in the ironstones. The influx of large quantities of illite, just as with detrital quartz, evidently diluted the available iron to such an extent that exploitable ore could not form. Therefore in a sense we are back where we started. It is hard to resist the conclusion that some iron was carried, perhaps as a colloidal solution or suspension, beyond the area where most of the terrigenous sediment was deposited. It could only concentrate on the sea bottom in areas of extremely slow sedimentation, probably off-shore shoals. Chamosite presumably formed authigenically in a reducing environment within fine-grained sediment but was evidently stable in slightly oxidizing conditions, as testified by the rich benthonic fauna Presumably it was carried to the surface by burrowing organisms and ooliths produced as a result of water agitation. Planktonic organisms such as diatoms could well have contributed to both the iron and silica required for the formation of chamosite, since opaline silica is readily soluble within sediments.

As regards the difficulty concerning the lack of modern analogies, one should bear in mind the following point. Because the world to-day has abnormally high relief terrigenous sedimentation rates on the continental shelf in the vicinity of river deltas are probably a good deal higher than in the shelf sea that covered north-west Europe in the early Jurassic. The concentration of iron coming from rivers need not have been appreciably higher than to-day, so long as sedimentation rates were slight. A comparison with the phosphate deposits that have formed recently off southern California may be apposite; these seem to owe their origin essentially to a conjunction of two factors: (*a*) a slow rate of sedimentation, (*b*) phosphate enrichment in sea-water due to upwelling. The significant point is that even in areas of upwelling phosphate concentration remains low compared with many other ions.

The well-known fact that iron concentrations in rivers are generally much higher than in the sea suggests that much may be deposited not far from the deltas. It would be instructive if studies could be made comparing the interstitial water composition of certain pro-delta and river-distant sediments.

Interpretation of Jurassic Palaeogeography

Distinction of the calcareous and ferruginous facies, and recognition of the probable association of the latter with river deltas, brings out with unexpected clarity important regional similarities and differences in the English Lower and Middle Jurassic. South-west England and Yorkshire show parallel contrasts during this time. The former area remained consistently within the calcareous facies, deeper water clays and calcilutites passing upwards stratigraphically into shallower water calcareous oolites. The Yorkshire Lias exhibits the first hints of a river delta near the base of the Carixian. Following a major marine transgression in the Lower Toarcian[20], when the calcareous facies temporarily returned, the pro-delta facies spread once more. In Middle Jurassic times, owing to shallowing of the sea as in the south-west, the delta itself began to cover the area. The Midlands form an intermediate area. In the Middle Jurassic, several oscillations are seen between calcareous facies (Inferior and Great Oolite limestones) and ferruginous/deltaic facies (Northampton Sand ironstone, Lower and Upper Estuarine Series), bound up possibly with the periodic migration of delta distributaries.

Major rivers evidently did not enter the sea in the area of South-west England, except locally for a short time during the Toarcian. It is tempting to suggest that the Yorkshire delta spread from a river draining the Scandinavian landmass (there is no good evidence supporting a Pennine landmass at the time, as shown in some palaeogeographic reconstructions). The main evidence favouring this is as follows. The only early Mesozoic remnants in Scandinavia are deltaic and ferruginous shallow marine deposits of Rhaetic and Lower Lias age. Also, most of the sandy deposits that spread into the north-west German Basin in Bajocian times came from the north or north-east[8]. Now that boreholes are being drilled below the North Sea off north-east England one may tentatively venture the prediction that they may show the Lias in this area to contain a higher proportion of sandy and ferruginous deposits than in Yorkshire.

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DISTRIBUTION OF CAESIUM-137 IN THE HUMAN ORGANISM AND IN THE HUMAN FOETUS*

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FOR some years the distribution of the fission product caesium-137 within the human and animal organism has been studied by several investigators[1–5]. Much of this research has been aimed at finding the ratio of caesium-137 activity in soft tissue to that in bone. During the past few years, different ratios have been established by different groups of workers. Moreover, Yamagata *et al.*[3] and Anderson *et al.*[5] measured the content of caesium-137 in rib bones as a function of the age of the individual. The results obtained by these authors revealed that the specific activity of caesium-137 per g bone ash is at a minimum at the age of puberty. Oberhausen[6], however, published data on whole-body caesium-137 related to potassium indicating an increase of body caesium-137 from 6 years of age up to the age of puberty and showed that it was constant from that time on.

From results of whole-body counting of adult foetuses, McDonald *et al.*[7] suggest that there is a placental discrimination for caesium-137. This is surprising, since no placental discrimination is known for potassium.

Because of the different results published by several authors concerning the activity ratio of caesium-137 in soft tissue to that in bone, the caesium-137 activity as a function of age and the behaviour of the placenta for caesium, we measured the distribution of caesium-137 within the human and animal organism. We also determined the caesium-137 activity of samples of human bone and tissue for different age groups. In order to discover whether or not the placenta is a barrier for caesium we analysed foetal, placental and soft tissue of adults. The parameters to which the content of caesium-137 is related are fresh weight and content of potassium, since caesium is known to follow to a certain extent the metabolic pathway of its periodic relative potassium.

After chemical processing, the caesium-137 activity was measured by both a low-level beta-ray counting technique and by gamma-ray spectrometry. With the latter method the limit of detection was 5×10^{-11} c. caesium-137, but activities as low as 3 pc. could be detected by the anti-coincidence method[8].

The samples were wet-ashed in all cases, since caesium is reported to be volatile at temperatures higher than 450° C (ref. 9) and to be incorporated into the glaze of porcelain crucibles during dry-ashing[10]. The analytical recovery and the recovery of activity were 85 per cent in a pilot experiment using caesium-137 spiked apatite.

The preparates mounted for counting did not contain analytically determinable amounts of potassium. Therefore, no interference with the low beta-ray counting rate of caesium due to traces of potassium was to be expected. The content of potassium in tissue was determined by means of flame-spectrometry[11,12]. All analytical reagents used in these measurements were redistilled in a quartz apparatus prior to use.

From investigations performed by Hood and Comar[10] the distribution pattern of caesium-137 in the animal organism following a single administration is known. To examine the distribution pattern following continuous ingestion of fall-out caesium, we measured the specific caesium-137 activity in the stag. From these investigations the ratio of the activities of femoral bone-marrow : femoral compacta and muscular tissue adjoining the femur was shown to be 1 : 2, 9 : 26 (ref. 8). In reindeer a similar ratio was found for the activity in femoral bone marrow to the activity in muscles by Miettinen and Paakkola[13].

About 6 years ago, Yamagata[1–3] reported that human ribs and muscles contain the same level of caesium-137 activity. Some results indicated an even higher activity in human bones than in muscles. About 3 years later, the muscular activity of caesium was reported by Harrison *et al.*[4] to be 3–4 times the activity found in bones separated from their marrow. Our measurements revealed that the activity of caesium-137 in skeletal muscles is 15 times the content in marrow-free bones. The content in ribs was 2–3 times the activity in femora[8].

In our investigations we dealt chiefly with the rectus femoris, the addition of the sartorius and vastus medialis muscles occasionally. Femora were carefully cleaned from their bone-marrow and both parts were processed separately. The activity ratio between marrow and compacta was 1 : 1·2 (ref. 8).

As mentioned earlier, the specific activity of caesium-137 in ribs was found by Anderson and Gustavson[5] to decrease from birth to puberty and to rise again in the later years of life. The rising slope in later life was confirmed by Yamagata[1–3]. These results could not be confirmed by our measurements covering samples of soft tissue as well as femur and ribs taken from 5–10-month foetuses, still-borns and adults up to an age of 80 years. As may be seen from Fig. 1, the caesium-137 activity related to potassium in liver is 130 pc./gK and in brain 50 pc./gK in all stages of development. Since the standard deviation is at least 50 per cent due to the relatively small amount of foetal tissue being available, there is no significant difference between foetal and adult tissue. This is also true for the other organs being analysed.

In the upper graph of Fig. 2, the content in femoral tissue is shown to be 200 pc./gK, being also independent of age. In the lower graph of Fig. 2, the content of caesium-137 in bone is plotted against the non-mineral substance of bone (representing the amount of potassium in bone). With this correlation the specific activity of caesium-137 found in foetuses, children and adults are of the same order of magnitude. The factor of 2 is probably not significant owing to the relatively small number of foetuses analysed.

As to the question of placental transfer, the specific caesium-137 activity correlated to the content of potassium was assessed in the muscular tissue of adult women, in placental tissue and in foetal muscular tissue. The ratio between these three parameters as shown in Fig. 3 was unity within the biological variability.

It may be concluded from the results that: (1) The results of Yamagata[1–3] as well as those of Harrison *et al.*[4] showed the specific caesium-137 content of human muscular tissue to be 1–3 times that of bone tissue (Table 1). Our results, however, show that the specific caesium-137 content of muscular tissue is about 15 times that of compact bone. In the stag, the caesium-137 content in muscular tissue is also significantly higher than

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Table 1. Activity Ratio of Caesium-137 (Bone/Muscular Tissue) measured in Human and Animal Organism

Author	Activity ratio bone : muscle tissue	Organism	Remarks
Yamagata *et al.*	⩾1	Human	Observed in 1960
Harrison *et al.*	1 : (2–3)	organism	1962/63
Own values	1 : 15		1963/64/65
Hood *et al.*	1 : 13	Rat	28 days after injection
Bretfeld	1 : 30		
Own values	1 : 9	Stag	Continuous uptake of fall-out ^{137}Cs with diet

Table 2. Specific Caesium-137 Content in Different Organs related to Muscular Tissue (=1·0)

Organ	Hood *et al.* (rat*)	Bretfeld (rat*)	Own values (man†)
Muscular tissue	1 0	1·0	1·0
Liver	0·22	0·32	0 65
Brain	0·29	0·21	0·25
Compacta	0 08	0·033	0 03

* 28 days after single injection.
† Continuous uptake of fall-out ^{137}Cs with diet.

that of bone tissue, though this ratio is somewhat lower than in man (Table 1). In cases of a single injection of caesium-137 into rats, results similar to ours for the distribution of caesium between muscular tissue and bone were published by Hood *et al.*[10] and recently by Bretfeld[14]. The results indicate a 13–30 times higher caesium-137 content in muscular tissue than that found in bone (Table 1). Besides this, the results show the activity ratio to be constant between the twentieth and seventieth days after injection. The conditions of a single injection may, however, be different from those of a continuous intake of caesium-137 in food. In order to find out whether or not both cases are comparable, we are undertaking animal experiments with continuous long-term feeding of caesium-137.

(2) For the distribution of caesium-137 between other organs such as liver and brain, our results indicate a specific caesium-137 content in brain of 25 per cent of that of muscular tissue and are in rather good agreement

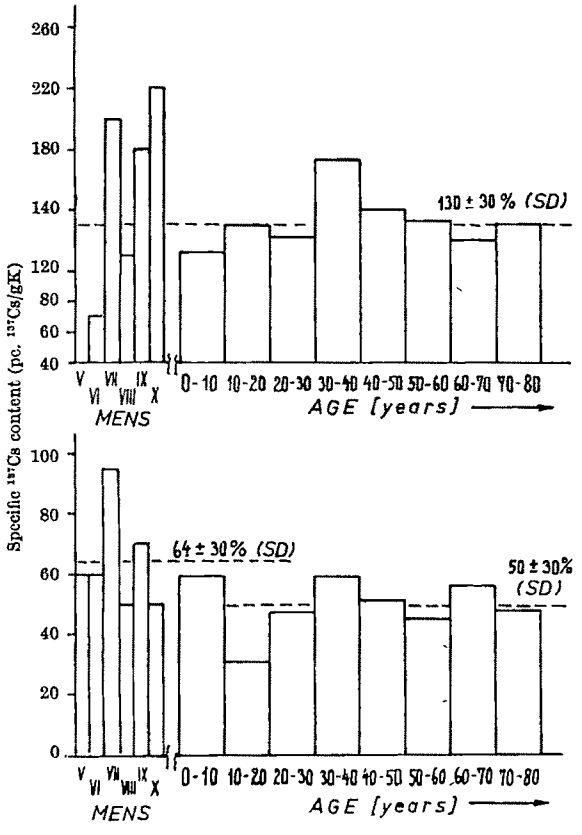


Fig. 1. Specific caesium-137 content of liver (top) and brain (bottom) as a function of the age of the individual

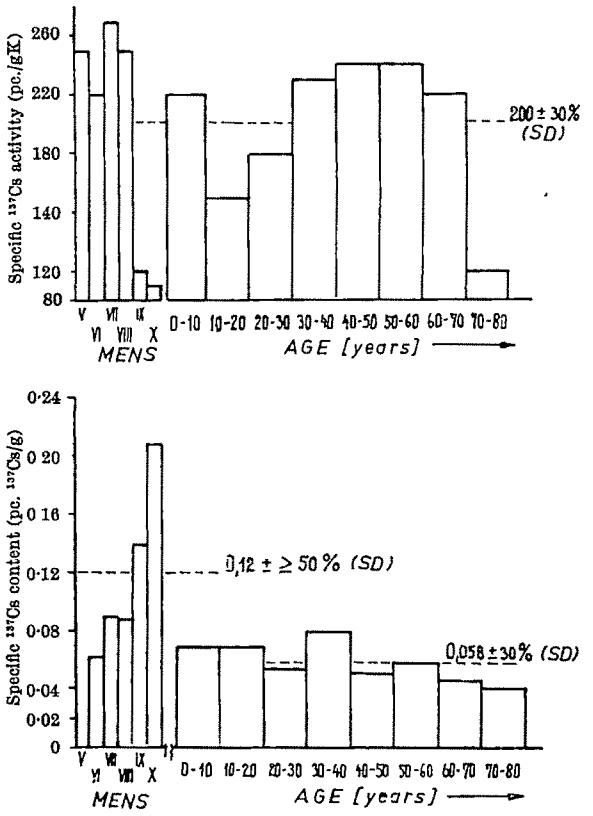


Fig. 2. Specific caesium-137 content of muscular tissue (top) and marrow-free bone (bottom) as function of age

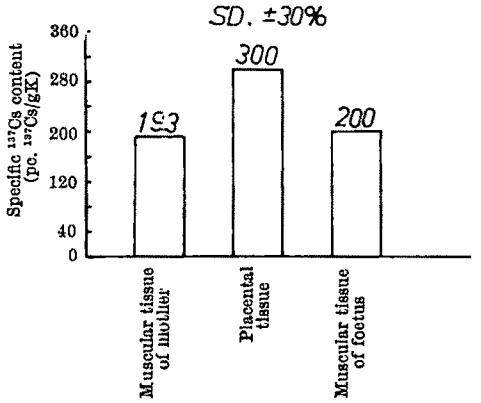


Fig. 3. Specific caesium-137 content of muscular tissue of mother and foetus and of placenta

with the results of animal experiments (Table 2). Though 2–3 times higher in man, the specific caesium-137 content in liver is also significantly lower than that in muscular tissue in man as well as in rat (Table 2).

(3) Contrary to results found by Anderson[5] and Yamagata *et al.*[1–3], no change with age in the specific caesium-137 content in different tissues including muscle and bone was observed in our cases.

From the fact that the specific activity of caesium-137 related to potassium in soft and bone tissues of adults is on the same level as the activities found in foetuses, it might be concluded that there is no significant placental discrimination for caesium-137 in the activity range under discussion. This conclusion is supported by the observation that the specific activity in muscular tissue taken from mother and foetus and the activity of the placenta proved to be on a comparable high level.

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POLONIUM-210 ACTIVITY IN THE LUNGS OF CIGARETTE SMOKERS*

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In order to contribute further information on the natural radiation burden applied to the human body, we measured the ^{210}Po content in different parts of the lungs of human cigarette smokers and non-smokers. These investigations became necessary, since in 1964 Radford and Hunt[1] were able to demonstrate that a considerable portion of ^{210}Po, naturally occurring in tobacco, is inhaled with cigarette smoke. The average activity of ^{210}Po assessed in some brands of cigarettes in our laboratory is summarized in Table 1. The determination of ^{210}Po was performed in the following way. The tobacco samples were wet-ashed using nitric, hydrochloric and perchloric acids. Then ^{210}Po was enriched by a co-precipitation with Bi_2S_3 and subsequent separation by silver-plating[2]. The processed samples were counted in a methane flow counter. The background of α counts in the proportional counter is of the order of 2–3 counts per h. On average, 50 per cent of ^{210}Po present in one cigarette was detected in the main smoke stream, 15 per cent in the cigarette-ash and 35 per cent was measured in the butt. For filter cigarettes the maximum ^{210}Po activity detected in the filter was 5 per cent. It can thus be seen that a filter has no noticeable protective effect against polonium inhalation. These results are in good agreement with the measurements of Radford and Hunt[1] and the findings of Hill[3]. According to Radford and Hunt[1] the main smoke stream amounts to approximately 50 per cent of the total smoke. Based on this value and on our measurements, the ^{210}Po activity inhaled on average per cigarette is of the order of 0·1 pc. This means that, for an individual smoking 20 cigarettes a day, the daily ^{210}Po inhalation rate is 2 pc.

Table 1. Polonium-210 Content in Various Brands of Cigarettes

Tested brand of cigarette	^{210}Po content/g cigarette tobacco (10^{-13} c/g)	^{210}Po content/ cigarette (10^{-13} c./cig.)	^{210}Po content/ash of the cigarette (10^{-14} c./ash cig)	^{210}Po content/ butt (10^{-13} c./butt)	^{210}Po content/ total smoke (10^{-13} c./tot. ms.)
'Prince of Wales' (filter)	4·5	4·2	6 3	1 7 (0·17 in the filter tip)	1·9
'Rothandle' (Pfalz)	5·0	6·0	7·2	1·9	3·4
'*H B*' (filter)	4·1	4·1	5·3	1·7 (0·21 in the filter tip)	1·9
'Lucky Strike' (United States)	3 4	3·4	5·5	1·2	1 6
'Lucky Strike' (United States)	3·2	3·2	5·7	1·1	1·5

The inhalation rate, due to polonium occurring in the open air, was estimated by Jacobi[4] to be 0·02 pc. per day. This activity is only one-hundredth the value being inhaled by a moderate smoker. This is the reason why considerable differences are to be expected between the ^{210}Po activities to be measured in the lungs of smoking and non-smoking individuals.

The ^{210}Po activities found in different parts of the lung tissue of both smoking and non-smoking individuals are summarized in Table 2. For these analyses, the parenchymal tissue, the bifurcation and the bronchial tissue were dissected prior to measurement. The lung tissue dissected from heavy smokers was shown to contain three to four times the ^{210}Po activity found in the lungs of non-smokers.

Table 2. Lung Tissue Levels of Polonium-210 in Smokers and Non-smokers

Lung parenchyma ^{210}Po content/g wet tissue (10^{-15} c./g)	Epithelium ^{210}Po content/g wet tissue (10^{-14} c./g)	Epithelium ^{210}Po content (10^{-4} pc./cm^2)	Bronchial tree ^{210}Po content/g wet tissue (10^{-15} c./g)	Bifurcation ^{210}Po content/g wet tissue (10^{-15} c./g)	Cigarettes per day	Age of subject (*a*)
Cigarette smokers						
14 1	—	—	8·6	—	30–40	21
7·8	—	—	4 7	—	20–40	42
2·5	—	—	—	3 1	30–40	55
3·4	—	—	—	4·1	40	51
6·0	15 3	18	8 8	3 6	40	33
7·7	1·4	10	5·1	—	20	40
5·1	2·2	3	3·2	—	40	32
7·6	1	1	5·1	—	40	67
—	—	—	10·5	—	30	63
12·0	2·3	5	12·0	—	40	50
—	4·5	7	3 2	6·2	30–40	31
18·5	8·3	11	20·8	—	40	52
2·8	7·5	8	5 0	—	30	64
5·7	7·0	11	5 4	6 8	30–40	60
Total average values:						
7·8	5 4	8	7·7	4 7		
Non-smokers (average values)						
2 5	—	—	2 0	1·2		

In nine lungs of smokers, the ^{210}Po activity was also measured in the epithelium of the bifurcation, the secondary bifurcation and parts of the trachea, using a chemical procedure of wet ashing and subsequent chemical separation by silver plating[2]. The specific ^{210}Po activity of the epithelium was about ten times the value assessed in the remaining lung tissue. As the area of the epithelium is known from the section, the specific activities are also given per square unit. As can be seen from Table 2, the values range from 1 to 18 $\times$ 10^{-4} pc./cm^2.

The measured ^{210}Po activities in the epithelium are close to the limit of detection, due to the small amounts of sample material available. Long sample and background counting periods were therefore necessary. Here the average total error was $\pm$ 30 per cent, whereas in all the other cases of ^{210}Po counting in lung tissue, the error was only $\pm$ 15 per cent.

The measured specific ^{210}Po activities of the epithelium both per g wet weight and per square unit are smaller than the activities published by Radford and Hunt[1]. According to these authors, the activity in an upper part bronchial bifurcation, dissected from 73-year-old heavy smokers, is reported to be 0·03 pc. per square unit, a value which is, on average, 40 times higher than our own activities. Concerning the specific activity correlated with wet weight, our values are also on a lower level than the activities published by Radford *et al.*[5], who found activities up to 1·25 pc. per g wet tissue in the epithelium of the segmental bifurcation dissected from smokers.

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Our activities per square unit measured in the epithelium of the bifurcation, the secondary bifurcation and in the lower part of the trachea are in good agreement with Jacobi's theoretical calculation of the ^{210}Po activity in the epithelial tissue, making allowance for the ciliary movement and for transfer of ^{210}Po from the alveolar zone into the bronchial tree[4]. The bronchial content for the segmental and terminal region is reported by him to be of the order of magnitude of 10^{-4} pc. per square unit. This calculation is based on the assumption that the rate of inhalation would be 2 pc. ^{210}Po per day, that the biological half-life of this nuclide in the lung would be 5 days, and that its deposition rate within the total respiratory tract would be 80 per cent with half this value deposited in the alveolar zone. According to Jacobi, the specific ^{210}Po activity in alveolar tissue is 6×10^{-15} c. per g wet tissue, and this activity is in good agreement with our findings for the specific ^{210}Po activity, measured in the parenchymal tissue.

Hill investigated the distribution of α-ray tracks in vacuum-dried epithelium samples dissected from the bronchial bifurcation of smokers. These measurements were performed using the stripping film method and showed no noticeable differences in the enrichment of α-ray tracks[5]. This means that the distribution pattern of ^{210}Po in bifurcation places shows no evidence of inhomogeneities. In other words, there is an equal distribution of ^{210}Po in the epithelium. His values were of the same order of magnitude as the theoretical values given by Jacobi, that is, 10^{-4} pc./cm^2 (ref. 6).

Jacobi[4] also calculated the burden applied to the human respiratory tract by naturally occurring radiation. He made special allowances for the dose absorbed in the range of the layer of so-called basal cells because this layer of the bronchial epithelium is especially important in carcinogenesis. For this calculation, the α-ray activity was assumed to be distributed homogeneously in the middle layer of the mucous layer situated over the bronchial epithelium. The calculation of the distribution pattern of the depth dose in the bronchial epithelium was based on the α-ray absorption within the mucous layer and on the stopping power of tissue as a function of α-ray energy.

According to Miller[7] and Altshuler[8], the thickness of the upper mucous layer is 10μ in the zone of the trachea and somewhat less in the region of the terminal bronchi. The average distance of the layer of basal cells from the mucous layer is 60–90μ within the trachea and 20–40μ in the region of the terminal bronchi. The distribution pattern of the α-depth dose emitted from ^{210}Po demonstrates that α-rays do not reach the critical layer of basal cells within the trachea, the main bronchia or the lobate bronchia, and therefore do not affect this layer at all. The critical regions for ^{210}Po are the terminal-, the subsegmental- and the segmental-bronchia.

On this basis, we calculated the α-ray dose absorbed in the critical zones using the activities determined in our laboratory. For this, the area activity of ^{210}Po determined in the bifurcation zone was assumed to be identical to the activities in the region of segmental or terminal bronchi, and the ^{210}Po activity was presumed to lie in the mucous layer. The calculated values are summarized in Table 3. The average dose-rate for the basal cell layer is 41 mrem per year or 1·0 rem over 25 years, respectively, in the subsegmental bronchi; and approximately 79 mrem per year, or 2·0 rem over 25 years, respectively, in the terminal bronchia. These values are small compared with the dose-rate given by Radford and Hunt, which is 165 rem over 25 years at the minimum.

Table 3. ALPHA DOSE-RATE IN BRONCHIAL EPITHELIUM

Object	Average activity (pc./cm^2)	Dose-rate (mrem/a) $QF = 10$
Basal cells of subsegmental bronchi	8×10^{-4}	41 (average) 86 (maximum)
Basal cells of terminal bronchi	8×10^{-4}	79 (average) 150 (maximum)

The ^{210}Po dose-rate based on our measurements for bronchial epithelium is small compared with a dose-rate in the range of 1–2 rem per year originating from the decay of naturally occurring radon and thoron and their short-lived daughter-products. This dose-rate was calculated by Jacobi in the basal cell layer of segmental and subsegmental bronchi[4]. This would seem to show that carcinogenesis caused by inhalation of ^{210}Po with the tobacco smoke is rather unlikely.

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RADIOSENSITIVITY OF IMMUNOLOGICALLY ACTIVATED CELLS

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NUMEROUS investigations have shown what appear to be clear-cut differences in the radiosensitivity of the immune response before and after exposure to antigen. Whole-body irradiation strongly inhibits the response if it is administered before the antigen and is relatively ineffective if given afterwards[1,2]. Radiosensitivity may fall sharply within a few hours of giving the antigen[1] or more slowly over some days[2]. With few exceptions[3] the production phase of the response has been found to be radio-resistant. These marked changes in the radiosensitivity of the response have led to the assumption that the cells mediating the response also change in radiosensitivity, but this does not necessarily follow.

Consider a model (Fig. 1) in which, before exposure to antigen, a few cells produce antibody at levels below the threshold of detection, the number of such cells being kept constant by a balance of a low mitotic frequency with a relatively long life. On antigenic stimulation, the cells increase rapidly, raising the level of serum antibody above the threshold of detection (Fig. 1*A*). Let the radiosensitivity of the cells remain constant so that a given dose of radiation destroys or inactivates the same fraction of cells whenever it is given in relation to the time of giving the antigen. Let the half-life of such irradiated, antibody-producing cells be short compared with the half-life of serum antibody.

If irradiation occurs before administration of antigen, the surviving cells may be envisaged as multiplying relatively slowly to restore the original number. Irradiation long before immunization allows time for complete restoration and the immune response will be normal (Fig. 1*B*). The shorter the interval between irradiation and immunization the less recovery will have occurred (Fig. 1*C*). In this simple model let the responses in such

cases differ from the normal only in the number of cells taking part, so that the curves describing the rise and fall in cell numbers and antibody levels will be normal in shape but displaced downwards (Fig. 1*C*). This results in a lower peak antibody titre and, because of the threshold below which antibody is not detectable, in apparent prolongation of the induction period and slowing of the rate at which antibody rises once it has passed the threshold.

When irradiation occurs late in the production phase serum antibody has already reached a high level (Fig. 1*F*). The number of antibody-forming cells falls rapidly after irradiation, the survivors continuing to rise and fall at the rates prevailing normally. If the surviving fraction is small, the rate of antibody production is at first negligible. The level of serum antibody therefore falls with approximately its characteristic half-life until production by surviving cells or their progeny becomes appreciable. The effects of irradiation after administration of antigen may therefore be partly masked by the relatively slow fall in the level of antibody already present in the serum. At least up to the time when peak titres are reached, the greater the interval between immunization and irradiation, the greater the contribution of already-formed antibody to subsequent levels (Fig. 1*D–F*).

The lower part of Fig. 1 shows the effects on peak titre, length of induction phase and rate of increase of antibody above the threshold when radiation is given at different times before or after the antigen in this model. These have been derived by inspection from the curves in the upper part of Fig. 1. It is seen that all these parameters, which are commonly used as indices of the radiosensitivity of the immune response, would change markedly during the response, even if there were no change at all in the radiosensitivity of the cells concerned.

For simplicity this model ignores effects on precursor cells not forming antibody and any radiation effects other than cell destruction. Moreover, it takes no account of possible homoeostatic mechanisms governing the number of cells producing antibody that might tend to counteract the immunosuppressive effects of radiation. A model so oversimplified cannot be expected to account quantitatively for experimental findings and there are important

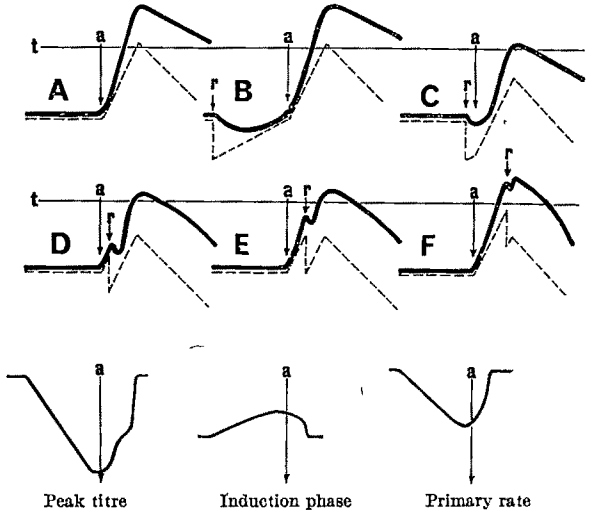


Fig. 1. Model of an immune response with constant cellular radiosensitivity. – – – – –, Log No. of antibody-forming cells; ————, serum antibody titre; *a* ↓, injection of antigen; *r* ↓, irradiation; *t*, threshold for detection of serum antibody. *A*, Normal response; *B—F*, responses with irradiation at different times in relation to administration of antigen (note that the fall in the log No. of cells is the same throughout). In the lower part of the Figure the horizontal axis (on the same scale as that in the upper part) shows the time of irradiation before or after administration of antigen, indicated by *a* ↓. The vertical axes show, respectively, peak titre, length of induction phase and the primary rate of increase in antibody above the threshold. These curves, therefore, which have been drawn through points derived by inspection from the curves in *A—F*, show what effect irradiation at different times would have on these parameters of the immune response in this model

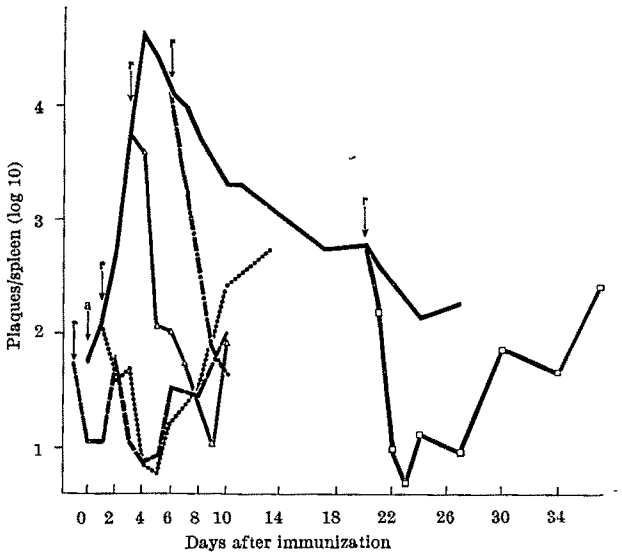


Fig. 2. Effect on numbers of haemolysin-forming cells of irradiation with 450 r. on days −1, +1, +3, +6 or +20. *a* ↓, injection of antigen; *r* ↓, irradiation; ————, controls, – – – – –, day −1 group, · · · · · ·, day +1 group; ——△——△——, day +3 group, ——·——·——, day +6 group; ——□——□——, day +20 group. Curves drawn through geometric means of each group, 5—10 mice per day per group

qualitative findings not accounted for; for example, the adjuvant effect of radiation in some circumstances. However, the overall similarity of the curves generated by the model to those found experimentally[1,2] suggested that it would be worth investigating directly the radiosensitivity of antibody-forming cells at different stages of the immune response. The following experiments were therefore performed.

Male *A2G* mice were injected intraperitoneally with 0·2 ml. of a 10 per cent suspension of formolized sheep red cells in saline. Groups of mice were killed at intervals and the numbers of haemolysin-forming cells in their spleens counted by Jerne's method[4]. Mice were irradiated either one day before giving the antigen or 1, 3, 6 or 20 days afterwards with 450 r. (65–75 r./min, 220 kVp, 15 m.amp, 0·5 mm copper and 1 mm aluminium added filtration, target distance 34·5 cm). The results are shown in Fig. 2. It appears that this dose of radiation causes a rapid fall in the number of antibody-forming cells whenever it is given. There is no evidence of an increase in radio-resistance after the antigen has been given, even late in the production phase of the response.

In view of the possibility that cells producing 19*S* haemolytic antibody might be atypical in radiosensitivity, further experiments were performed in which the numbers of cells forming haemagglutinin were counted. A modification of Zaalberg's method[5] was used; 1-ml. portions of spleen cell suspensions and 0·25 per cent sheep red cell suspensions in cold tissue culture medium '199' were mixed and stood overnight in the refrigerator in 13 × 150 mm 'Pyrex' test-tubes. These were then rotated at 28 r.p.m. for 10 min at room temperature in a plane 33° from the horizontal and the number of agglutinates counted in a Fuchs–Rosenthal chamber. The results are shown in Fig. 3. Again, radiation causes a rapid fall in the number of antibody-producing cells and there is no evidence that radio-resistance rises after the antigen is given.

There are two notable differences between the curves obtained here and those predicted by the model. First, irradiation a day before giving the antigen causes a smaller fall in cell numbers than irradiation after the antigen has been given. This finding is unexpected and difficult to explain. Secondly, cell numbers tend to rise toward control levels after the initial post-irradiation fall. This suggests the operation of homoeostatic mechanisms.

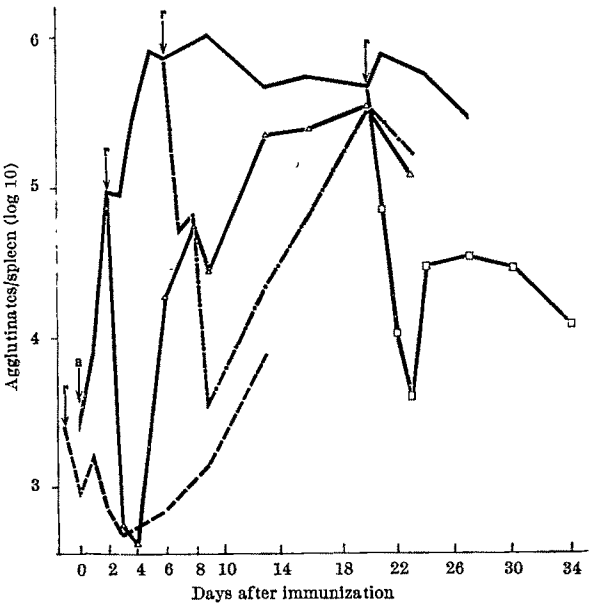


Fig. 3. Effect on numbers of agglutinin-forming cells of irradiation with 450 r. on days −1, +2, +6 or +20. *a* ↓, injection of antigen; *r* ↓, irradiation, ————, controls; – – – – –, day −1 group, ——△——△——, day +2 group; ——·——·——·——, day +6 group; ——□——□——, day +20 group Curves are drawn through the geometric means of each group, 5 mice per day per group

It is not possible to determine the surviving fraction of cells from simple inspection of the curves because part of the fall in cell numbers may have been due to damage to precursor cells which, in any event, were not forming antibody and were therefore not counted. However, if the proportion of cells functioning at the lowest point of the post-irradiation fall is assumed to be the surviving fraction, most of the curves obtained here would suggest that the fraction surviving a dose of 450 r. is about 0·5–1 per cent.

Now, when immunologically competent cells are irradiated before antigenic stimulation, the fraction surviving a dose of 450 r. is estimated variously as 0·4 per cent[6], 0·04–1 per cent[7], 0·6 per cent[8] and 0·3 per cent[9]. There does not, therefore, appear to be good evidence of a material change during the immune response in the radiosensitivity of the cells that mediate the response.

These results do not exclude the possibility that cell radiosensitivity may change to some extent at different stages of the response, especially at stages not examined in these experiments. They suggest, however, that the marked fluctuations in radiosensitivity found when certain end-results of the immune process are investigated may, to some degree, be experimental artefacts due to the delay with which changes in cell population are reflected in serum antibody levels and to the impossibility of following changes in serum antibody below the threshold of detection.

Many agents differ from radiation in the timing of their immunological effects, being relatively ineffective if given before the antigen and most effective if given some 1–4 days afterwards[10]. If the interpretation given here is correct it follows that cellular sensitivity to such agents cannot remain constant throughout the response but must change markedly. This possibility is under investigation.

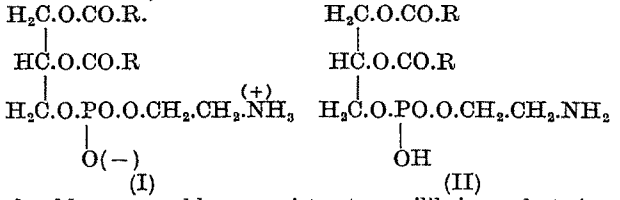
I thank the Medical Research Council and British Empire Cancer Campaign for Research for financial support, Dr. E. A. Wright for irradiation facilities and Miss D. Lyall for help.

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EVIDENCE FOR THE INVOLVEMENT OF IONIZATION IN THE POLYMORPHISM OF A PHOSPHOLIPID

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WHETHER a neutral α-phosphatidylethanolamine molecule exists as the zwitter-ion (I) or as the un-ionized tautomer (II) has been of interest for some time[1]. In a solution, both tautomers:



should presumably co-exist at equilibrium, but in a crystal, only one tautomer might be expected. However, although numerous infra-red spectra of solid α-phosphatidylethanolamines have been published, which have been interpreted to indicate the existence of at least three polymorphs[2], none gives a clear indication of tautomeric state. We have now prepared DL-α-(dipalmitoyl)-phosphatidylethanolamine in a crystalline form which, from infra-red spectroscopic evidence, appears to consist of the un-ionized tautomer (II). Other polymorphs appear to contain the zwitter-ionic tautomer (I), perhaps in combination with the un-ionized.

van Veen and Verkade[3] were the first to point out that discrepancies between spectra of α-phosphatidylethanolamines could be attributed to polymorphism. They published the infra-red spectra of two forms of the dipalmitoyl compound, which they termed *A* and *B* polymorphs. Later, Chapman and co-workers[2,4,5] agreed with the findings of van Veen and Verkade, but reported also a *C* polymorph which is formed at temperatures above the melting-point of the hydrocarbon chains; this means that the *C* polymorph is liquid-crystalline.

Starting with commercial preparations of DL-α-(dipalmitoyl)phosphatidylethanolamine, we have crystallized various specimens of which the spectra agree well with all three published types. However, we also obtained many spectra similar to the '*B*-spectrum'[3] but in which certain absorptions—most notably a peak near 1,550 cm^{-1}—were weakened. Gas chromatography showed that the spectral differences were not due to differences in fatty

acid composition, which was approximately 90 per cent palmitate in all samples. However, on thin-layer chromatograms of silica gel G developed[6] with chloroform–methanol–acetic acid–water (65 : 15 : 10 : 4, by volume) all the specimens gave two major zones in the region where α-phosphatidylethanolamine was expected to be. (There were also minor zones of higher and lower R_F which were apparently formed by palmitic acid and lysophosphatidylethanolamine respectively.) Attempts to distinguish between these zones with numerous spray reagents failed. But infra-red analysis of materials obtained by preparative layer chromatography showed that the slower zone (R_F 0·67) was formed by α-phosphatidylethanolamine, whereas the faster (R_F 0·78) was formed by the β-isomer. The identity of β-(dipalmitoyl)phosphatidylethanolamine was confirmed by comparing its chromatographic behaviour and infra-red spectrum (Fig. 9) with those of an authentic specimen obtained from the late Dr. Malkin's collection[7]. (Specimens were obtained from this collection through the courtesy of Prof. Wilson Baker.) Some other workers have also observed two major zones on thin-layer chromatograms of commercial α-phosphatidylethanolamine preparations[8,9]. Our evidence indicates that some preparations, claimed to consist of either the DL-α- or the L-α-isomer, contain up to 30 per cent of the 'unnatural' β-isomer, which is very disturbing in view of the widespread use of these preparations for biochemical and biophysical studies.

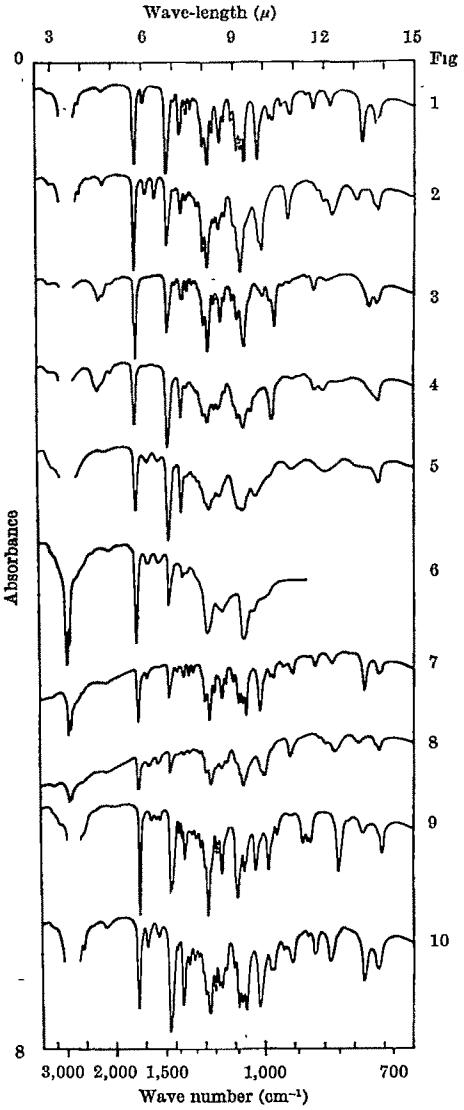
When the commercial preparations were subjected to column chromatography on silicic acid, using 12 per cent methanol in chloroform as eluent[10], they gave partly resolved peaks of (in order) the β- and α-isomers, and late fractions contained the α-isomer in a high state of purity. As the fractions were stored at −20° C and the solvent slowly evaporated, crystals were formed which gave the infra-red spectrum shown in Fig. 1. This contains no peak at all near 1,550 cm^{-1}. An alternative and more rapid method for obtaining crystals with the same spectrum was to prepare a near-saturated solution in boiling chloroform, add a trace of ammonium hydroxide, cool to −20° C with shaking, and desiccate the crystals that separated over phosphorus pentoxide. Crystallization from chloroform or ethanol by more conventional methods usually caused the appearance of a small peak near 1,550 cm^{-1}, the spectra resembling Fig. 10. However, cooling a highly concentrated, boiling solution in ethanol gave crystals with the spectrum shown in Fig. 2. This spectrum was also observed after crystals with the other spectra (Figs. 1 and 10) had been briefly heated to 140° C and cooled to room temperature.

It appears that the spectra published by van Veen and Verkade[3] represent impure polymorphs. Following their nomenclature, we attribute Fig. 2 to polymorph *A* and Fig. 1 to polymorph *B*. Hence, van Veen and Verkade's '*A*-spectrum' represents polymorph *A* contaminated with *B* and their '*B*-spectrum' represents polymorph *B* contaminated with *A*. Whereas Chapman and Collin[6] reported that DL-α-(dimyristoyl)phosphatidylethanolamine was relatively stable in the *C* polymorphic form at room temperature, this was not true of our purified dipalmitoyl compound. At 140° C a microcrystalline film of our compound gave a spectrum characteristic[2] of the *C* polymorph, but after being left for only 1 h to cool to room temperature it gave the spectrum of the *A* polymorph (Fig. 8). We were only able to obtain crystals of the *C* polymorph with some stability at room temperature by slowly evaporating a solution in pure dioxan (see Fig. 5).

Storage for a week at −196° C, or for 30 min at other temperatures up to 126° C, did not change the spectrum (taken at room temperature) of either the *A* or the *B* polymorph. These findings appear to conflict with those of Chapman[2].

When some of the β-isomer was deliberately added to the α- it again became impossible to crystallize the *B* polymorph in pure state, and when an equal quantity was added it was only possible to obtain crystals with the spectrum of the *A* polymorph. This may partly explain why all previously published[2–4,11–16] spectra of crystalline, saturated α-phosphatidylethanolamines appear to represent mixtures of polymorphs. Other possible explanations include the presence of different impurities, the use of other crystallization techniques, and the use[2,4,12,13,16] of the potassium bromide disk technique for infra-red spectroscopy: this is notorious[4,17,18] for producing artefacts. However, our evidence does not preclude the possibility that true polymorphs other than *A*, *B* and *C* may exist, nor that these could be hybrids of *A* and *B*. (That the *A* and *B* polymorphs have similar stability might favour the existence of hybrids.)

The *A* and *B* polymorphs gave satisfactory analyses for carbon, hydrogen, nitrogen and phosphorus, were readily interconvertible by the methods described, and gave the same spectrum (Fig. 6) after dissolving in tetrachloroethylene. Spectra taken on Nujol mulls (Figs. 1 and 2) were indistinguishable from those taken on microcrystalline films (Figs. 7 and 8).



Figs. 1–10. 1, *B* Polymorph of DL-α-(dipalmitoyl)phosphatidylethanolamine, as Nujol mull. 2, *A* Polymorph (Nujol). 3, Deuterated *B* Polymorph (Nujol). 4, Deuterated *A* Polymorph (Nujol). 5, *C* Polymorph (Nujol). 6, Solution of *A* or *B* Polymorph (or of β-(dipalmitoyl)phosphatidylethanolamine) in tetrachloroethylene. 7, *B* Polymorph, as microcrystalline film. 8, *A* Polymorph, as microcrystalline film. 9, β-(Dipalmitoyl)phosphatidylethanolamine (Nujol). 10, *B* Polymorph of DL-α-(dipalmitoyl)phosphatidylethanolamine (compare with van Veen and Verkade (ref. 3)) contaminated with *A* Polymorph (Nujol)

Crystallization from pure chloroform in the presence of deuterium oxide, or exposure of the *B* polymorph to deuterium oxide vapour for 10 days, produced deuterated *B* polymorph (see Fig. 3); when this was exposed to water vapour the spectrum shown in Fig. 1 was restored. The *A* polymorph underwent deuterium- or hydrogen-exchange roughly ten times more slowly than the *B*, and tended to yield deuterated *B* polymorph as well as deuterated *A*. (Another physical difference observed was that the *A* dissolved in chloroform more rapidly than the *B*.) Deuterated *A* polymorph (see Fig. 4) was best obtained by heating deuterated *B* polymorph.

The spectra depicted in Figs. 1 and 2 show substantial differences which cannot be ascribed merely to differences in the packing of hydrocarbon chains. Moreover, many absorptions that are not common to both spectra are shifted by deuteration, thus clearly implicating the polar parts of the molecules.

The $NH_3^{(+)}$ group gives rise to at least two peaks, attributed to deformation modes[19–21], in the region 1,485–1,660 cm^{-1}. For example, the spectra of glycine polymorphs[22], α-phosphatidylserines[23], and α-phosphatidylethenolamine solutions (see Fig. 6)[24], all show such peaks, one occurring fairly near 1,640 cm^{-1} and another near 1,550 cm^{-1}. The NH_2 group normally gives rise to only one peak in this region, usually[19–21] above 1,600 cm^{-1}, so that a peak near 1,550 cm^{-1} in the spectrum of a pure α-phosphatidylethanolamine should indicate $NH_3^{(+)}$ groups specifically. This conclusion is supported by Baer and Blackwell's finding[24] that *N*-methylated α-phosphatidylethanolamine derivatives give no such peak.

The absence of any peak near 1,550 cm^{-1} in Fig. 1 is evidence that our *B* polymorph contains no $NH_3^{(+)}$ groups, and, hence, that it consists of the un-ionized tautomer (II). Broad-based absorption in the N—H stretching region and peaks at 1,645, 810 and 746 cm^{-1}, all sensitive to deuteration, are consistent with the presence of NH_2 groups[19,20]; and peaks at 2,550 and 1,015 cm^{-1} can be attributed respectively to the stretching[14] and deformation[23,25] vibrations of POH groups. In the spectrum (Fig. 3) of the deuterated *B* polymorph, the appearance of a peak at 2,195 cm^{-1} and a shoulder at 1,275 cm^{-1} can be attributed to ND_2 stretching[19] and deformation[26,27] vibrations, respectively, and a shoulder at 2,000 cm^{-1} and a peak at 735 cm^{-1} to POD vibrations[19]; enhancement of the peak at 965 cm^{-1} may result[26,27] from the shifting of a C—N stretching vibration from near 1,015 cm^{-1}. Since deuteration changes the pattern of peaks near 1,080 cm^{-1} attributable[28] to POC groups it appears that these are hydrogen-bonded. However, deuteration does not perceptibly change the peak at 1,730 cm^{-1} and shoulder at 1,745 cm^{-1} attributed to C=O stretching vibrations. The peak is at anomalously low frequency for phospholipids and if, as it seems, this is not due to hydrogen bonding, it may be due to nucleophilic interaction between amino and carbonyl groups, visualized $H_2N : \underset{\downarrow}{\overset{\downarrow}{C}}=O$.

Analogous interpretations of the spectra of the *A* polymorph (Fig. 1) and its deuteration product (Fig. 4) indicate the zwitter-ionic tautomer (I). However, several features of these spectra (such as peaks attributable to POH and POD stretching) suggest that the un-ionized tautomer may also be present, perhaps in equal quantity. Since the shapes of the peaks near 720 cm^{-1}, assigned to CH_2 rocking vibrations, are different in Figs. 1 and 2 (also in Figs. 7 and 8, which were obtained without Nujol), we conclude that the hydrocarbon chains are packed differently in the *A* and *B* polymorphs. This accords with some results obtained by X-ray diffraction[2,28]. (It appears that the designation *A* was applied to the same polymorph independently by workers using X-ray[26] and infra-red[3] methods.)

The spectrum of the liquid-crystalline *C* polymorph (Fig. 5) shows less fine structure than those of the *A* and *B* polymorphs but, as expected, more than that of the solution (Fig. 6). Peaks near 1,550 cm^{-1} indicate that the *C* polymorph and the solution contain at least some zwitter-ionic tautomer.

Since the crystalline state of phospholipids in biological membranes is thought to be of vital significance[2,29] the question may be raised whether its involvement with ionization could facilitate ion transport.

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INHIBITION OF DNA SYNTHESIS BY ULTRA-VIOLET LIGHT UNDER CONTROLLED TEMPERATURE

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THE dose response curve for the inhibition of DNA synthesis by ionizing radiations[1–7] or by ultra-violet light[8,9] has in most cases been claimed to be a complex multiphasic curve. In the case of the *in vitro* experiments this curve has been interpreted in terms of two components; one component, the S_1, predominates at the lower doses, and the other, the S_2, predominates at the higher doses[4]. In many cases, however, irradiations have been performed at room temperature (20°–25° C), and the subsequent incubations with labelled precursors of DNA performed at the normal incubator temperature of 37° C. In other cases the precise temperature conditions have not been stated (Table 1). In the experiments for which the temperatures have not been stated, it is probable that the irradiations would have been performed at room temperature and the subsequent incubations at

37° C. This is because it is unlikely that the effort would be made to perform irradiations at 37° C and this procedure then left unmentioned. In two cases[10,11], however, care was taken to ensure a uniform temperature of 37° C throughout the irradiation and labelling procedures, and the resulting dose-response curve was not multiphasic but followed a simple one-hit curve. These results of Smith[10] and of Manasek *et al.*[11] make it possible to explain the multiphasic curve which has been obtained using ionizing radiations as an artefact arising from the temperature changes during the experiments. On account of this possibility it is necessary to determine the dose response curve for the inhibition of DNA synthesis by ultra-violet light under controlled temperature conditions. The result of this determination is described in the following paragraphs.

Cultures of both mouse *L* strain and human amnion, *FL*, strain were used in these experiments. *L* strain was grown in Eagle's basal medium supplemented with 10 per cent bovine serum and the *FL* strain in nutrient mixture *F*10 supplemented with 20 per cent calf serum (all growth media and sera were obtained from Grand Island Biological Co.). Eagle's medium is thymidine-free but *F*10 contains thymidine at 3×10^{-6} moles/l. In order to prepare ^{3}H-thymidine (^{3}HTdr) solutions of known specific activity, ^{3}HTdr was added to Eagle's medium or to specially prepared Tdr-free *F*10. Monolayer cultures were grown on quartz coverslips which formed one window of small culture chambers similar to those described first by Perry *et al.*[12]. Cultures in the exponential phase of growth were irradiated through the quartz coverslip without disturbing their normal environment of growth medium which was at the optimum *p*H, and fully oxygenated. The ultra-violet light source was a 15-W General Electric germicidal lamp, and a constant flux of approximately 20 ergs/mm^2/sec at 2537 Å was used throughout the experiments. All the irradiations and all the subsequent incubations in ^{3}HTdr were performed in a warm room at

Table 1. Temperature Conditions of Irradiation and Incubation in Experiments to determine the Dose-response Curve for the Inhibition of DNA Synthesis *in vitro*

Reference	Cell type	D_{37} S_1 (krads)	D_{37} S_2 (krads)	Temperature conditions: Irradiation	Incubation
Ionizing radiations					
* Berry *et al.* (ref. 1)	Ehrlich ascites (anoxic)	10	30	—†	37° C
* Berry *et al.* (ref. 2)	Ehrlich ascites (anoxic)	10	30	—†	37° C
	Ehrlich ascites (oxygenated)	0·5	13	—†	37° C
	Human bone marrow	0 5	13	—†	37° C
* Huntley and Lajtha (ref. 3)	Mouse spleen lymphocytes	0·5	13	—†	—‡
Jasinska and Michalowski (ref. 19)	Rat bone marrow	0·5–1	10	25° C	37° C
	Ehrlich ascites	0·5–1	10	25° C	37° C
* Lajtha *et al.* (ref. 4)	Human bone marrow	0·5	13	—†	—‡
Maass and Künkel (ref. 5)	Yoshida sarcoma	0 5–1	30	—†	—‡
Manasek *et al.* (ref. 11)	Hamster spleen lymphocytes	monophasic 7		37° C	37° C
	Hamster spleen lymphocytes	4	28	20° C	37° C
Painter (ref. 6)	HeLa	0·6	2	—†	—‡
Painter and Rasmussen (ref. 7)	HeLa	0·5	30	25° C	37° C
Smith (ref. 10) (X-rays and α particles)	HeLa	monophasic 90		37° C	37° C
	Mouse fibroblasts	14	25 α/μ^2	37° C	37° C
Ultra-violet light		S_1 (ergs/mm^2)	S_2 (ergs/mm^2)		
Cleaver (ref. 9)	*L* strain	1,100	30,000	25° C	37° C
Rasmussen and Painter (ref. 8)	HeLa	200	1,000	—†	37° C

† Temperature not stated in original paper, assumed to be room temperature, 20°–25° C.
‡ Temperature not stated in original paper, assumed to be incubator temperature, 37° C
* All references marked thus originate from the same research group and the data cited may in some instances be from the same experiment.
Where the D_{37} values are not stated in the original papers, approximate estimates have been made from the published diagrams. In the case of ultra-violet light the approximate D_{37} values are incident flux, not absorbed dose.

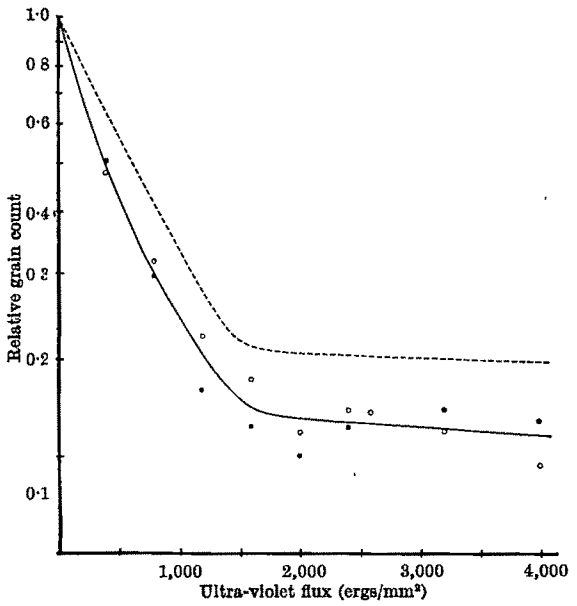


Fig. 1. Incorporation rates of ^{3}HTdr into DNA after exposure to ultra-violet light. ○, *FL* strain, 1·25 μc./ml., 3 c./mmole, ^{3}HTdr; ●, *L* strain, 2·5 μc./ml., 3 c./mmole, ^{3}HTdr. Each point represents the mean of duplicate experiments. The dotted line represents the dose-response curve obtained using *L* strain cells, in which irradiation was at room temperature, 20° C, and labelling at 37° C. This curve has been taken from a previous publication (ref. 9) in which a different dose rate (47 ergs/mm^2) and different ^{3}HTdr concentration (5 μc./ml., 2 c./mmole) were used to those used in the experiments described here

37° C. Controls were maintained on every quartz coverslip by shielding a region completely from the incident light. Immediately after the irradiations the culture chambers were opened and the medium replaced with culture medium containing ^{3}HTdr (Schwartz Bioresearch). The ^{3}HTdr was used at 2·5 μc./ml., 3 c./mmole (Fig. 1), 2·5 μc./ml., 0·36 c./mmole (Table 2) for the *L* strain, and at 1·25 μc./ml., 3 c./mmole for the *FL* strain. After allowing the cultures to continue growth for 10 min in labelled medium they were rinsed in Earle's balanced salt solution and fixed in acetic acid–alcohol (1 : 3) and then air dried. All the cultures were treated with 2 per cent perchloric acid at 4° C for two periods of 1 h to ensure complete removal of the acid-soluble pool. It was assumed that the ^{3}H-labelled material which was not removed by this fixation and acid hydrolysis had been incorporated into DNA[13,14]. Autoradiographs were then prepared using Kodak '*NTB*3' liquid emulsion and after exposure at 4° C for periods of 2–26 days these were developed with Kodak '*D*19', fixed, washed and afterwards stained through the emulsion with Ehrlich's haematoxylin. Grain counts were made over fifty labelled cells in both the irradiated and control regions of each culture, cells being regarded as unlabelled if there were five grains or less over the nucleus. The average grain count/labelled cell was taken as a measure of the amount of 3HTdr which had been incorporated into DNA during the labelling period in that region of the culture. For short labelling times of the order of 10 min, the average grain count can then be taken as a measure of the average rate of DNA synthesis at the time of labelling[15]. Cultures labelled under identical conditions, but with the autoradiographs exposed for different periods of time, gave control grain counts/labelled cell/day exposure which were distributed with a standard error of 10 per cent. A similar standard error has also been obtained in earlier experiments using Kodak '*AR*10' emulsion[16].

Table 2. Ratios of Average Grain Counts in Irradiated to Control Cultures as a Function of Incubation Time in ^{3}HTdr

(2·5 μc./ml., 0·36 c./mmole for *L* strain; 1·25 μc./ml., 3 c./mmole for *FL* strain) (expressed as a percentage)

Incubation time	30 min	1 h	2 h	3 h	4 h
L strain (400 ergs/mm^2)	45	29	22	21	20
FL strain (400 ergs/mm^2)	37	27	14	10	8

A series of cultures were irradiated with a total flux of ultra-violet light that ranged between 400 and 4,000 ergs/mm², labelled with ^{3}HTdr for 10 min and fixed. Grain counts on autoradiographs of these cultures were determined in the manner already described. The results are shown in Fig. 1, and it can be seen that the responses of the two cell types, which have been determined under almost identical conditions, can be represented by the same dose-response curve. (The difference between the concentrations of ^{3}HTdr solutions used for labelling the two cell types is insufficient to make differences in pool sizes a significant factor in these results[17,18].) It was found by Jasinska and Michalowski[19] that the inhibition of DNA synthesis in rat bone marrow cells and in ascites cells by X-rays could also be represented on a single dose-response curve, when both cell types were used under the same conditions. It is consequently possible that the differences in the D_{37} values (Table 1) which have been obtained by different workers is due to differences in experimental techniques rather than differences between cell types. One factor which is particularly important in this respect is the duration of the labelling period after irradiation, as the following discussion will indicate.

The incorporation of ^{3}HTdr into tissue culture cells in the exponential phase of growth proceeds at a constant rate for several hours and this results in a linear increase in the average grain count in autoradiographs as a function of the incubation time[16]. After irradiation with ultra-violet light the rate of DNA synthesis is not constant, but varies in a manner which depends both on the total flux and the time elapsed after irradiation[6]. This results in a non-linear increase in the average grain count in irradiated cultures as a function of the incubation time. Consequently, the ratio of the average grain counts in the irradiated to the control cultures, which can be used as a measure of the irradiation effect, will depend on both the total flux of ultra-violet light and the time for which the cultures are incubated in ^{3}HTdr between irradiation and fixation. In Table 2, this dependence is illustrated for the case of increasing incubation time in ^{3}HTdr, for both the cell types, after a single exposure to ultra-violet light.

It can be seen from Table 2 that the longer the incubation time in ^{3}HTdr after irradiation, the greater is the relative reduction in average grain counts and the higher the apparent sensitivity. This is probably a major reason for the lower D_{37} values obtained by Rasmussen and Painter[6] than those obtained by Cleaver[9], for example (Table 1). In the former experiments incubation times of up to 2 h were used, whereas times of only 10 min were used in the latter.

This dependence of the D_{37} values on the duration of incubation in labelled precursors makes it difficult to apply target theory to the results of these experiments, and of the experiments referred to in Table 1. In those instances in which target theory has been applied, the values obtained for the target dimensions and molecular weight can only be regarded as very rough approximations[7,10].

No difference was noted between the fraction of cells that were labelled in the irradiated and control regions of either cell type, even in autoradiographs which were deliberately over-exposed. These observations differ from those of Rasmussen and Painter[6] in which they observed that almost 100 per cent of the population in cultures of HeLa and of Chinese hamster cells were labelled after ultra-violet irradiation. This phenomenon of almost 100 per cent labelling has been interpreted in terms of a repair system which operates after irradiation with ultra-violet light[6]. The results described here indicate, however, that this repair mechanism does not operate in every type of mammalian cell, and is absent from both *L* and *FL* strains.

Since, in these experiments, no change in the fraction of labelled cells was detected, and the labelling time was too short for there to be any significant redistribution of the populations of cells in the various phases of the cell cycle during the experiments, the results shown in Fig. 1 are taken as representative of the response of DNA synthesis in *S* phase cells to ultra-violet light at 37° C over the dose range indicated, in both cell types.

The dose-response curve which has been obtained in the experiments described here appears to be genuinely multiphasic when determined under carefully controlled conditions of exponential growth and uniform temperature. A comparison between this dose-response curve and the one previously reported, for which irradiations were performed at room temperature and labelling at 37° C (9, Fig. 1), indicates that both procedures result in essentially the same dose-response curve. The results obtained for ultra-violet light are in contrast to those obtained for ionizing radiations by Smith[10] and by Manasek *et al.*[11]. These workers found that if both irradiation and labelling were performed at 37° C, the dose-response curve followed a single one-hit curve. The dose-response curve obtained when ionizing radiations are used may then be multiphasic only when irradiation and subsequent labelling are performed at different temperatures, whereas a multiphasic curve may be obtained after irradiation with ultra-violet light irrespective of temperature conditions.

These observations make it necessary to consider at least two main problems:

(1) The temperature conditions during irradiation and labelling do not seem to affect the shape of the dose-response curve for irradiation with ultra-violet light, whereas these conditions may affect the curve obtained when ionizing radiations are used. One possible explanation for this difference is that irradiations with ultra-violet light are brief; the highest flux in Fig. 1, for example, corresponds to only about 3·5 min irradiation. If cells are grown and labelled at 37° C, the precise temperature during irradiation is then unimportant. This is because there is insufficient time for the growth rate of the cells to respond to a brief change in temperature during the period of irradiation Irradiation with X-rays, however, involves periods of 30 min and longer, which is sufficiently long for the growth rate of the cells to respond to temperature changes. Consequently, during irradiation at room temperature with ionizing radiations the growth rate of the cells will fall from the normal rate at 37° C, and the radiation sensitivity may then be different from that of cells maintained at 37° C throughout.

(2) The second problem is that of finding an explanation for the precise shape of the multiphasic dose response curve obtained using ultra-violet light. A similar multiphasic curve has been obtained in a number of *in vivo* experiments with ionizing radiations[8,20–22], and in these cases temperature effects do not seem to afford a possible interpretation. Although many explanations have been offered for the shape of the dose-response curve, no definitive experiments have made it possible to choose between them, and in the case of *in vitro* irradiation with ionizing radiations temperature effects must be added to the list of possible explanations which have been discussed in detail elsewhere[1,2,4,6,9,20,22]. Temperature changes may not be the complete explanation for the shape of the dose-response curve, however, because a biphasic curve has been obtained by Mak and Till[23] from experiments in which cell cultures were maintained at 37° C throughout the experimental procedure.

A final point should be made, however, concerning the relationship between the dose-survival curves following irradiation with ionizing radiations or ultra-violet light and the dose-response curves for the inhibition of DNA synthesis by these radiations. The value of the D_{37} dose for the survival of cells irradiated in the *S* phase is about 200 rads, in the case of X-rays, or about 40 ergs/mm², for the flux of ultra-violet light[24]. If these values are compared with the D_{37} values shown in Table 1 for the inhibition of DNA synthesis, it is apparent that the dose range over which significant inhibition is observed

is one over which the surviving fraction will be much less than 1 or 2 per cent. Consequently, the major portion of the dose-response curve for the inhibition of DNA synthesis represents the residual DNA synthesizing ability of cells which are unable to recover from the effects of irradiation. This pathological condition may involve different processes to those which are involved in DNA synthesis in undamaged, viable cells. Such processes may involve, for example, the initiation of new growing points for the polynucleotide chains[7,25] or end addition of labelling molecules to the sites of damage[26].

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PROLONGED WHOLE-BRAIN REFRIGERATION WITH ELECTRICAL AND METABOLIC RECOVERY

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THERE is good evidence that the physical phenomenon of temperature reduction in itself confers protection on certain organs[1,2] during protracted periods of circulatory arrest, presumably by markedly curtailing intrinsic metabolic activity. While the brain *in situ* appears to enjoy this protection to a limited degree at moderately lowered temperatures[3,4], its response as an intact organ to extended periods of ischaemic refrigeration at temperatures approaching 0° C is not known. It is true that small tissue aggregates of brain incubated at 0·5° C for up to 24 h have been shown to be capable of resuming measurable, although reduced, metabolic activity on rewarming[5,6]. Unfortunately these and all *in vitro* studies of cerebral metabolism are limited because damaged tissue (in the form of slices or homogenates) is used[7], and because the experimental conditions necessarily prevent use being made of the intrinsic circulation of the tissues[8].

This article reports some experiments designed to examine the recovery properties of the whole brain following prolonged storage at 2·0 ± 1·0° C without circulation. The intrinsic cerebral circulation was utilized for rapid cooling, for rewarming and to provide metabolic support for the brain. During the period of refrigeration, the blood volume of the brain was filled by a non-cellular solution.

The canine brain, exposed within an isolated head preparation, was selected for the experimental model. The following parameters were examined: electrical activity, oxygen consumption and carbon dioxide production, and topographical appearance of the brain.

Dogs were anaesthetized with sodium pentobarbital and endotracheally intubated through a tracheotomy permitting mechanical ventilation following immobilization with gallamine triethiodide. Intra-arterial pressure measurements were made from a femoral artery. After mechanically fixing the head and body, all the muscles of the neck were surgically divided in a continuous ring at C_2 and the individual spinal muscles were scraped from the bony surface of C_2. The jugular veins and the carotid sheath structures were preserved. A laminectomy of C_1 and C_2 was performed, the dura opened and the anterior spinal arterie(s) destroyed. Both vertebral arteries were obliterated in their canals in C_1, leaving only the carotid arteries to provide circulation to the head. All scalp tissues were ablated and the entire dorsal surface of skull, except a thin bony strip over the sagittal sinus, was removed. The sagittal sinus was cannulated with a small polyethylene tube.

The isolated head preparation was differentially cooled following heparinization (2 mg/kg) utilizing a simple perfusion circuit incorporating a pre-calibrated Mark pump and a Mayo heat exchanger interposed between a cannulated femoral artery and bilateral metal 'T' cannulae in the carotid arteries. When intracerebral temperatures reached 20° C, the entire head was encased in a plastic reservoir filled with ice water to increase the efficiency of cooling. Within 20 min, and invariably before cardiac activity had ceased, brain temperatures of 6°–8° C had been achieved. The spinal cord was ligated and divided and the spinal column separated at C_2. The carotid arteries, previously closed behind the cannulae, were divided and the jugular veins and vagi severed. The isolated cephalic preparation was immediately perfused by means of its carotid cannulae with 250 c.c. of ice-cold heparinized Ringer's lactate solution, thus removing the remaining cerebral blood volume and rendering the cortical surface a pale white colour. The brain temperature was simultaneously reduced further to 3° C. The arterial cannula system was closed, the dura was opened over the brain and the isolated head was completely immersed in a special reservoir containing Ringer's lactate solution at 2° C. Two canine preparations were used for each of the following ischaemic refrigeration periods: 2, 4, 12 and 18 h; 1, 2, 8 and 15 days.

At a predetermined time, the isolated preparation was removed from the reservoir and connected by means of its cannula system to a large donor animal previously

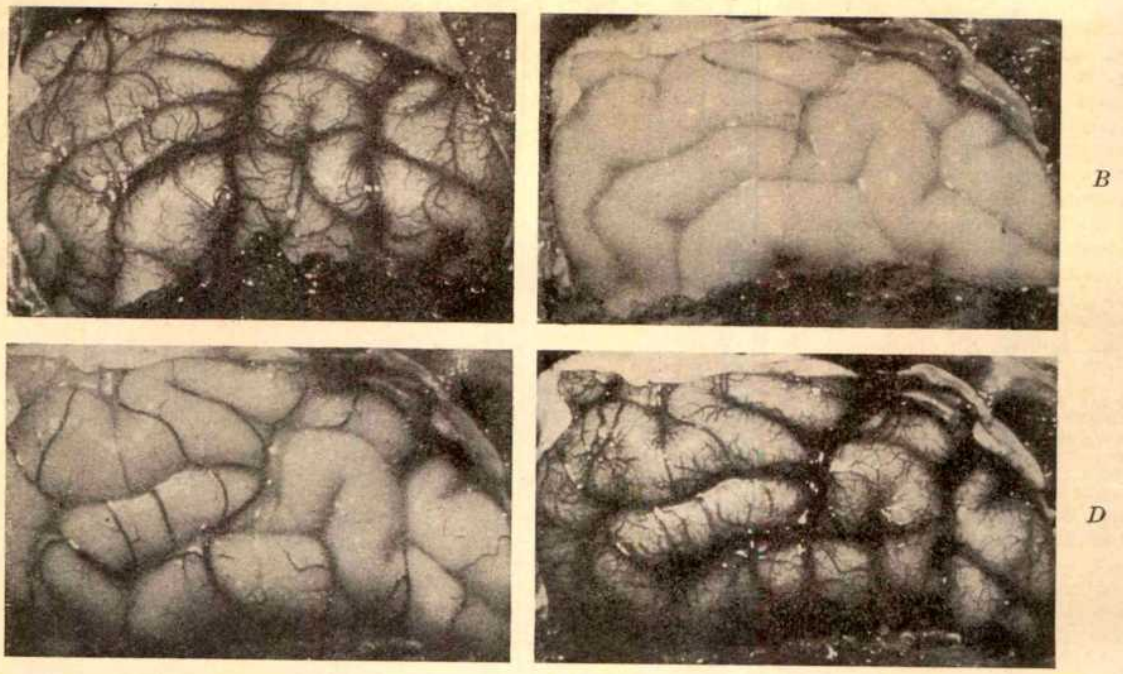


Fig. 1. Lateral view of the cerebral cortex of dog; (*A*) Normal morphology of cortex and vasculature prior to cooling. (*B*) Cadaveric appearance of brain surface following cooling and perfusate 'washout' of blood volume (intracerebral temperature 3° C just before ischaemic storage refrigeration). (*C*) The first appearance of blood in cortical arteries during initial donor perfusion rewarming. (*D*) Complete cerebral revascularization during late rewarming perfusion (intracerebral temperature 24° C). Note similarity of morphology of the cortical surface, arteries and veins of brain stored for 15 days with its pre-storaged (normal) appearance in (*A*)

anaesthetized with sodium pentobarbital and endotracheally intubated. The same simplified perfusion equipment was interposed between a cannulated femoral artery of the donor animal and the 'T' cannulae in the carotid arteries of the isolated head. The brain was gradually rewarmed by perfusing its cerebral circulation with donor blood at a temperature approximately 5° C higher than the intracerebral temperature. This temperature differential was consistently adjusted in an upward direction by altering the temperature of the donor blood during its passage through the heat exchanger. At intracerebral temperatures of 20° C, six electrodes were positioned in brain and connected to a Grass 'Polygraph' with electroencephalogram preamplifiers. Periods of 30–40 min were required to rewarm the brain to more than 30° C; at this temperature, blood samples were simultaneously removed from the arterial line and the jugular vein or the sagittal sinus for measurements of Po_2 and Pco_2.

Arterial pressures were continuously monitored within the aorta of the donor animal and the extracorporeal perfusion line. Temperatures were recorded from the oesophagus of the donor animal, from the perfusion line distal to the heat exchanger, and from the brain by means of suitable thermo-probes. Precalibration of the Mark pump provided satisfactory control of perfusion flows which, for cooling, were gradually reduced from 120–100 c.c./min to 40–50 c.c./min. For rewarming, the rate of flow was gradually increased, depending on the brain temperature and the pressure in the perfusion line. At the beginning, the flow rate was of the order of 40–50 c.c./min, and was increased to 150–200 c.c./min as brain temperatures reached 30° C or higher in order to maintain perfusion line pressures of between 100–150 mm mercury systolic.

In all storage preparations, including the two refrigerated for 15 days, excellent revascularization of brain was observed during rewarming perfusion (Fig. 1). The anatomical differentiation of cortical arteries and veins was readily apparent as the pale white colouring of the cool brain gave way to the more normal appearance of the cerebral cortex with active perfusion by warm donor blood (Fig. 1). Cerebral oedema became manifest in each brain during the rewarming cycle, gradually developing with increases of temperature above 20° C. Oedema was most pronounced in those brains stored for the longest periods of time. The onset of brain oedema could be delayed or temporarily reduced by introducing small

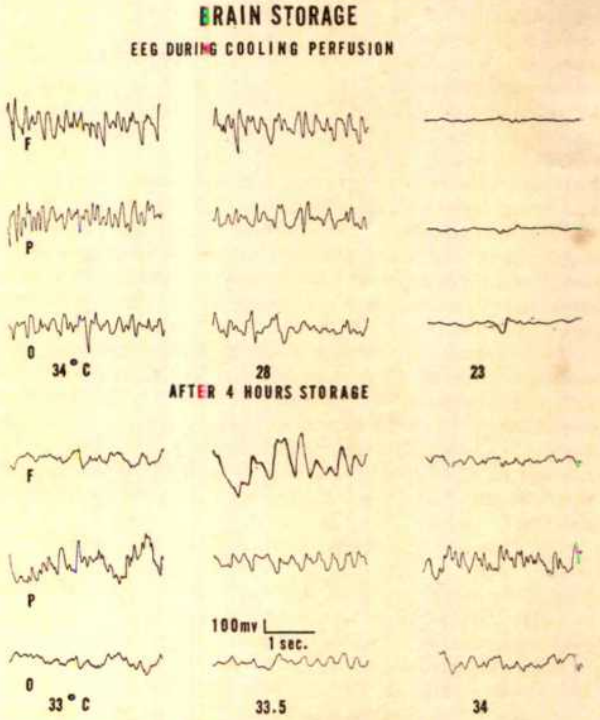


Fig. 2. Electro-cortical brain activity from paired frontal, parietal and occipital surface electrodes during cooling perfusion and on rewarming after 4 h of hypothermic storage. Note the isoelectric activity in all leads at 23° C. On rewarming, the tracings at 34° C show reduced activity in the frontal and occipital lobes. The parietal lobes, on the other hand, contain good activity of about 10 c/s at 30–50-mV amplitude with some large slow spikes of 150-mV appearing

amounts of urea (750 mg) directly into the arterial perfusion line. (Presumably the urea led to an acute increase in the osmolality of the perfusion blood, thus producing a diffusion gradient across the blood-brain barrier.)

With brains stored for 2 h and 4 h, cerebral electrical activity was restored at intracerebral temperatures of 22° C or more (Fig. 2). Prolonged maintenance perfusion of the rewarmed brain (34° C) for more than 6 h invariably resulted in the disappearance of electroencephalographic activity and the development of irreversible cerebral oedema. Brains refrigerated for periods of time between 4 and 12 h were not examined, and no recognizable cerebral electrical activity has been recorded from brains stored for 12 h or more. Furthermore, pupillary light reflexes could easily be elicited, and persistent rhythmic gasping observed in the isolated heads refrigerated for 2 h and 4 h; these were not observed after 12 h or more of ischaemic hypothermia.

The resumption of metabolic activity by the refrigerated brain was indicated by the development of significant arterio-venous O_2 and veno-arterial CO_2 differences on rewarming. Isolated heads stored for 12 h or less (4 h and 2 h) averaged A-Vo_2 and V-Aco_2 differences of 5·1 vol. per cent and 4·1 vol. per cent respectively at 34° C. Since samples were taken from the jugular vein, these values are indicative of cephalic and not of pure cerebral metabolism. Brains stored for longer periods (18 h, 1, 2, 8, 15 days) demonstrated substantially identical values for A-Vo_2 and V-Aco_2 differences, which averaged 2·6 vol. per cent and 2·3 vol. per cent respectively when measured at 30° C. These values are based on sagittal sinus blood, and thus they represent oxygen consumption and carbon dioxide production after rewarming across the brain alone.

While it can scarcely be argued that the brain is normal when rewarmed after prolonged hypothermic storage, these investigations indicate that the intrinsic circulation of brain remains intact, even after 15 days of refrigeration, permitting reconstitution of an active, functioning cerebral arterial and venous circulation. Brains refrigerated for short periods of time (up to 4 h) demonstrate that cerebral tissue stored at extremely low temperatures (2°–3° C), and when entire blood volume has been replaced by a non-cellular perfusate, will reconstitute a recognizable electroencephalographic pattern on haematogeneous rewarming. Examination of haematoxylin- and eosin-stained microscopic sections of stored and rewarmed brain revealed that both glial and neuronal cellular elements are preserved.

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PROGESTAGEN INHIBITION OF NORMAL SEXUAL BEHAVIOUR IN THE MALE GUINEA-PIG

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LIKE the female of many species, the female guinea-pig, when injected with androgen, is easily induced to display male-like sexual behaviour[1]. High progesterone levels, however, resulting from pregnancy, pseudo-pregnancy or exogenous sources will protect the female from this 'induced masculinization'[2,3]. These findings raise questions as to possible effects of progesterone on normal males. Will progesterone or synthetic progestagens inhibit masculine sexual behaviour in the male as well as in the androgen-treated female? How do the effects of progestagen treatment compare with those of castration? These questions are of practical, as well as theoretical, interest in view of present-day tests of progesterone and progesterone-like substances as male contraceptives[4]. With non-standardized indices of behaviour some reports indeed indicate that progesterone can affect sexual performance[4,5].

To answer these questions, sixty-one mature male guinea-pigs 6–12 months old kept in individual cages were observed and tested with an oestrous female for a 10-min period each week. Observations were continued through three 10-week periods constituting respectively a control phase, an experimental phase, and a recovery phase.

During the control phase, all subjects were tested and handled in a similar fashion in order to obtain a base line of sexual performance for each animal. The experimental phase made it possible to observe the immediate effects of the various treatments. To prepare for this phase (within 6 h subsequent to the tenth test of the control phase), animals were assigned to one of six groups. Animals assigned to three of these groups were castrated. Beginning the following day, each subject in group 1 (progesterone-intact) was given two 25-mg injections of progesterone in cotton seed oil with 10 mg injections daily thereafter throughout the experimental phase; group 2 (MPA-intact) was given the same dosage of the synthetic progestagen 'Depo-provera', a brand of medroxyprogesterone acetate (MPA); group 3 (progesterone-castrate) was given the same dosage of progesterone; group 4 (progesterone-TP-castrate) was given the same dosage of progesterone with the simultaneous addition of 5 mg of the androgen testosterone propionate (TP) on the first day and 1 mg daily thereafter. This dosage of TP has been shown to be sufficient to maintain precastration levels of sexual activity in male guinea-pigs[6] and permits consideration of behavioural effects due to the interactions of hormones in known proportions. This would not be possible in intact animals with individually varying androgen output. Groups 5 and 6 served as intact and castrate controls respectively. Animals in these groups were given two initial 0·5 ml. and subsequent 0·2 ml. daily injections of cotton seed oil, the injection vehicle. Tests during the recovery phase indicated the nature and duration of post-treatment behaviour characteristics. During this phase all injections of progesterone and MPA were discontinued and the castrate groups (3, 4 and 6) received replacement

dosages of testosterone propionate, 5 mg on the first day and 1 mg daily thereafter. The intact groups (1, 2 and 5) received equivalent dosages of the injection vehicle. Injectates were uniformly prepared to contain 50 mg hormone/ml. oil. All injections were given intramuscularly daily and administered alternately into the right and left gluteal and hamstring regions.

The behaviour characteristics recorded included sniffing, nuzzling, mounting, intromission and ejaculation. Each of these components contributed a weighted value to a total sex score. (These behaviour components and scoring procedures have already been described elsewhere[6,7].)

The differences among the groups were statistically significant. During the experimental phase all groups except control-intacts showed marked and significant declines in sexual activity (Table 1). For most groups, an immediate decrease was evident. Between the final test of the control phase and the first test of the experimental phase (an interval of 1 week; 100 mg of progestagen) the sex score of the control-castrate group declined from 4·7 to 1·7, that of the progesterone-castrate group from 5·2 to 1·4, that of the progesterone-TP-castrate group from 4·7 to 1·8, and that of the intact group receiving MPA declined from 5·2 to 1·4. The progesterone-intact group dropped less, but significantly, from 6·3 to 3·4. At the end of the following week return toward control levels was seen, but after that, through the remainder of the experimental phase all groups except the control-intact showed a downward trend in their level of sexual activity.

Table 1. HORMONAL INHIBITION OF MALE SEXUAL BEHAVIOUR

Group	*N*	Sex score I Control phase	II Experimental phase	III Recovery phase	Significance* Phase I versus Phase II	Phase II versus Phase III
Control-intact	14	4·9	4·3	3·9	N S.	N.S.
Progesterone-intact	11	5 5	2·9	3·6	0·005	0 025
MPA-intact	8	4·1	2·1	2·4	0·01	N.S.
Control-castrate	10	5 1	1·9	3·6	0·005	0 025
Progesterone-castrate	7	5·1	1 6	3 8	0·01	0·01
Progesterone and TP-castrate	11	4·8	2·1	2 4	0 005	N.S.

* Wilcoxon matched-pairs signed-ranks test.

Over-all findings from intact and castrate control groups were as expected; no significant effects due to the placebo were seen in intact animals, but castration significantly reduced sexual activity. In the critical experimental groups it was obvious that both progesterone and the synthetic progestagen, MPA, are capable of inhibiting normal masculine sexual behaviour to an extent comparable with castration. The effect of castration and progesterone combined produced the largest drop in activity but this was not statistically more injurious to libido than was either treatment alone. The results gained with the castrate group receiving simultaneously androgen and progesterone showed that 1 mg of testosterone daily was generally insufficient to overcome the inhibiting effect of 10 mg of progesterone daily.

The decline in sexual behaviour scores generally reflects a decrease in incidence of the higher components of the male sexual pattern, that is, mounting, intromission and ejaculation. Ejaculation frequencies were most markedly affected.

In all experimental groups at least a 50 per cent reduction was found in the frequency of ejaculation. Groups injected with progesterone or medroxyprogesterone acetate experienced a decrease in ejaculations of more than 75 per cent.

During the recovery phase, the cessation of progestagen treatment, replacement therapy with TP, or both were followed by a significant return toward control phase levels of sexual behaviour (Table 1). In the progesterone-intact and the progesterone-castrate groups a rise toward normal levels of sexual activity was evident within a week. Cessation of treatment with MPA was not followed by a similar recovery until the last 3 weeks of the investigation. Thus, although both progesterone and MPA are capable of inhibiting male sexual activity, the effects of progesterone seems relatively slow to appear and quick to disappear, while the effects of MPA develop quickly and persist longer.

No group displayed as high a level of sexual performance during the recovery phase as it had during the control phase. This may not be unexpected for the progestagen-treated animals where a longer interval for recovery might be needed. Concerning the control males, Grunt and Young[6], Antliff and Young[8] and Jakubczak[9] also previously noted a decrease in the sexual behaviour scores of normal guinea-pigs during long-term experiments. They suggested that this is possibly related to advancing age. Analysis and comparison of the components of the sexual pattern for the recovery period with those found during the control phase reveal that these differences are qualitative as well as quantitative. While the low frequency of mounting seen during the experimental phase returns toward control phase levels, the frequency of intromission and ejaculation remains low. These latter behaviour components have been shown to be more labile than mounting when tested in respect to such factors as age[9] and castration and androgen therapy[10,11]. A differential sensitivity in the neural tissues mediating these behavioural patterns has been postulated.

Histological studies of the testes of the intact groups 1, 2 and 5 were conducted within a week following the end of the recovery phase. In addition, the last day of the experimental phase, testes were removed from 3 sets of 2 males each, treated in a manner identical to the three intact groups. Thus comparisons of gonads immediately after the experimental treatment and after recovery were possible without disturbing the actual experimental animals during the test sequence.

The testes of males treated with progesterone for 10 weeks showed few histological differences from controls. Nevertheless, the mean weight of the testes obtained from the progesterone-treated males was 2·79 g while that from the control males was 3·99 g. A substantial decrease in testes weight due to progesterone has also been reported for humans[4]. Despite this weight loss, as in humans[4], the tubular contents and interstitial cells appear unaffected by therapy except for a decrease in the absolute number of mature sperm. This is in contrast to findings in the rabbit[5] and ram[12], where general disorganization and degeneration of tubule contents have been reported.

Testes from males treated for 10 weeks with MPA, on the other hand, are severely affected. The mean weight of the testes from this group was 1·25 g; less than half that of the controls. Under the microscope these testes showed general disorganization of the tubules and a lack of mature spermatozoa. Occasional tubules were spared, however, and all stages of spermatogenesis could be seen. Mature spermatozoa could be found in the epididymides of all animals. Following the recovery phase of 10 weeks no significant differences in testis weight or histological appearance were discernible between the groups. Ericsson and Dutt[12] report similar effects of MPA on the ram testis.

These data indicate that progesterone and MPA are both capable of temporarily reducing the level of sexual performance in the male guinea-pig. It is not clear from these results where these progesterones act to bring about this inhibition. The seeming lack of serious effect of progesterone on the testes found in these experiments (and reported elsewhere for humans[4]) implies that we are dealing either with a pituitary feed-back problem or a direct nervous system effect. Additional experiments will be needed to clarify this point.

In view of its ability to inhibit androgen-induced masculine behaviour in the progesterone-rich female[2,3] as well as in the male, progesterone is thus seen to be capable of affecting sexuality in both sexes so that

androgen is ineffective in its role of activating the neural tissues mediating masculine sexual behaviour patterns. With recent findings[13–15], confirming the unique part played by androgen in the differentiation of the hypothalamus with respect to structuring both male and female sexual behaviour, it is perhaps not surprising that a single hormone, progesterone, is capable of antagonizing an androgenic action in both sexes.

Progesterone, testosterone propionate and 'Depo-provera' were supplied through the generosity of Dr. S. S. Stubbs of the Upjohn Company, Kalamazoo, Michigan. Appreciation is extended to Barbara Tullis, Herbert Cook and Milton Palanker for assistance at various times during the course of this study.

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BEHAVIOURAL SIGNIFICANCE OF HYPOTHALAMIC TEMPERATURE-SENSITIVE CELLS

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THE existence of thermally sensitive neurones in the hypothalamus has been inferred since the classic demonstration that heating the pre-optic region in the cat produces the appearance of sweat on the foot pads and panting[1]. Furthermore, electrical stimulation of this region leads to cutaneous vasodilatation and a decrease in rectal temperature[2]. These neurones, considered physiologically important in the central control of body temperature, have been shown to be sensory by single-unit investigations in which an increased frequency of firing accompanied local hypothalamic heating and a decrease accompanied cooling[3]. A change in the temperature of the blood perfusing the hypothalamus might thus provide a stimulus for activation of thermoregulatory mechanisms[4].

It has not been clear, however, whether sensations of warmth or coldness accompany activation of these cells. Such perceptual alterations might be revealed in a number of ways[5,6], but the participation of cutaneous receptors is hard to rule out. Vasodilatation, which accompanies hypothalamic heating, and vasoconstriction, which accompanies cooling, would be expected to affect cutaneous temperature.

In recent investigations of behavioural temperature regulation, rats were trained, in a cold environment, to press a lever to obtain radiant heat[7]. The rate of response in these investigations varied inversely with the ambient temperature. This technique has been used in the present investigation in conjunction with hypothalamic heating to establish a situation in which temperature-dependent behaviour can be observed while hypothalamic and peripheral temperatures are clearly separated.

Bilateral heating coils and a thermistor were chronically implanted by stereotaxic means in the hypothalamus of six rats. The maximal diameter of each heater was 1·0 mm, and the distance between heaters 1·5–3·0 mm. Heat was generated by the passage of direct current while the degree of hypothalamic heating was monitored by a thermistor (*VECO* 32*A*7) placed less than 1 mm from one heater. In some cases, subcutaneous thermistors were also placed at the nape of the neck. Electrical connexions were made with Winchester plugs, the sockets of which were fixed to the cranium after implantation of heaters and thermistor.

The unrestrained animals were placed in a temperature-controlled chamber and coupled to the recording equipment with flexible cables which permitted heating and recording over extended periods. The rats were trained to respond to cold ambient temperatures by pressing a lever that turned on an infra-red heating lamp. The intensity and duration of heat re-inforcement obtained for a single lever-press could be varied by the experimenter as desired. Training was continued until stable rates of bar-pressing were obtained (at least three sessions of 7 h each). The hypothalamus was then heated by way of the heating coils while the animal pressed the lever to obtain peripheral radiant heat.

Four animals with anterior hypothalamic-preoptic placement of heaters showed a decrease in the rate of responding when this region was heated to 40·0°–40·5° C for 20–30 min. The percentage decrease in rate of responding during the initial heating period, in comparison to the period immediately before heating, was 48, 63, 72 and 89 per cent for these rats. Increased rate of responding during hypothalamic heating was never observed. Two animals with more posterior placements (level of the paraventricular nuclei) showed no consistent behavioural effect during central heating.

When the experiment was repeated during subsequent sessions a diminished effectiveness of central heating was observed. That is, hypothalamic heating produced a decrease in rate of responding for peripheral heat, but not to the extent previously seen. This may be attributed to tissue damage as a result of heating[5], or to experience in this situation.

An activation of heat-loss mechanisms was evident in addition to the behavioural effect of central heating. Hypothalamic temperature fell below the pre-heating level on cessation of central heating, indicating a decrease in whole-body heat content. This decrease could not be entirely due to the diminished rate of responding, since the magnitude of the heat-loss was greater than when the animal was exposed to the same ambient temperature with no opportunity to obtain peripheral heat (during the extinction period).

These results are illustrated in Figs. 1 and 2, which were obtained from the animal having the most marked effect from central heating. Fig. 1 shows the suppression of responding during the period when the hypothalamus was heated to 40·5° C. Hypothalamic temperature fell

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to 34·2° C on cessation of heating, a decrease from the pre-heating value of 38·3° C. The amount of heat lost to the environment during hypothalamic heating should be compared with the amount normally lost when temperature-regulating mechanisms are intact. It should be noticed that during extinction, when the heat lamp was disconnected from the circuit, hypothalamic temperature decreased only 0·4° C in the first 20 min. The temperature decrease during hypothalamic heating was 4·1° C for a comparable period. Hypothalamic heating therefore increased heat loss more than tenfold over that observed during exposure to the same ambient temperature. From previous investigation[6], it can be inferred that the extent of heat loss observed in this experiment was due to peripheral vasodilatation and a decrease in heat production.

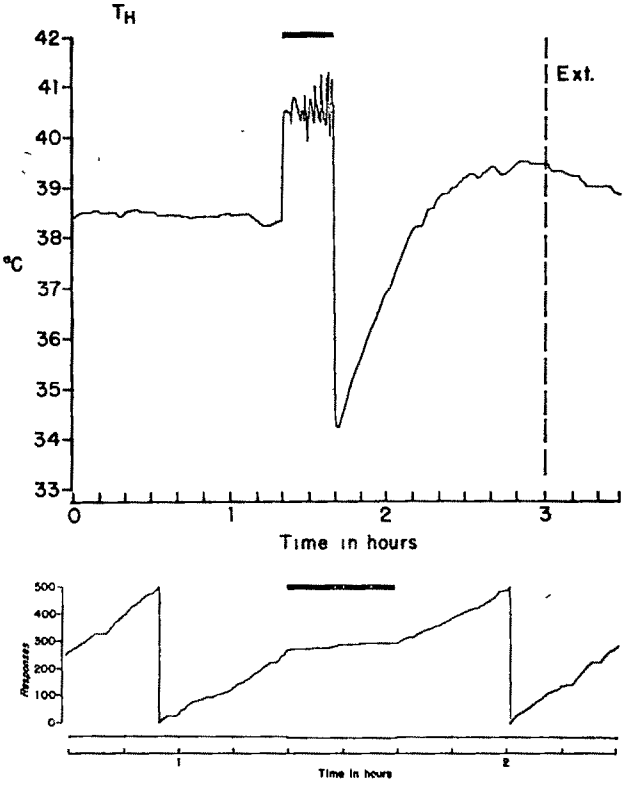


Fig. 1. Suppression of responding for peripheral heat during local hypothalamic heating (black bar). T_h, Hypothalamic temperature. Ambient air temperature, 0° C. Reinforcement, 2 sec at 200 W. Average temperature during hypothalamic heating is 40·5° C. Rate of responding is indicated by the slope of the curve in the lower record. The dotted line indicates the point at which the heat lamp has been disconnected (extinction period). Note that, although the lower time scale is expanded in length, it corresponds with the upper scale

Immediately after the heating period, the rise in hypothalamic temperature is too fast to be due to heat reinforcement alone and must therefore reflect a reinstatement of peripheral vascular tone and metabolic heat production. A rebound effect is evident in that hypothalamic temperature rises to 39·5° C at 70 min after cessation of heating, a level higher than that observed normally. The animal groomed and sniffed at various parts of the cage during heating, and a number of bar-presses were apparently made inadvertently during this activity. There was no sign of discomfort as judged by the behaviour of the animal.

When the experiment was repeated over a period of several days, the effect of hypothalamic heating on behaviour diminished, as shown in Fig. 2. Hypothalamic heating still decreased the rate of responding but not to the extent previously seen. Subcutaneous temperature fell steadily, reaching 29·8° C at the end of the 30 min

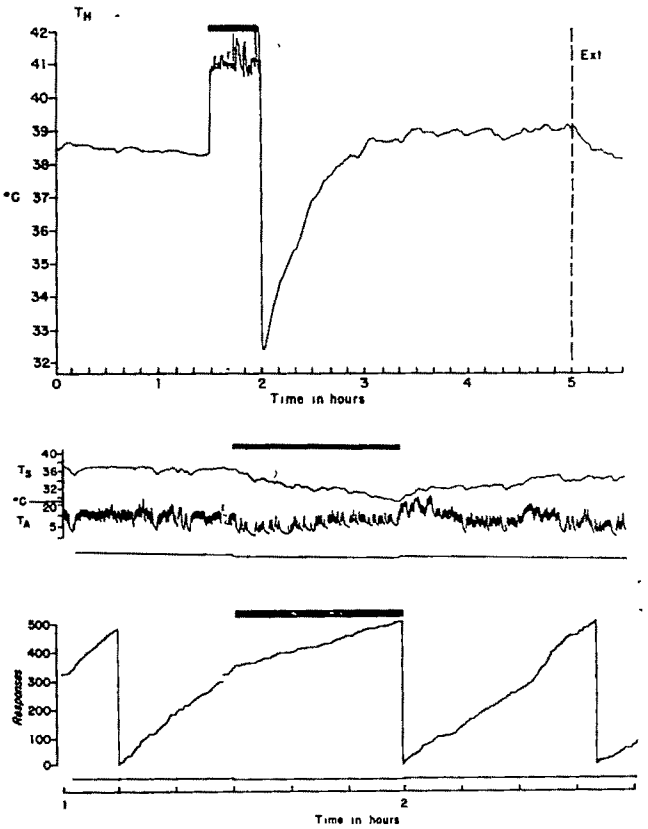


Fig. 2. Decrease in rate of responding for peripheral heat during local hypothalamic heating. T_s, Subcutaneous temperature; T_a, temperature measured by thermistor placed between heat lamp and the animal (each reinforcement produces a sharp increase in temperature, which then falls slowly until the next reinforcement). Ambient temperature, 0° C. Reinforcement, 2 sec at 200 W. Average temperature during hypothalamic heating is 41·0° C. The lower time scale refers to the lower records and, although expanded in length, corresponds to the upper record also

heating period. The rate of fall of subcutaneous temperature, and the final low value attained, would seem to contradict a strong thermal drive contribution from cutaneous receptors during hypothalamic heating. Rate of responding should actually increase during the heating period to compensate for falling peripheral temperature if these receptors are influential.

The relatively large size of the heaters produced hypothalamic damage, and central heating may have further increased the amount of this damage. Nevertheless, the animals maintained normal body temperature in a neutral environment, and worked for heat in a cold environment. Heater implantation and hypothalamic heating, therefore, did not preclude either temperature regulation or adaptive responsiveness. It is concluded that, under the conditions used here, an animal may respond as though the environment were warm if the hypothalamus is warm, even though deep body and subcutaneous temperatures may be low.

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INFECTIVITY OF GROSS AND RAUSCHER LEUKAEMIA VIRUSES AND THEIR NUCLEIC ACIDS

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TWO reports[1,2] have demonstrated infectivity of ribonucleic acid extracted from tissues infected with mouse leukaemia viruses, the Graffi agent and the Maloney virus. The present investigations were undertaken to reproduce these observations using the viruses of Gross and Rauscher; however, they failed to do so. Cell-free extracts of neoplastic tissue from mice with leukaemia induced by Gross and Rauscher viruses were found to reproduce the disease in inoculated mice, while nucleic acids extracted from the same neoplastic tissues did not result in disease.

The mice used in these experiments were from *AKR/J* and *C3HeB/FeJ* inbred strains maintained in this laboratory. The Gross virus used was passage *A*. This was supplied by Dr. Ludwik Gross of the Bronx Veterans Administration Hospital, Bronx, New York, in the form of a *C3H* mouse with virus-induced leukaemia, and has been maintained in our laboratory by inoculation of suckling mice with extracts prepared by Gross's method[3] without filtration. The preparations for virus inoculation and nucleic acid extraction were from animals developing lymphoma after the fifth such passage.

The Rauscher virus (*P*-805) was generously supplied by Dr. Frank J. Rauscher of the National Cancer Institute. It was from a crude 10 per cent extract of spleens collected from *BALB/c* mice 28 days post-intraperitoneal inoculation of virus. This material was shipped in the frozen state and stored at −70° C until used. Animals with Rauscher disease for use in these studies were inoculated when 1–2 days old with *P*-805 virus in an undiluted form. All the tissue extracts containing Rauscher virus were prepared by Gross's method[3] without filtration; nucleic acid preparations were made from the same animals with disease developing after inoculation of the original spleen extracts obtained from Dr. Rauscher.

Homogenates of tissues from animals with either Gross virus-induced leukaemia, or Rauscher disease, were used for extraction of the nucleic acid. The deoxyribonucleic acid (DNA) was prepared by the method of Kirby[4] with cold phenol and *p*-amino salicylic acid. To minimize nuclease activity 5×10^{4} M versene (disodium ethylenediamine tetraacetate) was added to the tissue homogenate and the whole procedure was carried out at < 4° C. To remove the ribonucleic acid (RNA) from these preparations, 100 per cent isopropyl alcohol was added to the phenol extract to give a 20 per cent alcohol content by volume. This precipitated the RNA. After centrifugation for 1 h at 16,000*g*, the sedimented RNA was removed and the alcohol content of the supernatant was raised to 37·5 per cent to precipitate the strands of DNA. The DNA was redissolved and precipitated two or more times and dissolved in saline or phosphate buffer for inoculation. The final material used for injection contained DNA in concentrations of 800 μg/c.c. as measured by the diphenylamine reaction. RNA contamination was 140 μg/c.c. as measured by the orcinol test. The control material was made by treating this solution with DNase (20 μg/c.c. for 20 min) at room temperature.

The RNA was prepared by the cold phenol method of Gierer and Schramm[5]. The final RNA concentrations in these inocula were 900–1,000 μg/c.c. The control inocula were incubated with RNase (20 μg/c.c.) for 20 min at room temperature. These RNA preparations had no measurable DNA contamination.

The cell-free extracts were administered intraperitoneally in 0·1–0·2 ml. amounts in 1–3-day-old mice of both strains. The nucleic acid preparations were given by three routes: (1) intraperitoneal (0·2 ml.) in animals 1–3 days old; (2) intrathoracic in 0·05 ml. amounts in 1–2-day-old animals; (3) intracerebral in 0·02 and 0·03 ml. volumes in new-born and 22–29-day-old animals.

Extracts of tissues from animals with Gross virus-induced leukaemia inoculated into suckling *C3HeB* mice produced at 2–4 months of age a lymphoblastic leukaemia in 100 per cent of the animals. Pathologically the virus disease resembled closely the spontaneous *AKR* lymphoma; the thymus was always involved as were the liver, spleen, lymph nodes, Peyer's patches and kidneys. The lymphoid tissue was in most instances completely replaced by lymphoblasts and cellular infiltrates were seen in the liver and kidneys. Transplantation of these virus-induced leukaemias was accomplished in isogenic strains. Adult *C3HeB* mice inoculated with viable cells developed leukaemia in 3–4 weeks.

Rauscher virus preparations when inoculated intraperitoneally into 0·1 c.c. amounts into suckling *AKR* and *C3HeB* mice produced disease in 100 per cent of the animals in 15–31 days. This disease was like that described by Rauscher[6] and was characterized by hepatosplenomegaly but no striking lymph node enlargement. The animals were all anaemic with marked anisocytosis, poikilocytosis and basophilia of the red cells. The peripheral blood nucleated cell count was 25,000–100,000/mm³. The differential count revealed many nucleated red cells in all stages of development as well as undifferentiated cells which were fragile and frequently smudged on the coverslip blood film. Microscopically the spleens were infiltrated with these same cell types in almost equal numbers, that is, erythroid precursors and undifferentiated cells. The immature cells resembled reticulum cells. The spleen also contained large areas of haemorrhagic necrosis. The lymphoid tissue of the spleen was virtually absent. The liver was heavily infiltrated with the erythrocyte precursors and reticulum cells; again each infiltrate was composed of equal percentage of both cell types. Lymph nodes were usually normal but some showed infiltrates of the abnormal cells found in the liver and spleen. The thymus demonstrated marked cortical atrophy. Morphologically the disease could be called an erythroid leukaemia.

However, when transplantations were carried out with viable cells obtained from the liver and spleen, and using isogeneic strain combinations, 10^{7} cells inoculated subcutaneously in suckling mice, or by either route in adult animals, did not result in local tumours or generalized disease. This was in marked contrast to similar cell transfers made in adult animals with Gross virus-induced leukaemic cells, where all the inoculated adult animals developed local tumours after subcutaneous inoculations and generalized disease 21–30 days after intraperitoneal inoculation of 10^{7} cells.

The nucleic acids were prepared by the methods already described from tissues of animals with Gross and Rauscher virus-induced leukaemias. As can be seen from the results summarized in Tables 1 and 2, inoculation of mice with the nucleic acid did not result in disease. Cell-free extracts of the same tissues used for nucleic acid extraction were given to suckling mice as a bioassay for the presence of virus in the tumours. All these extracts had potent oncogenic properties as indicated in Table 3. In each instance the disease produced was characteristic for the virus inoculated. All the animals given nucleic acid and their controls were killed 7 months later, a time well beyond the latent period of disease produced by these viruses when administered to suckling mice.

Table 1. RESULTS OF INOCULATION OF DNA EXTRACTED FROM TISSUES OF ANIMALS WITH GROSS AND RAUSCHER VIRUS-INDUCED NEOPLASMS

Mouse strain	Material inoculated*	No. neoplasms/No. mice
AKR	DNA (Rauscher)	0/22
AKR	DNA (Rauscher) + DNase	0/20
C3H/eB	DNA (Rauscher)	0/13
C3H/eB	DNA (Rauscher) + DNase	0/23
C3H/eB	DNA (Gross)	0/41
C3H/eB	DNA (Gross) + DNase	0/39

* Inoculations made intracerebrally at 22–30 days, or intrathoracically at 1–3 days.

Table 2. RESULTS IN INOCULATION OF RNA EXTRACTED FROM TISSUES OF ANIMALS WITH GROSS AND RAUSCHER VIRUS-INDUCED NEOPLASMS

Mouse strain	Material inoculated*	No. neoplasms/No. mice
AKR	RNA (Rauscher)	0/29
AKR	RNA (Rauscher) + RNase	0/30
C3H/eB	RNA (Rauscher)	0/18
C3HeB	RNA (Rauscher) + RNase	0/16
C3H/eB	RNA (Gross)	0/60
C3H/eB	RNA (Gross) + RNase	0/23

* The inoculation routes were intracerebral at 1 or 25 days intrathoracic at 1–3 days and intraperitoneal at 1–3 days.

Table 3. RESULTS OF INOCULATION OF CELL-FREE EXTRACT OF TISSUES OF ANIMALS WITH GROSS AND RAUSCHER VIRUS-INDUCED NEOPLASMS

Mouse strain	Material inoculated*	No. neoplasms/ No. of mice	Latent period (days)
AKR	Rauscher	20/20	7–28
C3H/eB	Rauscher	9/9	12–21
C3H/eB	Gross	9/9	90–150

*All inoculations were given intraperitoneally to 1–2 day old mice. All extracts tested were from tissue used to make the nucleic acid preparations.

The findings of these investigations demonstrate the high degree of infectivity of cell-free extracts of tissues of animals with both Gross and Rauscher viral leukaemias, when inoculated into suckling mice of the *AKR* and *C3HeB* strains. The disease produced is characteristic for each virus. These agents are representative of two distinct classes of leukaemia virus based on the type of disease produced. The Gross virus results in a lymphoid neoplasm with cellular homogeneity which has a 2–4-month latent period and when it develops is readily transplanted by cells to susceptible hosts. The Rauscher disease (developing 2–4 week after inoculations) is characterized by a proliferation of erythroid and reticulum cells which are seen in the spleen, liver and peripheral blood. This disease is not transmitted by cells in that cell inoculation of adult mice does not result in local tumour formation, or generalized disease. The phenomenon of lack of development of solid cell-transmitted tumours is similar to observations in Friend virus-induced leukaemia, which closely resembles the Rauscher disease, where it was found that successful grafting only occurred late in the disease when the liver and spleens were massively infiltrated with leukaemic cells[7]. The lack of transplantability of cells of the early disease suggests that the initial disease characterized by the dual infiltrate is a pre-neoplastic state characterized by a cellular reaction to infection with the virus rather than a true neoplasia.

Nucleic acids extracted from these same neoplastic tissues did not produce disease in inoculated hosts. This lack of infectivity of nucleic acids could have been the result of nuclease activity during preparation, or after administration of the nucleic acids or to a physical alteration of the nucleic acid molecule resulting from the extraction procedure *per se*. The intracerebral route was used to minimize nuclease activity, and confidence in the phenol method of extracting infective nucleic acid from virus-infected tumour tissues was gained from results of previous experiments where DNA prepared from polyoma virus-induced tumours was shown to be highly oncogenic when administered to suckling mice by the intrathoracic route[6]. Despite this finding, a fundamental alteration of these viral nucleic acids resulting from their extraction by phenol would seem to be a likely explanation for the observed loss of infectivity. Their large helical RNA, much less stable than polyoma DNA, was probably broken into smaller fragments which were not infectious, for as Rubin[9] has suggested, the avian and murine leukaemia viruses are probably large RNA viruses.

These observations of loss of infectivity in nucleic acid preparations indicate a similarity of the Gross and Rauscher viruses to the larger, more complex animal viruses (poxviruses, herpes viruses and myxoviruses) from which infectious nucleic acid has not been completely extracted. In the case of myxoviruses, Maassab[10] has reported extraction of infectious RNA from influenza virus-infected cells, but not from preparations containing only virus. His work, however, has not been confirmed. Careful studies of Rous sarcoma virus by Weil *et al.*[11] could not demonstrate infectious RNA. This virus is a large RNA virus which like the leukaemia viruses is formed at the cell membrane[9]. A difference between the Gross and Rauscher viruses and those of Maloney and Graffi, from which infective nucleic acid has been extracted with methods similar to those reported here, is demonstrated by this investigation. The positive results of these two investigators might be explained by slight differences in the technique used in the extraction, or by contamination of their preparations with virus.

Cell-free extracts of tissues of mice with leukaemia induced by Gross and Rauscher viruses were found to be highly effective in producing the specific disease characteristic for each virus when administered to suckling *C3HeB/FeJ* mice. Nucleic acids extracted with phenol from the same neoplastic tissues failed to cause disease. These results suggest that the nucleic acid of these two classes of mouse leukaemia viruses is not phenol extractable.

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ATTEMPTS TO INDUCE LEUKAEMIA BY INCUBATION OF MOUSE THYMUS CELLS WITH LEUKAEMIC CELL NUCLEIC ACID

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PREVIOUS investigations carried out in this Laboratory have shown that nucleic acids extracted from cells of leukaemic *AKR* mice and inoculated into new-born animals resulted in an increase of the incidence of leukaemia of host type in $(AKR \times C3H)$ F_1 hybrid mice[1]. Investigations using labelled DNA[2] have shown that this macromolecule can traverse the cell membrane *in vitro*, and Weil[3] has shown *in vitro* incorporation of viral DNA

into cells, utilizing hypertonic treatment to alter membrane permeability. The present study was undertaken to add to the previous *in vivo* investigations by attempting to produce *in vitro* leukaemic transformation of normal thymus cells. Two approaches were undertaken: (1) incubation of normal cells with leukaemic cell nucleic acid and inoculation into neonatal hosts of a low incidence leukaemia strain; (2) incubation of normal cells with leukaemic cell nucleic acid in hypertonic medium and inoculation into thymectomized adult animals with regenerating thymus grafts. The latter approach was used because of the well-known but poorly understood relationship of the thymus to the development of spontaneous leukaemia in mice. It was reasoned that the regenerating graft might be populated with the inoculated, nucleic acid treated cells, these cells then being in an environment more conducive to leukaemogenesis.

Most of the mice used in these studies were from inbred colonies of *C3HeB/Fe* and *AKR/J* mice maintained in this laboratory. However, the host animals in the second experiment were *AKR/J* females obtained directly from the Jackson Memorial Laboratory, Bar Harbor, Maine. The animals were fed Wayne 'Lab-Blox' and water *ad libitum* and housed in plastic boxes with white pine shavings. The leukaemia incidence in our *AKR/J* colony is 70–80 per cent. No leukaemias have been observed in the *C3HeB/Fe* mice which were routinely kept under observation until they were 14 months old.

The nucleic acids were extracted from the spleen, lymph node and thymus cells of leukaemic *AKR* and normal *C3HeB* mice by a modification of Kirby's cold phenol *p*-aminosalicylic acid method described previously[4]. The preparations contained both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in concentrations of 500–1,000 μg/ml. of DNA and 100–200 μg/ml. of RNA.

In the first experiment, suspensions of adult thymus cells, thymus cells of 5–6 day-old mice, or 10–19 day embryos were prepared by trypsinization from mice of the *C3HeB/Fe* strain. The cells were suspended in 5–10 c.c. of medium 199 or Eagle's medium. They were placed in 15 ml. culture tubes or 60 mm Petri dishes. In some of the initial experiments horse serum or foetal calf serum was added to a final concentration of 10 per cent. Serum was not utilized in later experiments because of the possibility of its nucleases inactivating the added nucleic acid. 0·5 ml. of nucleic acid was added to these cell suspensions at zero time. The experimental cells were incubated in nucleic acid extracted from leukaemic tissues of *AKR* mice and the control cells were incubated in nucleic acid from normal lymphoid tissue of *C3HeB* mice. The incubation time was 5–24 h at 37° in an incubator with 5 per cent carbon dioxide in air. The cell suspensions were agitated from time to time. At the end of the incubation period the cells were injected intravenously and, in a few instances, intraperitoneally into new-born *C3HeB/Fe* mice. In each instance approximately 1–2 million cells were inoculated. Eosin tests for viability (utilizing the principle that viable cells do not stain with dilute solutions of eosin) showed that more than 90 per cent of the cells were viable after 4–5 h of incubation and 50 per cent of the viable after 18–24 h. The animals were killed when 14 months old and only animals living longer than 6 months, the usual lowest age of onset of spontaneous *AKR* leukaemia, were included in the experiment.

The second experiment was of somewhat different design. The recipient animals and all the tissues used were from *AKR* strain mice. The test animals were thymectomized using 'Nembutal' and 'Fluothane' anaesthesia when 5–6 weeks old, and a week later were grafted subcutaneously with both thymuses from 1–3-day-old *AKR* mice. The incision for grafting was made in the left groin and the grafts were inserted by a subcutaneous tunnel on to the left anterior chest wall. The thymic lobes were placed in Tyrode's solution with 100 u./c.c. of penicillin and streptomycin prior to grafting. Ether anaesthesia was used for the grafting procedures. All the host animals were females; the donor thymuses were from both sexes as were the incubated cells.

When mice grafted with neonatal thymus were killed at 2-day intervals, it was found that the thymus underwent an initial regression with disappearance of cortical lymphocytes. There was a return of the lymphocytic population at 11–14 days of age. After 19 days the graft resembled normal thymus. On the tenth day after thymus grafting, a time when the graft had been shown to be reconstituting its lymphocyte population, the thymectomized and grafted mice were inoculated intravenously with normal thymus cells from 3-week-old *AKR* mice. The 0·25 ml. inoculum consisted of 1–8 million cells; one group of mice was given cells which had been incubated in hypertonic buffer containing leukaemic cell nucleic acid and the second group of animals received cells which were incubated in hypertonic buffer without nucleic acid. The method of incubation followed closely that of Weil[3], and was as follows: the cells were teased from the whole organ in Tyrode's solution. They were gently centrifuged and the supernatant decanted. Hypertonic sodium chloride (0·55 M) in 0·03 M *tris* buffer at *p*H 7·8 was added to the cells which were then centrifuged at a low speed. The supernatant was decanted and 1 ml. of the nucleic acid solution (1 : 10 dilution from our original preparation) was added. The cells were mixed with the nucleic acid and incubated 15 min in a water bath at 37°. At the end of incubation 9 ml. of Tyrode's solution was added; the solution was then mixed, centrifuged, and re-suspended in 2–3 ml. of Tyrode's solution for inoculation. The hypertonic treatment increased the eosin permeability from 5–10 per cent to as much as 100 per cent in some instances. Because the hypertonic treatment in the first experiment resulted in an increased number of eosin-permeable cells, all succeeding cell suspensions contained mixtures of cells incubated in both hypertonic and isotonic medium. Whether eosin permeability meant death in all these instances is questionable, for when the hypertonic incubated cell suspensions were placed in the refrigerator for 5–10 min the number of eosin permeable cells decreased.

Incubation of *C3H* Cells with *AKR* Leukaemic Cell Nucleic Acid–First Experiment

In this experiment 59 animals received cells incubated with *AKR* leukaemic cell nucleic acid. One of these animals developed a lymphoma when 10 months old. This animal had received 10^6 cells, 90 per cent eosin-negative, by the intravenous route at about 24 h after birth. These cells had been incubated for 5 h in the leukaemic nucleic acid prior to inoculation. The suspending medium was Eagle's with 10 per cent horse serum. The lymphoma was characterized by massive enlargement of peripheral nodes, spleen, and liver. There was no enlargement of the thymus or mesenteric nodes. Microscopically the tumour was a reticulum cell sarcoma. There were 56 animals receiving cells incubated in normal *C3H* lymphoid cell DNA. No lymphomas were seen in this group.

Incubation of Normal *AKR* Cells in Leukaemic Cell Nucleic Acid in Hypertonic Medium–Second Experiment

The design of this experiment was to determine if nucleic acid from leukaemic cells could effect a leukaemic transformation of normal isogenic thymic cells. It was assumed that if this occurred it would happen during, or shortly after, the time of incubation and the transformed leukaemic cells would produce disease as does a leukaemic cell transfer, that is, within 2 months, and that the regenerating thymus would provide a suitable environment for these cells. Leukaemic transformation,

however, did not occur. All the experimental animals were completely normal 2 months after the cell inoculations.

Observation of the animals up to the age of 12 months revealed some interesting and unexpected findings. The normal spontaneous incidence of leukaemia was strikingly reduced in the experimental mice. Fourteen mice were thymectomized, isografted with neonatal thymus, and inoculated 10 days later with a 3-week-old thymus cell suspension which had been incubated in leukaemic nucleic acid in a hypertonic medium. Four of these animals (28 per cent) developed lymphocytic leukaemia when 9–12 months old. The disease was morphologically identical to the spontaneous *AKR* disease and involved all the lymphoid organs. In three animals the graft was also involved and in the fourth it was completely normal. The remaining ten mice in this group were killed when 12 months old and no evidence of lymphoma was found. The grafts were viable and normal in appearance and were 5–10 mm in diameter. A second group of thirty mice were prepared in the same way but given a mixture of cells incubated with nucleic acid in hypertonic and isotonic medium. Three of these animals (10 per cent) developed typical lymphocytic leukaemia involving the graft as well as other organs. The remaining twenty-seven mice when killed at 12 months of age were completely normal with normal-appearing thymus grafts. The animals in both these groups had complete thymectomies.

A third group of mice were also thymectomized, and grafted with neonatal thymus. Ten days later these animals received thymus cells from 3-week-old animals incubated in hypertonic and isotonic medium without nucleic acid. None of these animals developed lymphoma and all were completely normal when killed at 12 months of age. The thymus grafts were all fully viable, and of normal morphology, and the thymectomies were complete.

Thirty-four intact *AKR* control mice were raised at the same time and under the same conditions as the experimental mice. Twenty-four, or 70 per cent, of them developed thymic lymphomata at a mean age of 11·2 months (range 6–12 months). The animals were observed until 12 months of age. These results are summarized in Table 1.

Table 1. Incidence of Lymphoma in Thymectomized-grafted *AKR* Mice given 3-week-old Isogeneic Thymus Cells 10 Days Post-grafting *

Treatment of cells	No. of mice	No. of lymphoma	Age of onset in months
Incubated with nucleic acid in hypertonic medium	14	4(28%)	9, 10, 12, 12
Incubated with nucleic acid in hypertonic and isotonic medium	30	3(10%)	9, 11, 11,
Incubated in hypertonic and isotonic medium	31	0	
Intact controls	34	24(70%)	6–12 mean 11·2

* All animals either died of lymphoma or were killed when 12 months old.

An analysis of the type of inoculum given these animals is presented in Table 2. As already mentioned, a portion of the cells was mixed with dilute solutions of eosin just before inoculation and cells staining with this dye were considered not to be viable. In the first group of experiments (inocula 1 and 2) the cells were all stained with eosin after the hypertonic treatment, hence in the subsequent inocula thymus cells incubated in DNA in isotonic solution were added to provide some viable cells. Two of six and two of eight mice developed late lymphomata after the inocula, which did not contain any 'viable' cells by the eosin test. Two of thirteen animals developed the disease when given a mixture of 'viable' and 'non-viable' cells, incubated in leukaemic DNA. In the 31 mice receiving inocula 5, 6 and 7, which did not contain nucleic acid but consisted of varying cell doses and 'viabilities', no lymphomata were seen.

These investigations show that *in vitro* incubation of normal isogenic thymus cells with nucleic acid extracted from leukaemic tissues of mice with spontaneous disease does not result in a direct leukaemic transformation of the cell or in a transformation to a precursor cell of leukaemia. If direct transformation had occurred in both of these studies one would have expected leukaemia development within 2–3 months after inoculation of the 'transformed' cells. If the nucleic acid incubation had effected a change to a precursor cell in the *C3HeB/Fe* mice in the first experiment the development of late leukaemias occurring after 6 months of age might have been seen. The one reticulum cell sarcoma developing is of interest since no others have been seen in the *C3HeB* mice, but certainly one cannot make any statements relating it to the neonatal inoculation of nucleic acid-treated cells.

The hypertonic treatment of the cells utilized in the second experiment has been shown by Weil[3] to result in effective *in vitro* incorporation into cells of DNA from polyoma virus. Therefore we can assume that the NA entered these cells but did not have any direct biological effect as measured by the studies utilized.

The suppression of the incidence of leukaemia in the *AKR* mice in the second study is of considerable interest. These animals were thymectomized, isografted, and then inoculated with normal thymus cells treated in a hypertonic medium with and without nucleic acid. The results show a general suppression of leukaemogenesis in the nucleic acid and control groups, therefore it is unlikely that the nucleic acid incubation was responsible for the lack of development of leukaemia. The leukaemias seen in the animals receiving cells treated with nucleic acid may well have been due to the lack of viable cells in the inoculum. This point will be discussed later.

It is an established observation that thymectomy results in a striking decrease in the incidence of lymphocytic leukaemia in *AKR* strain mice[5]. Subcutaneous isografts of 4-week-old thymus into thymectomized *C58* mice, another strain with a high natural leukaemia incidence which is reduced by thymectomy, have been shown to restore the incidence to that of intact controls[6]. Such studies on *AKR* mice have not been reported, but in experiments utilizing intact F_1 hybrid mice where the dam was of the *AKR* strain, subcutaneous grafts of 1-week-old *AKR* thymus into these $(AKR \times C57BL)F_1$ mice resulted in an incidence of lymphoid leukaemia of 20 per cent compared with 9 per cent in the controls[7]. In $(C3H \times AKR)F_1$ hybrid mice, not thymectomized but grafted with 4–5-week-old *AKR* thymus, the lymphoid leukaemia incidence was 60·9 per cent as compared with 27·7 per cent in the control animals[8]. These observations indicate that the presence of an *AKR* thymus promotes leukaemogenesis and strongly suggest that subcutaneous grafts of this organ should restore the high leukaemia incidence in thymectomized *AKR* mice. In our experiments, however, this was not the case. Isogenic 1–3-day-old thymus was used for grafting the thymectomized hosts, while in the aforementioned reports thymus grafts from older mice were utilized. However, the histological changes observed postgrafting in our studies were like those observed by Law[8] for subcutaneous grafts of thymuses from 4–5-week-old *AKR* donors as well as like these described by Dukor *et al.*[9] occurring in the subcutaneous isografts of new-born thymus in thymectomized hosts. Miller[10] also has shown that in virus-inoculated mice, in which thymectomy abolishes leukaemia development, grafts of neonatal thymus in 4–5-week-old recipients result in the same high percentage of leukaemias as seen in virus-inoculated, intact controls. These observations make it unlikely that the age of the thymus graft played an important part here.

Another possibility might be that the grafting postponed the age of onset of leukaemia and that, if the animals had been observed longer, a high incidence might have been seen. There is, however, no support for this in the experi-

ments in which *AKR* (ref. 8) and *C58* (ref. 6) grafts were used and in which the time of onset of leukaemia occurred earlier than, or at the same time as, that of the controls.

An explanation of the inhibition of leukaemogenesis seen in the isografted cell-inoculated mice might be that an altered pattern of regeneration of the thymus graft was promoted by the inoculation of viable cells on the tenth day after grafting and that this resulted in suppression of leukaemia development. The phenomenon of altered regeneration of the thymus has been observed in irradiated *C57BL* mice given intravenous inoculations of viable bone marrow cells[11]. The animals treated in this manner had a much lower incidence of leukaemia than did irradiated mice of the same strain[12]. It was also found to be true in the case of subcutaneous grafts of thymus in thymectomized, irradiated mice[13], that is, the thymectomized, irradiated *C57BL* mouse when grafted with isogenic thymus subcutaneously has a considerably higher leukaemia incidence than the thymectomized, irradiated and thymus isografted mouse that has also been given intravenous doses of bone marrow cells from a single femur of an isogenic animal. Therefore, the reduction of leukaemia incidence is the result of an altered and probably more rapid regeneration of the thymus presumably promoted by the prompt regeneration of the irradiated haematopoietic tissue by stem cells in the marrow inoculum.

Table 2. Cell Viability as determined by Permeability to Eosin in the Various Inocula

Treatment of cells	Inocula	No. of cells inoculated	Percentage of unstained cells*	Lymphoma/Mice inoculated
Incubated with nucleic acid in hypertonic medium	1	6×10^6 on successive days	Zero	2/6
		2×10^6 on successive days	Zero	
			Zero	
	2	3×10^6	Zero	2/8
Incubated with nucleic acid in hypertonic and isotonic medium	3	2×10^6	75%	2/13
	4	1×10^6	35%	1/17
Incubated in hypertonic and isotonic medium	5	$4{\cdot}7\times10^6$	75%	0/14
	6	8×10^6	80%	0/9
	7	$2{\cdot}7\times10^6$	20%	0/8

* Cells placed in a dilute solution of eosin and counted under the microscope prior to inoculation.

In the experiments reported here, as is shown in Table 2, the leukaemia incidence was lowest in the animals receiving the inocula with the greatest numbers of viable cells, on the tenth day after grafting. Dukor *et al.*[9] have shown that the regeneration of the thymus after subcutaneous grafting was accomplished initially by donor cells, but at the end of the second week the donor cells began to be replaced by host cells. Complete host cell repopulation was accomplished by day 21. Inocula of additional viable thymus cells in our experiments, then, were given at the time when regeneration of the original graft was nearly complete and when the host cells were beginning to repopulate it. This could have resulted in an altered pattern of regeneration of the thymus, one not compatible with leukaemogenesis. These observations suggest that the sequence of events occurring in the thymus under these experimental conditions differ in some important way from those occurring naturally in the *AKR* thymus.

Nucleic acids extracted from leukaemic tissues of *AKR* mice were incubated *in vitro* with cells of mice of a low incidence leukaemia strain (*C3HeB/Fe*) and inoculated intravenously into new-born mice of the same strain. Similar nucleic acid preparations were incubated with normal *AKR* thymus cells *in vitro* in a hypertonic medium. These cells were then inoculated intravenously into thymectomized adult *AKR* mice with regenerating thymic isografts. No early leukaemias were seen in either group, indicating that direct nucleic acid-mediated leukaemic transformation did not occur. The absence of late leukaemias in the *C3HeB* mice also indicated that there was no transformation to a precursor cell of leukaemia when the cells were incubated with leukaemic cell nucleic acid.

The marked depression of leukaemia incidence observed in thymectomized, isografted *AKR* mice inoculated with *AKR* thymus cells could have been due to a more prompt thymic graft regeneration resulting from the administration of viable thymus cells.

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MICRO-AEROBIC CAPABILITIES IN LAND PLANTS: OBSERVATIONS ON SURVIVAL AND GROWTH OF PLANTS SUBMERGED IN FRESH AND SALINE WATERS

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INVESTIGATIONS in this laboratory have shown that seed germination and seedling growth of many common vascular land plants occur with little or no oxygen[1–4]. In a number of cases, the rates of germination or seedling growth at reduced oxygen pressures have exceeded the rates in air. In the course of this investigation, it was recognized that the vigour of micro-aerobic plant growth may reflect a similar micro-environment of the seed and pre-emergent seedling in Nature. Air compositions beneath the soil surface, for example, may be radically different in their partial pressures of oxygen and carbon dioxide (P_{O_2}, P_{CO_2}) and in respect to the presence of hydrogen and methane from those above the soil surface[5,6]. The extent of such differences will depend on the structure, water table, drainage, and organic matter of the soil, and on the microflora and fauna present.

Among the factors which determine the P_{O_2} of the soil, the presence of water is of major importance. P_{O_2} will vary from that in relatively more aerobic, well-drained soil through heavier, poorly drained substrata,

muck, marsh and swamp-lands, to free-standing fresh, brackish, or sea-waters. Even shallow waters can be anaerobic or nearly so. Thus, Wetzel[7] showed that 5 cm below the surface of a pond which he investigated, the oxygen level at no time exceeded 0·21 vol. per cent and most of the year ranged from 0·0 to 0·01 vol. per cent. The bottom mud, only 15 cm in depth, was always anaerobic and rich in hydrogen sulphide.

Vascular plants native to aquatic habitats are usually treated as a group apart which requires a buoyant medium, perhaps as a consequence of loss in lignifying capacity. However, plants such as *Elodea* and *Salvinia* can live in such water, which serves primarily as a substratum and the atmosphere of importance is the gas phase which they contact. Others, *Typha* and *Alisma*, for example, may be rooted in anaerobic bottom sediments, extend through a micro-aerobic liquid phase, and emerge into the gaseous atmosphere[8–11].

Within the context of the foregoing discussion, the existence of micro-aerobic capabilities among vascular plants native to mesic or xeric conditions raises questions about the commonly held theories relating excess water and poor aeration to plant damage[12] and suggests that even submergence should not be a barrier to the survival and distribution of many species.

General observations on germination and growth. Aerated water at 20°–30° C, and 1 atm. pressure, saturates at about 0·8 vol. per cent oxygen; oxygenated water contains approximately 4 vol. per cent. In order to facilitate comparisons, we have generally treated submerged systems as analogues of plants in air. That is, the ambient fluid phase, whether liquid or gaseous, is the 'atmosphere' into which the plant, resting on a supporting surface, extends.

Submergence trials were generally carried out in 6-l. battery jars approximately 30 cm in depth. Aeration or oxygenation was carried out using sintered glass tubes. Dissolved oxygen was determined by a Beckman immersible oxygen electrode. No other special conditions or techniques were used.

Determinations of nitrogen, phosphorus, sulphur and ash were carried out by Micro-Tech Laboratories, Skokie, Illinois; halogen analyses were run by Schwarzkopf Laboratories, Long Island City, New York. Other analyses were carried out in this laboratory using routine procedures.

Preliminary experiments showed that several species germinated well when submerged in aerated water (oxygen about 0·8 vol. per cent), but in stagnant water (oxygen < 0·1 vol. per cent) only rice showed appreciable activity (Table 1). Of the species tested, only the beet required an oxygen-saturated medium. Cucumber and rye germinate well in a gaseous anaerobic medium, but not at all when submerged[3,4]. Failure of gas phase anaerobes to perform when submerged could be in part a result of accumulation of bicarbonate (carbon dioxide) under stagnant conditions.

Table 1. Germination Percentage of Submerged Seeds after 6 Days

Species	Oxygen vol. per cent		
	< 0·1	0·8	4
Beet (*Beta vulgaris*)	0	2	41
Cucumber (*Cucumis sativus*)	8	100	96
Marigold (*Tagetes* sp.)	0	56	100
Rye (*Secale cereale*)	0	100	100
Rice (*Oryza sativa*)	90	89	83
Barley (*Hordeum vulgare*)	0	66	—
Pole bean (*Phaseolus vulgaris*)	0	72	—
Lettuce (*Lactuca sativa*)	0	68	—

It appears from our limited number of experiments that the ambient medium *per se* is a factor in anaerobiosis, that a low volume percentage of oxygen in water is sufficient for many submerged species to grow, and that rice is an example of a true sub-aquatic anaerobe.

A comparison of growth in gaseous and aqueous 'atmospheres' was made on rye seedlings (Table 2). After 10 days in argon (oxygen < 0·005 per cent) the seedlings produced 10–20 mm shoots, some with primary leaves emerging from the coleoptiles. A gas-phase optimum oxygen level which was sub-atmospheric was evident. In the aqueous phase an optimum was also evident, but at a decidedly lower oxygen level. Clearly the 'operational' range of oxygen levels for the growth of rye is lowered and narrowed quite markedly in aqueous as contrasted with gaseous systems.

Table 2. Comparison of Rye grown in Approximately Equivalent Volume Percentages of Oxygen in Air and Water

Oxygen concentration Vol. per cent	Shoot height after 10 days (mm) 'Atmosphere'	
	Gaseous	Aqueous
< 0·1	15	< 1
0·8 (air-saturated water)	58	75
4·0 (oxygen-saturated water)	136	38
20·9 (air)	90	—

Root initiation in *Coleus blumei* cuttings has been compared. Air controls were set up with 4–6 cm plants extending out of 2 cm of water, whereas submerged whole plants were beneath approximately 20 cm of aerated or stagnant water. By the tenth day, 10–12 roots per plant were present in controls, 5–7 in aerated water, and none in stagnant water.

Other experiments have shown shoot growth in bulbs of *Narcissus* but, unexpectedly, comparable responses were observed in submerged specimens whether aerated or not.

Constitution of air-grown and submerged rye. Winter rye was selected for extended comparative investigations of the submerged state. The selection of rye was prompted by its tolerance to unusual or exotic environmental conditions[13].

About one hundred plants each of 10-day-old air-grown and aerated underwater-grown rye were subdivided for microscopic and chemical analyses. The microscopic examination was carried out using fresh free-hand sections of leaves (Table 3). This revealed that both epidermal and internal (mesophyll) cells were larger in the submerged group; that a distinct cuticle was not noticeable, if present at all; and that intercellular spaces were not noticeably different in magnitude, but submerged plants had somewhat reduced numbers of chloroplasts per cell. The enlarged state of leaf cells suggested a higher degree of succulence, or a 'waterlogged' condition. When samples of submarine rye were transplanted into ordinary conditions in air, they showed a pronounced tendency to collapse within a few days, and generally lacked the mechanical integrity of air-grown plants. This change was not, however, a result of 'waterlogging', as their dry matter content shows (Table 4).

Table 3. Overall Growth and Cellular Comparisons: Air versus Aerated Underwater-grown Rye at 10 Days

Measurement	*a* Air-grown	*b* Submerged	*b*/*a* (per cent)
Shoot height (mm)	90 ± 12	75 ± 15	83
Root height (mm)	72 ± 10	100 ± 17	139
Epidermal cell diam. (μ			
Upper	29·8 ± 1·2	50·5 ± 10·0	169
Lower	26·4 ± 1·5	39·0 ± 2·8	148
Epidermal hair cell			
Length (μ)	109·6 ± 15·1	136·0 ± 14·7	124
Width (μ at mid-point)	9·5 ± 1·0	10·4 ± 1·2	109
Nucleus (μ diam.)	5·4 ± 0·5	7·4 ± 0·8	137
Mesophyll cell diam. (μ	27·3 ± 2·0	33·4 ± 3·6	122
Chloroplast (No./cell)	29·2 ± 3·0	22·4 ± 2·9	77

We found that submerged plants were richer in organic nitrogen, phosphorus and sulphur. The biochemical significance of this difference is not yet known. Increased protein nitrogen has, however, been noted previously in bean plants grown in 5 per cent oxygen[2]. In contrast to such differences, the linear rates of growth of controls and submerged plants are comparable. The analyses of organic materials have been limited to the components listed in Table 5. The chlorophyll content is slightly reduced in submerged plants, but less so than the number

Table 4. Analytical Comparisons: Dry Matter and Elementary Constituents in Air versus Aerated Underwater Rye After 10 Days' Growth

Analysis	*a* Air-grown	*b* Submerged	*b*/*a* (per cent)
Dry weight (per cent)			
Shoot	8·5	7·8	92
Root	7·8	8·5	109
Total N (mg/g dry wt.)			
Shoot	47·2	58·9	125
Root	22·2	33·8	152
Organic P (mg/g dry wt.)			
Shoot	5·4	6·9	128
Root	4·6	6·8	148
Organic S (mg/g dry wt.)			
Shoot	4·2	5·5	131
Root	1·3	2·7	200
Ash (mg/g dry wt.)			
Shoot	60·5	113·8	187
Root	275·3	173·2	63

Table 5. Analytical Comparisons: Organic Components in Air versus Aerated Underwater-grown Rye at 10 Days

Analysis	*a* Air-grown	*b* Submerged	*b*/*a* (per cent)
Chlorophylls (mg/g dry wt.)			
a	5·0	4·5	90
b	2·4	2·2	92
	Total 7·4	6·7	91
Cell wall (mg/g dry wt.)			
Shoot			
Cellulose	365	270	70
Non-cellulosic polysaccharides	200	154	77
Root			
Cellulose	406	510	123
Non-cellulosic polysaccharides	167	129	77
Glucose (mg/g dry wt.)			
Shoot	58·1	71·0	122
Root	40·0	17·7	44

of chloroplasts per cell. The chlorophyll *a* : *b* ratio is unchanged.

Microscopic examination also suggests that the cell walls of air and submerged groups differ. In the shoot, only a slight reduction in cellulosic and non-cellulosic constituents is evident, but in the root, there is a shift to a higher proportion of cellulose relative to total dry matter and total cell wall. The glucose content of the submerged shoot is also increased (perhaps a reflexion of reduced wall synthesis) but is quite reduced in the root, perhaps as a consequence of increased cellulose synthesis.

Tissue spot tests (Table 6) show that submerged tissues are poorer in catalase and richer in peroxidase than those grown in air, a response also found in comparing beans grown in air and 5 per cent oxygen[2].

Table 6. Histochemical 'Spot' Tests on Whole Air and Aerated Underwater-grown Rye Seedlings

Reagents	Test	Response: Air-grown	Response: Underwater-grown
M/10 hydrogen peroxide	Catalase	+ + +	+
M/100 hydrogen peroxide M/100 pyrogallol	Peroxidase	+	+ + +
Saturated ammonium molybdate	*ortho*-diphenols	+)	–
1 per cent phloroglucinol in hydrochloric acid			
(*a*) In fresh tissue	Lignin + precursors	+	+
(*b*) In alcohol-extracted tissue	Lignin (polymer only)	+	–
1 per cent glucose-1-phosphate + potassium tri-iodide	Phosphorylase	+	+
Potassium tri-iodide	Starch	+	–

Fresh tissues, especially roots, show a positive lignin test, but the phloroglucinol colour reaction is also given by coniferaldehyde and related precursors (monomers)[8]. The precursors are soluble in alcohol and in water, and after extraction, submerged tissues are negative, but the solution is phloroglucinol-positive, indicating the presence of unpolymerized precursor. Ultra-violet spectra of extracts made with cold 4 per cent sodium hydroxide confirm that there is more soluble phenol in submerged roots. Again, the response of submerged rye resembles that of other species grown in sub-atmospheric oxygen levels[2].

Roots of both groups can convert glucose-1-phosphate into amylose (phosphorylase-positive), but the submerged roots are devoid of starch.

Preliminary experiments also showed that rye seedlings grow nearly as well in aerated 1–3 per cent sodium chloride solution as in distilled water (Table 7). Increased salinity is partially reflected in altered fresh weight, and reduction in non-halogen-containing dry matter. Work in progress also shows that solutions of 1 per cent sodium bromide and sodium iodide can support reasonably good seedling growth. In addition to accumulation of appreciable water-soluble halide ion, these plants contained up to several hundred p.p.m. of water-insoluble—presumably organically combined—chlorine, bromine or iodine. Plants have also been grown submerged in solutions of calcium, magnesium and potassium salts.

Table 7. Effect of Sodium Chloride on Growth and Dry Matter in Submerged Aerated Rye

Measurement (10 days)	Sodium chloride (weight per cent) 0	1	3
Length (mm)			
Shoot	97	110	75
Root	80	71	64
Fresh weight (mg/plant)	110	170	140
Dry matter (mg/g fresh wt.)			
Organic	135	109	105
Inorganic (–chlorine)	18·8	13·8	15·5
Chloride	0·2	4·2	5·5

The results of these and related experiments will be detailed separately when completed.

Survival of air-grown plants under water. So far, submerged growth initiated by way of seed germination has been considered. The tolerance of air-grown and air-adapted plants might conceivably fall below that of seedlings actually grown under these novel conditions. Although a direct comparison has not been made, several species have been tested for survival in aerated water (Table 8). In order to heighten the contrast, a number of zerophytes were included, and two of these, both *Euphorbia* species, showed the highest tolerance. After 9 months

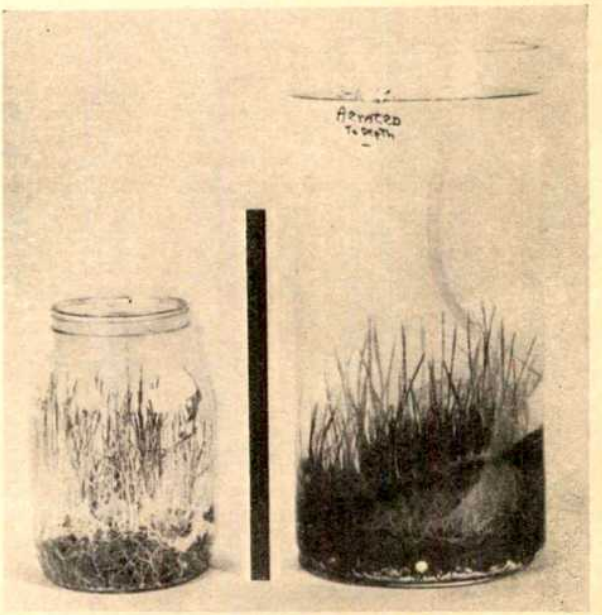


Fig. 1. Comparison of rye grown from seed in air (left) and under aerated water after 10 days

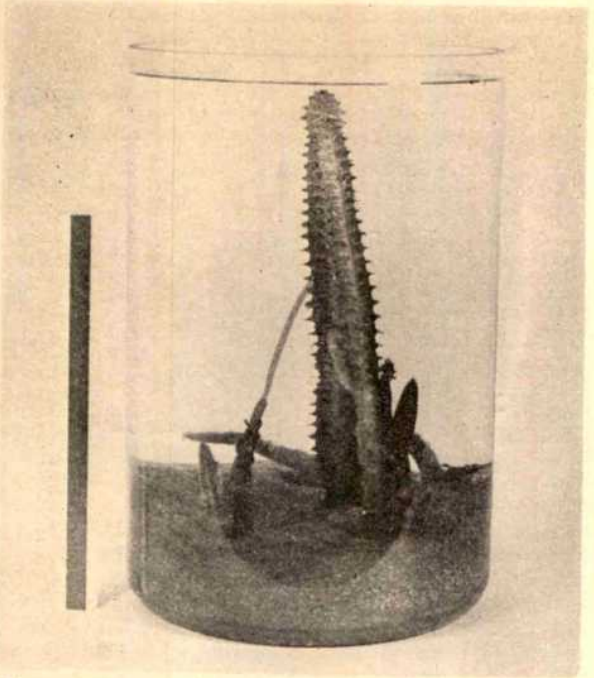


Fig. 2. *Euphorbia hermentiana* after 2 months' submergence in aerated water

of continuous submergence, *E. hermentiana* (Fig. 2) was still essentially unchanged except for the appearance of new leaves as much as 2 cm in length above the water line and many new roots varying from 4 to 40 cm in length within the sand substratum. The same species can also produce leaves, but no roots, when allowed to remain unplanted and unwatered under ordinary laboratory conditions.

Table 8. Tolerance of Selected Plants to Submergence in Aerated Water

Species	Condition after various submergence periods (Months)		
	1	3	9
Euphorbia hermentiana	+*	+	+‡
Euphorbia xylophylloides	+	+	−
Cereus peruvianus	+	−	−
Senecio gregori	+	−	−
Cotyledon sp.	+	−	−
Gasteria hybrida	+	−	−
Coleus blumei	−†	−	−
Hedera helix	−	−	−

* +, Normal turgor, colour; no sign of necrosis; no surface microbial growth.
−, Colour changes, necrosis, and microbial growth marked.
† Root growth still evident after 1 month.
‡ Both root and leaf growth evident.

It is of interest that *E. hermentiana* tolerates gas phase anoxia well, and shows appreciable viability for periods of up to 2 months in atmospheres rich in normally noxious gases such as ammonia[14].

For comparative purposes, the tolerance to submergence of several invertebrates was tested. The earthworm, *Lumbricus terrestris*, remains continuously active in aerated distilled water for at least one week, dies within hours under stagnant conditions, and survives in 1–3 per cent sodium chloride solutions for 3 days. The millipede, *Iulius*, which is relatively tolerant of low oxygen levels in the gas phase dies[15] in 1·5 days in aerated water, but remains active for more than 3 days in oxygenated water. The beetle, *Passalus* sp., and the spider, *Miranda aurantia*, die within hours in oxygenated water.

Von Brand reports species of nematodes, gastrotrichs, rotifers and oligochaetes capable of surviving periods of 2–4 months under anaerobic or near-anaerobic conditions[16]. In contrast, other obligate water forms, the molluscs and echinoderms, show little tolerance to anaerobic or micro-aerobic conditions, surviving for hours generally, rarely for several days. Crustaceans show somewhat better performance, as do aquatic insect larvae.

The biological significance of submergence studies. In some respects, the experiments described and discussed above are highly artificial, and perhaps somewhat surrealistic, in so far as they apparently place ordinary land plants and 'ordinary' (simulated) aquatic or marine conditions together in unnatural or unreal combination. This is not true for rice, of course, but applies to rye, *Euphorbia* and most other forms used. This criticism is misdirected, to some extent, since many mesic and even xeric environments are subject to periodic flooding or submergence by fresh or brackish waters. There is thus an autecological aspect to these experiments, inasmuch as they offer an explanation for the tolerance which some species show to submergence, even in saline waters.

Further ecological significance may be derived from a comparison of the micro-aerobic capabilities of specified land forms and their actual distribution in poorly aerated soils or shallow standing waters. *Typha*, as noted earlier, fits such considerations well, as does rice, but rye and *Euphorbia* do not occur naturally in environments which they could apparently tolerate. This suggests that such forms are not excluded from water-logged soils or other watery environments *per se*. The analysis of the factors responsible for their distribution is beyond the scope of this article, whereas the elimination of an often-invoked factor—soil oxygen supply—is.

Beyond the ecological and micro-ecological areas, this investigation has a bearing on the general fields of stress physiology and exo-biology. In order to define, at the various levels of bio-organization, the extreme parameters of survival, one not only has licence, but is required, to venture into organism-environment combinations that have no close natural counterpart. In the present case, plants normally associated with a two-part system—supporting substratum plus gaseous atmosphere—are placed in an analogous situation in a liquid 'atmosphere'. This work emphasizes further that a hardy species will show high performance under a multiplicity of stress conditions. That is, stress tolerance is not necessarily a matter of specific 'fit' into or selection for a particular condition, but may also be a reflexion of a general state of plasticity of the genome. Again, both rye and *Euphorbia* qualify as competent multi-stress forms whereas a species such as *Coleus* has low tolerance toward extremes of atmosphere, temperature, and their combinations.

From an experimental physiological-biochemical point of view, the plant in its submerged environment can be treated uniquely in terms of growth regulation and metabolism. A new mode of application to the 'aerial' plant of hormones, nutrients and metabolites is now possible. Translocation–transpiration–stomatal relations and cuticular exchange processes must be totally altered.

Evidence suggesting that the 'norms' of regulatory processes are changed with modified Po_2 (ref. 17) has been extended with observations on geotropism. Although the coleoptile is invariably orientated in an 'upward' direction, many roots are randomly disposed relative to gravity when rye is germinated under water. In an extreme case, a 100-mm negatively geotropic shoot was accompanied by several positively geotropic roots of 5–20 mm and one negatively geotropic root more than 250 mm in length. Cucumber seedlings showed even more striking disturbances, including geotropic reversals.

The metabolic effects of submergence are clearly indicated by the changes in chemical composition in rye, many being reminiscent of the effects of growth in a micro-aerobic gas phase.

The cultivation of land forms under water is thus a reasonable extension of and useful addition to previous environmental approaches centred about the gas phase.

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VERTEBRATE EPIDERMAL MELANIN UNIT

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MELANIN pigmentation of mammalian epidermis involves the formation of melanin granules within melanocytes and their transfer to malpighian (receptor) cells. The total intensity of epidermal pigmentation is related to the qualitative and quantitative attributes of melanin granules within both types of cells. The close integration of melanocytes and malpighian cells in pigmentation of mammalian skin has been stressed in the concept of the 'epidermal melanin unit' developed by Fitzpatrick and Breathnach[1]. The 'epidermal melanin unit' has been defined as a melanocyte with an associated pool of malpighian cells, the size of which may be variable. In this view, factors which influence any component in the 'epidermal melanin unit' might be expected to modify the function of the entire system. For example, ultra-violet light may act to stimulate melanogenesis, not by a direct action on melanocytes, but rather, indirectly, by inducing proliferation of malpighian cells, thereby providing more vehicles for melanin transport[1].

Genetic mechanisms appear to influence the size of mammalian 'epidermal melanin units' by their effects on melanocyte morphology. Dilute black (*ddBB*) murine melanocytes have shorter melanotic dendritic processes than more highly branched non-dilute (*DDBB*) melanocytes. Owing to the reduction in dendritic processes, the dilute melanocytes appear to maintain contact with fewer malpighian cells and accordingly have fewer vehicles available for melanin transport than comparable non-dilute melanocytes[2,3].

The 'epidermal melanin units' of mammals may overlap, with two or more melanocytes sharing some malpighian cells in common, or they may be discrete with no apparent overlap in their populations of epidermal cells. The overlapping of 'epidermal melanin units' is determined by the morphology of melanocytes and their population density.

As yet, there has been no attempt to extend the concept of the 'epidermal melanin unit' to other classes of vertebrates. Based on the definition provided by Fitzpatrick and Breathnach, it would appear that such units not only are present in mammalian skin but also exist in the skin of certain reptiles[4] and amphibians[5]. Their general occurrence elsewhere among vertebrates awaits clarification. This communication documents the existence of 'epidermal melanin units' among amphibians and stresses the importance of the comparative approach to an understanding of epidermal cell–melanocyte interactions in pigmentation of the skin.

Although there are numerous conflicting reports on the part played by epidermal melanocytes in colour changes in amphibians, our investigations indicate that under appropriate conditions the responses of the epidermal melanocytes of certain amphibians are entirely predictable[6]. This information is derived largely from investigations on the leopard frog, *Rana pipiens*, but similar investigations on several other species of amphibians (for example, *Xenopus laevis* and *Diemyctylus viridescens*) have yielded comparable results.

When adult *Rana pipiens* are placed on a white background and illuminated from above, there is an aggregation of melanin granules within epidermal melanocytes; this is in contrast to the dispersion of melanin granules found in the epidermal melanocytes of frogs placed on a black background under similar lighting conditions. *In vitro* investigations also indicate that the distribution of melanin granules within epidermal melanocytes is influenced by some of the agents (for example, caffeine, commercial ACTH, 'Pituitrin') with known action on pigment mobilization within dermal melanophores[7]. However, melatonin is a noteworthy exception; though effective in causing melanin granule aggregation in the dermal melanophores at extremely low concentrations it has no demonstrable influence on the epidermal melanocytes even at extremely high concentrations.

The epidermal melanocytes of frogs possess the dual capacity to synthesize melanin granules for transport by epidermal cells and to mobilize melanin granules intracellularly in a manner adaptive to the colour of the background. The population of epidermal cells actively receiving melanin granules from an epidermal melanocyte is determined by the extent to which melanin granules are distributed throughout its highly branched processes. In animals adapted to a neutral background, or as captured in the field, fewer epidermal cells receive melanin granules from a given epidermal melanocyte than in animals adapted to a black background. In contrast, the melanocytes of frogs adapted to a white background release less melanin and the newly released melanin granules are frequently restricted to those epidermal cells immediately adjacent to the melanocyte perikaryon which contains the aggregated melanin granules. In frogs adapted to a neutral background, regions of the epidermis where the density of epidermal melanocytes is low are characterized by many 'epidermal melanin units' which are discrete, having little or no functional overlap. Each melanocyte may possess its own distinctive constellation of epidermal cells for pigment transport; a halo of non-pigmented epidermis often separates the individual units (Fig. 1*A*).

When frogs are adapted to a black background for an extended period of time, functional overlap of 'epidermal melanin units' is observed in regions of the epidermis previously characterized by discrete 'epidermal melanin units' (Fig. 1*B*). This results when melanin granules are dispersed throughout the more distal regions of the branched processes of the melanocytes and consequently are distributed to a greater number of epidermal cells. This overlapping of functional units results in the seeding of previously non-pigmented epidermal cells with melanin granules; some epidermal cells receive melanin granules from the dendritic processes of more than one epidermal melanocyte.

Environmental and, consequently, hormonal stimuli appear to determine the effective population of epidermal cells for melanin transport within an 'epidermal melanin unit' by influencing the distribution of melanin within the dendritic processes of the melanocyte. This is most clearly demonstrated in animals adapted to a white background for extended periods of time. Owing to the perinuclear aggregation of melanin, few melanin granules pass into the dendrites and less melanin is found outside the

confines of the epidermal melanocyte. Therefore, in epidermal regions where 'epidermal melanin units' normally overlap, discrete units may now be found and are seen to be separated from other 'epidermal melanin units' by pigment-free epidermal cells (Fig. 1*C*). These results are clearly the reverse of those found in dark-adapted animals.

Our investigations indicate that there is a direct relationship between the distribution of melanin granules within melanocytes and the rate of melanogenesis. The dispersion of melanin granules within melanocytes is associated with a high rate of melanogenesis and release of melanin granules for transport by epidermal cells. The aggregation of melanin granules within epidermal melanocytes is associated with a lower rate of melanogenesis and little transport of melanin granules within epidermal cells. The patterns of melanin transport within the epidermis suggest an intermediate rate of melanogenesis where there is partial dispersion of melanin granules. Thus, it is possible that the hormones which regulate the distribution of melanin granules within melanocytes may also regulate melanogenesis. This finding confirms the earlier suggestion of Bábàk[8] that morphological colour change (quantity of pigment) in vertebrates is of necessity dependent on physiological colour change (distribution of pigment). The dispersion of melanin granules by hormones increases the opportunity for transfer of melanin granules to epidermal cells. Therefore, the rate at which melanin granules are carried away from melanocytes may also be a decisive factor in determining the rate at which they are synthesized. It remains to be established whether hormones act directly on the melanocyte or indirectly through the epidermal cells among which the melanocyte resides.

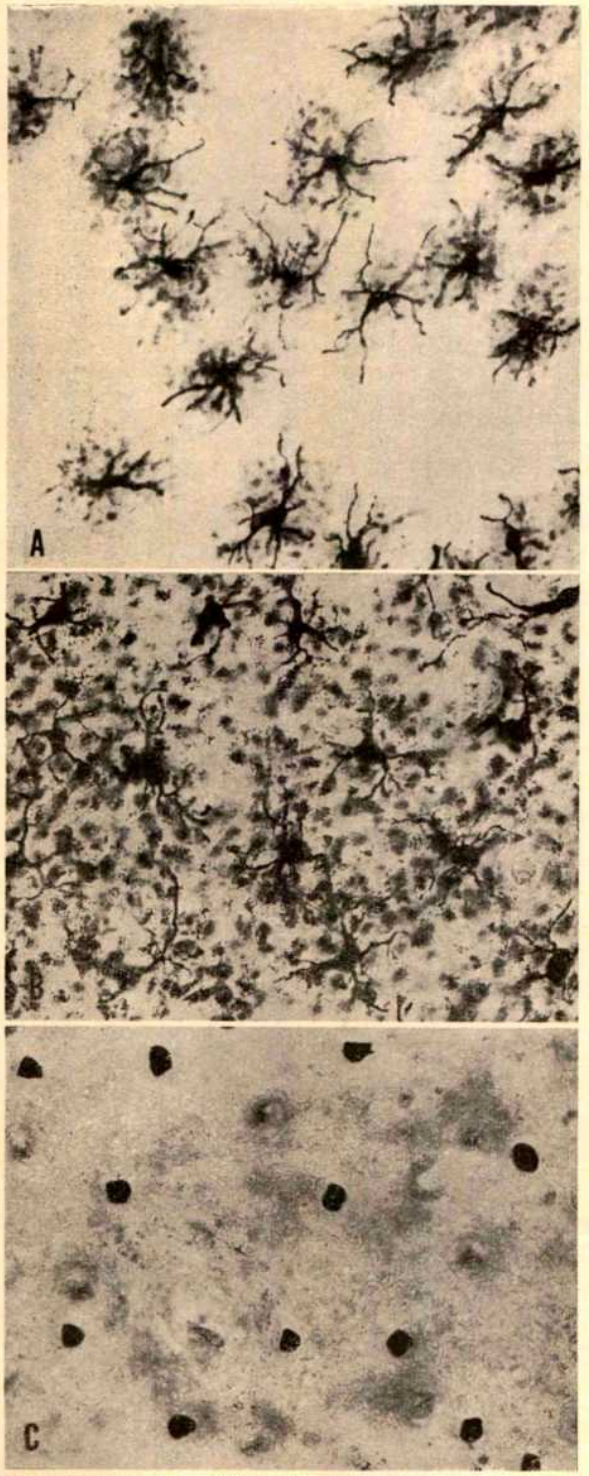


Fig. 1. Photomicrographs of the 'epidermal melanin units' of frog (*Rana pipiens*) epidermis: *A*, Little functional overlap among 'epidermal melanin units' in skin adapted to a neutral background; *B*, 'black-adapted' skin with 'epidermal melanin units' exhibiting functional overlap; *C*, 'white-adapted' skin illustrating perinuclear aggregation of melanin granules within melanocytes and absence of functional overlap among 'epidermal melanin units' (×600)

Lerner and co-workers[9] have postulated that hormones may influence melanogenesis within mammalian melanocytes by initial actions on the intracellular distribution of melanin granules. The rapid darkening of human subjects treated with melanocyte-stimulating hormone suggests that the dispersion of melanin granules within melanocytes may precede the onset of new melanin formation[9]. Snell[10], in a number of recent publications on mammalian pigmentation, has provided cytological evidence on the action of certain hormones on melanocyte morphology, melanin synthesis, and the release of melanin granules to surrounding malpighian cells. Snell has suggested that mammalian epidermal melanocytes react to these hormones in a manner similar to the dermal melanophores of amphibians. At present, there is no clear evidence that hormones bring about a reversible mobilization of melanin granules within mammalian melanocytes. The findings in amphibians reported herein suggest that such an action may well be demonstrated and indicate the importance of further work in this area.

Traditionally, the term melanophore has been used to designate a pigment-retaining cell with the capacity to disperse or aggregate its melanin granules[11]. The term melanocyte has generally been restricted to the neural crest-derived, melanin-forming cells of mammals and birds; melanocytes frequently secrete melanin granules into surrounding cells and until recently have been considered to lack 'melanophore action'. The 'epidermal melanocytes' of amphibians possess both capacities and indicate the need for a re-examination of this facet of pigment cell terminology. The vertebrate 'epidermal melanin unit' is a useful addition to pigment cell nomenclature and should lead to new light being shed on the integrated action of the diverse cell types associated with integumentary coloration.

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INTRA-CELLULAR SITE OF EPIDERMAL KERATIN SYNTHESIS

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At present it seems likely that the component molecules of fibrous proteins are biosynthesized in the same way as other proteins, in the sense that they have specific amino-acid sequences and molecular weights[1]. Experiments with many small proteins suggest that immediately after synthesis they spontaneously assume their native, three-dimensional arrangement[2]. As a result of the relative positions of positively and negatively charged, polar and non-polar side-chains, the native structure is the most stable in the physiological conditions inside or outside the living cell. The native structures of the components of fibrous proteins usually involve more than one protein molecule, each in a specific configuration and a definite position with respect to the others. Examples are the three chains of the tropocollagen molecule[3], the triplet of α-helices forming the protofibril of wool keratin[4], and the component molecules of myosin[5]. It is difficult to imagine that these native structures arise spontaneously when the component molecules come together *in vivo*. In the light of the available evidence of the mechanism of fibrous protein synthesis, and the preliminary examination of epidermal keratin biosynthesis described here, we suggest that fibrous proteins acquire their native structure at the time of synthesis. If the component molecules could be synthesized at the same place within the cell, and if the moment of starting and the rate of synthesis of the components could be controlled, then the native fibrous protein might well be the result. Our present experiments are designed to discover the intracellular machinery which synthesizes epidermal keratin and perhaps gives it its native structure. It may be larger and more elaborate than the polyribosomal aggregates found to synthesize small protein molecules.

Epidermal Sub-cellular Particles and Macromolecules

The skin on the ventral surface of the sheep's ear is relatively free from hair follicles. This makes the separation of dermis and epidermis easier and means that our examination of epidermal keratin synthesis is not confused by concurrent hair keratin synthesis. After the animals were killed about ten ears were kept in ice for about 2 h. The ears were cleaned with mild detergent, 0·5 per cent sodium deoxycholate (DOC) at room temperature, or wiped with cotton wool soaked with ether. Slivers of epidermis plus some dermis were cut with a razor and floated, epidermis upwards, on 0·01 per cent trypsin (Seravac Laboratories, once crystallized) in isotonic buffer (Oxoid 'Dulbecco *A*') adjusted to *p*H 7·8, for 30–45 min at room temperature. After this time the epidermis could be separated from the dermis, which was rejected, and washed in isotonic buffer. This and subsequent operations were carried out at 0° C. The epidermal preparation consists of dividing cells, which are the lowest layer, cells filling with epidermal keratin fibrils, and the fully hardened stratum corneum[6]. The preparation was washed quickly in hypotonic buffer (10^{-2} M *tris*(hydroxymethyl)aminomethane (*tris*), 10^{-2} M potassium chloride, $1{\cdot}5 \times 10^{-3}$ M magnesium chloride, *p*H 7·4) (ref. 7) and then allowed to swell for 5 min in 1·5 ml. of the same buffer. The preparation was homogenized by three stokes of the loose pestle and one of the tight in a Dounce homogenizer, and then centrifuged for 5 min at 1,250*g* to remove unbroken cells and tissue. The supernatant was made 0·5 per cent in DOC to remove ribosomes and ribosomal aggregates from the small amount of endoplasmic reticulum in epidermal cells, and if possible to prevent them from adhering to lipid droplets which contaminate the preparation. Fig. 1 shows the schlieren pattern 10 min after this supernatant from slow centrifugation had reached 50,740 r.p.m. in the Spinco model *E* ultracentrifuge. The pattern shows, from left to right, the lipid layer at the meniscus, a sharp peak due to epidermal macromolecules, proteins and nucleic acids, and then two small peaks caused by ribosomal sub-units and a sharper single ribosome peak.

Characterization of Ribosomes

The sedimentation coefficients of ribosomes and ribosomal sub-units are affected by the presence of macromolecules which alter the viscosity of the solution, and by temperature. Ribosomes and sub-units were prepared free from contaminating macromolecules by a method used by Earl and Korner[8]. All operations were carried out at 0° C. Epidermis was prepared as before, and washed three times by decantation after slow centrifugation with 0·2 M sucrose, 0·05 M *tris*, 0·005 M magnesium acetate adjusted to *p*H 7·6. The preparation was homogenized in the same buffer by five strokes of the loose pestle, and the homogenate was centrifuged for 10 min at 11,000*g* in a Spinco *SW*39 swinging bucket rotor in order to remove unbroken cells and tissue, nuclei, mitochondria and membranes. DOC was added to a final concentration of 0·5 per cent, and 4·25 ml. of this supernatant were carefully layered on to 5 ml. of a buffered, strong sucrose solution (1·0 M sucrose, 0·02 M *tris*, 0·1 M potassium chloride, 0·04 M sodium chloride, 0·005 M magnesium acetate, *p*H 7·6). Ultracentrifugation in a model *E*, *C* rotor at 105,000*g* for 4 h caused the ribosomes and sub-units to sediment through the lower, strong sucrose solution, while the macromolecules remained in the upper layer. The upper layer was removed using a Pasteur

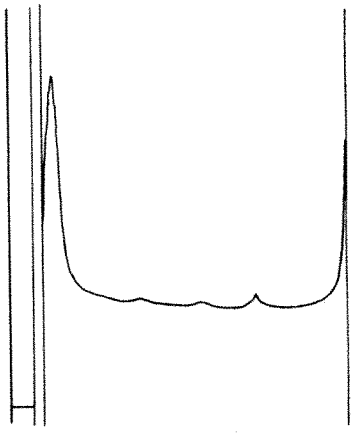


Fig. 1. Cell-free preparation from epidermis: schlieren pattern 10 min after reaching 50,740 r.p.m. Bar angle, 45°; 4° C. Sedimentation left to right

pipette. Then the lower layer was removed, and the pellet gently washed with an approximately isotonic buffer (0·02 M *tris*, 0·1 M potassium chloride, 0·04 M sodium chloride, 0·005 M magnesium acetate, *p*H 7·6) and re-suspended in the same buffer by carefully and repeatedly filling and emptying a hypodermic syringe with the solution. Ultracentrifugation in an analytical cell at 37,020 r.p.m. at two low concentrations of ribosomes allowed measurement of the sedimentation coefficients at 20° C, and s^0 was calculated assuming $1/s$ is proportional to concentration. The result, s^0 = 74 Svedberg units, is consistent with other mammalian ribosomes.

Incorporation Experiment

Epidermis was prepared as before and washed four times in isotonic buffer to remove trypsin. The pieces were then incubated at 37° C in 10 ml. Glaxo tissue culture medium 199 for about 30 min. The preparation was concentrated into about 1 ml. of medium by centrifugation at 1,250*g* for 2 min. Then, still at 37° C, 30 μc. of a carbon-14 labelled mixture of amino-acids (protein hydrolysate–^{14}C from *Chlorella vulgaris*, Radiochemical Centre, Amersham) were added in 0·3 ml. isotonic buffer. After 1 min excess cold isotonic buffer was added to stop amino-acid incorporation and to wash off excess labelled amino-acids. All subsequent operations were carried out at 0° C. Washing was repeated three times. The preparation was then washed quickly in hypotonic buffer, allowed to swell 5 min in the same buffer, and then homogenized in 2·5 ml. of the buffer. The homogenate was centrifuged 5 min at 1,250*g*, DOC was added, and 1 ml. was layered on to a 25 ml. linear sucrose gradient, 5–20 per cent sucrose dissolved in hypotonic buffer, in a cellulose nitrate tube. A further 1 ml. of the supernatant from homogenization was treated with 1 μg. ribonuclease (B.D.H.) for 10 min at 0° C, and layered on to a second gradient. After centrifugation for 90 min at 24,000 r.p.m. Spinco model *L–2*, *SW*25 rotor, the tubes were punctured with a hypodermic needle, and fifty-drop (about 1 ml.) fractions were collected. The fractions were diluted to 3 ml. and their optical densities were measured at 260 mμ using a Unicam *SP*500 spectrophotometer. Carrier protein (0·25 mg egg albumen in 0·5 ml. 4 N potassium hydroxide) was added to each fraction, and after 10 min the fractions were made 5 per cent in trichloroacetic acid in order to precipitate the protein. The precipitates were filtered off on 'Millipore' filters, the filter papers gummed to planchets, and the radioactivity of each fraction was measured using an end-window Geiger–Müller counter (Ericsson Telephones, Ltd.). Fig. 2 shows the result of the experiment without added ribonuclease. The optical density peak at about fraction 21, due to single ribosomes, had little radioactivity associated with it. In neither the optical density nor the counts per minute curves is there evidence of small polyribosomal aggregates usually found in rapidly dividing cells or in cells synthesizing small protein molecules. After 90-min centrifugation at 24,000 r.p.m. an aggregate of five ribosomes would be expected to appear in about fraction 10. The pellet was washed from the bottom of the cellulose nitrate tube; as before, carrier protein in potassium hydroxide was added, and total protein was precipitated by trichloroacetic acid. On a 'Millipore' filter the count of this precipitate was 365 per min. At present we are trying to discover the significance of the radioactivity in the pellet (i) by investigating the effect on it of different times and speeds of centrifugation of the supernatant from homogenization before layering on to the sucrose gradient; (ii) by collecting the pellet on a cushion of concentrated sucrose at the bottom of the sucrose gradient[9] instead of washing it from the tube; (iii) by fractionation of the pellet on sucrose gradients at lower speeds and for shorter times. When ribonuclease is added to the homogenate before density gradient centrifugation it has little effect on the result shown in Fig. 2 or on the radioactivity found in the pellet.

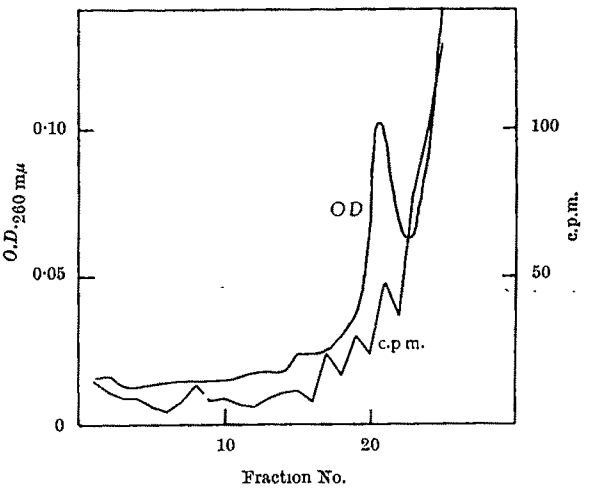


Fig. 2. Optical density and radioactivity of fractions from density gradient centrifugation of a homogenate from epidermis which had been incubated with carbon-14-labelled amino-acids

Discussion

The active units in the reticulocyte cell cytoplasm for the synthesis of haemoglobin sub-units are aggregates of four, five, or six ribosomes linked by a messenger–RNA strand[10]. In rapidly dividing cells, for example fibroblasts in culture dividing to form a monolayer on glass, similar small polyribosomal aggregates are the site of protein synthesis[11]. When the monolayer is complete, and rapid cell division has ceased, protein synthesis continues and the main product is now probably collagen[12]. Polyribosomes can no longer be detected in the cell homogenates. *In vivo* experiments confirm that collagen is not formed at small polyribosomal aggregates, but in subcellular fractions with *s*-values greater than 400, which are insensitive to ribonuclease treatment[13]. In adult rat heart muscle, small polysomes have been shown to be the site of protein synthesis[8]. However, the synthesis of heart muscle protein in chick embryo appears to be similar to collagen synthesis. Small polysomes are not detected; protein synthesis appears to occur in large ribonucleoprotein complexes which are disrupted by proteolytic enzymes[9].

There is evidence, therefore, that the fibrous proteins of muscle and collagen may be synthesized at large ribonucleoprotein complexes. We suggest that the native structure of fibrous proteins may arise as a result of concurrent synthesis of more than one polypeptide chain at these complexes. We are continuing to investigate the possibility that epidermal keratin may also be synthesized at a large ribonucleoprotein complex.

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LETTERS TO THE EDITOR

ASTRONOMY

Evidence that the Dark Areas on Mars are Elevated Mountain Ranges

In the communication under the above title[1], Dr. Wells makes a good point in reference to Lowell's conclusion regarding Martian topography. It agrees in part with the phenomena I have often observed, namely, that the migration of portions of some maria and gradual shading off of some low-cloud or frost areas implies very gradual slopes[2]. But even in the case of abrupt topographic differences, I think Lowell was over-optimistic in his value of 2,500 ft. It must be remembered that the maximum phase occurs at about twice the oppositional distance. At that time the terrain slopes away from the observer at about 45°. Furthermore, the colour contrasts of both maria and deserts show a marked drop at about half the radius from the centre of the planet's disk, apparently from the Martian atmosphere. There is also the troublesome factor of irradiation. Mars has some twilight effects, further lessening the contrast. The total of these effects and the limitation of detail under the best 'seeing conditions' makes an abrupt topographic difference of 20,000 ft. or less impossible to detect.

I cannot reconcile the statement that the dark areas are elevated land. On the contrary, our observations show that the formation of localized clouds on Mars is a common occurrence, not a rare one. Many desert areas behave this way, such as: Elysium, Hellas, Isidis-Neith, Aeria, Edom, Nix Tanaica, Nix Olympica, Ogygis, and some other 'islands in the south'. In addition, there are some other areas exhibiting less whiteness and persistence. Meteorologically, the aforenamed regions must be the high-elevation areas and this is the basis for my topographic mapping of Martian desert-relief. The white areas strongly avoid the maria. Radiometric measurements show the maria to be warmer, which could be due to lower reflective power alone, but it is of the right sign to favour lower topographic altitude. The temperatures of our dark mountain forests are certainly lower than those of the brighter lowlands.

Recently, I presented evidence for the absence of folded mountain ranges on Mars[3]. Such mountains are of geosynclinal origin, in which erosional sediments are deposited into sedimentary rocks. There are good reasons why such conditions never prevail on Mars. On the other hand, the desert areas which are observed to whiten must represent plateaux. While most of these are round or oval in outline, at least two (Elysium and eastern Aeria) are definitely angular, suggesting fault-block terrain.

The relatively small number of minute white spots suggests that volcanism has operated on a much lesser scale than on the Earth. To my mind, and as I suggested in 1950, the round dark oases represent asteroid impact craters, which are much more numerous than the small white spots[4]. Fifteen years later this was confirmed by the *Mariner* 4 pictures.

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Dr. Tombaugh's communication raises some interesting points about the topography of Mars. In view of the brevity of my earlier communication[1] I could only mention the most salient features of the proposed model for the dark maria. A more detailed discussion is now in preparation for publication.

One can scarcely doubt the rarity of Martian cloud phenomena if a comparison is made with some standard, say, one week's cloud occurrence on Earth. It is of interest to note that only one possible indication of the presence of a cloud was recorded by *Mariner* 4 although, admittedly, less than 1 per cent of the Martian surface was photographed.

Furthermore, the phenomena in the desert areas to which Dr. Tombaugh refers are quite different from those I gave as examples of lee-wave clouds. He has listed areas of possible hoar frost deposits on the surface which could imply elevated plateaux. (In my previous report I gave one example each of a possible mountain and plateau revealed by surface deposits of snow or hoar frost.) It is questionable that these areas participate in surface frost formation as frequently as has been observed. A powerful means of distinguishing between surface hoar frost and white clouds is by use of the polarimeter. For the past decade such observations at the Pic-du-Midi and Meudon have revealed that atmospheric clouds are as frequent a cause of brightening, particularly in the equatorial regions mentioned. The few instances that Dr. Tombaugh and others[2] have mentioned need not, of course, imply that all the bright areas (nearly three-quarters of Mars is bright desert area) are elevated. Since dust storms are considerably widespread occurrences, it would seem more natural that low areas would be dust covered and bright. Hence if the dark maria are depressions, why do they exhibit themselves at all? One would think that they would have been filled with dust and obliterated long ago. Application of Öpik's[3] 'rejuvenation' theory at this point would find even more stringent demands.

Figs. 29–31 of a recent publication by Dollfus[4] show the initial developments of the great dust storm of 1956. The dust clouds formed and spread along the bright regions between the dark maria without transgressing the boundaries. Later, of course, most of the maria became enveloped in some of the dust clouds. This phenomenon is certainly not consistent with the maria as depressions.

It may be that Dr. Tombaugh's "white areas strongly avoid the maria", but the white clouds (atmospheric phenomena) to which I have referred behave otherwise. They form at the interface of a bright and dark area, although they remain over the bright region and do not seem to penetrate the boundary into the mare. It was this peculiar 'stabilization' that caused me to suggest the similarity to lee-wave clouds on Earth. These Martian clouds are stationary but not unduly persistent, dissolving after several hours to re-form again the next day. One of the finest examples of these phenomena is depicted in Plates 33 and 34 in an article by Dollfus[5], where he describes them as: ". . . of the same nature as the bright formations observed at the top of the mountains surrounding the polar cap . . . and are probably also due to surface elevations". The bright formations in the polar regions to which Dollfus refers are ". . . bright, small clouds [which] appear in late spring each day at the same locations"[6].

In view of the mounting interest in the wind-blown formation theory of the dark maria beginning with McLaughlin[7], amended by Kuiper[8], and re-modelled by Rea[9], it appears that the interpretation of the dark maria

as elevations is finding increasing favour. At a recent symposium[10], Rea stated: ". . . the idea that the dark areas are depressed I find difficult to accept. Specifically, I know of no good evidence suggesting the dark areas are depressed".

The radiometric measurements mentioned by Dr. Tombaugh are worth considering; nevertheless, the difference in temperatures between the dark and bright areas is what would be expected on the basis of albedo differences alone provided, of course, that the thermal inertia of both regions is low and of comparable magnitude. The evidence derived chiefly from Sinton's[11] scans is still somewhat tenuous since they were averages of a few observations and not throughout an entire Martian year. Hence, it is not definitely known whether the dark areas maintain their higher temperatures over the complete Martian orbit or merely during the interval at oppositional passage. Observational bias would naturally disappear if the data extended over enough oppositions to make a statistically complete orbit.

Finally, I did not suggest any mechanism of mountain formation on Mars. To what extent crustal folding did or did not play a part does not preclude the existence of mountains. There are a considerable number of tectonic mountain ranges on the Moon.

To conclude, I find it strange that Dr. Tombaugh cannot believe the dark areas to be elevated when he himself states: ". . . the migration of portions of some maria . . . implies very gradual slopes". One can see that the nature of the topography of Mars is for the present of a highly speculative character and will remain so until the advent of orbiting reconnaissance probes.

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The Radio Source Cassiopeia *A*

The high-resolution observations of the radio source Cassiopeia *A* by Ryle, Elsmore and Neville[1] show for the first time details of the structure of the source that suggest an interpretation of a feature of the nebulosity connected with the source the significance of which was not clear. The nebulosity is essentially a circular shell with an outer radius of 2·0 min of arc, but it shows a flare in position angle 70° that extends to a distance of about 3·8 min of arc from the centre[2]. The long streaks of nebulosity plotted by Ryle *et al.* at the east side of the source (α = 23h 21m 21s; δ = + 58° 33·5′ and α = 23h 21m 31s; δ = + 58° 33·1′) are the brightest features of the flare. They and the fainter details of the flare extend radially while the features of the shell show no strongly preferential direction.

The diffuse fast-moving condensations that form the shell are all inside of the shell shown by the source. The only filament plotted by Ryle *et al.* on the outer edge of the radio shell (α = 23h 21m 0s; δ = + 58° 31·1′) does not belong to the expanding shell, but is one of the semi-stationary filaments. The only part of the expanding nebulosity that extends beyond the radio shell is the flare; its outward motion is large, as one would expect, but could not be measured because all details are quite diffuse and change their shape rapidly. Relatively small negative radial velocities show that the flare moves in a direction at an angle of about 80° from the direction of vision, almost in the plane of the sky.

The contour map of the source shows a gap in the radio shell exactly coincident with the flare. If the shell structure of the source results from the compression of the surrounding interstellar medium by the expanding material, both the gap and the flare can be interpreted as due to locally very low interstellar density. If the material ejected in the direction of the flare encounters very low density only, compression will be negligible and the ejected material will move outward with negligible deceleration.

Two models are to be considered for the interpretation of the nebulosity: (*a*) one shell was ejected with high velocity which still persists almost unchanged in the flare while the rest of the nebulosity has been slowed down to its present velocity of expansion; (*b*) two shells with different velocities were ejected. The flare is a surviving part of a shell of material ejected with high velocity. In all other directions, this shell has encountered interstellar material of higher density, has compressed it and thus has formed the radio shell. A second shell of material ejected with lower velocity has followed the fast shell which has swept up the interstellar material so that the slow shell moves without deceleration. The circular shell of the nebulosity is the result of the slow ejection; its velocity of expansion has remained essentially unchanged.

Model (*a*) requires that the circular nebulosity has been slowed down considerably. In that case the age of 256 years estimated on the assumption of constant velocity of expansion[2] is too high. To explore the size of the error, we assume that the velocity v_s of the decelerated shell of radius r_s decreases as:

$$v_s = v_0/(1 + br_s^n) \qquad (1)$$

Integration gives:

$$v_0 t = r_s + br_s^{n+1}/(n + 1) \qquad (2)$$

The original velocity, still shown by the flare, is v_0. If r_f is the radial extent of the flare, $T = r_f/v_0$ is the true age; $\tau = r_s/v_s$ is the age determined on the assumption of expansion with constant velocity. Elimination of b from equations (1) and (2) leads to:

$$\tau = T[(n + 1) - nr_s/r_f] \qquad (3)$$

For the simple model of a shell slowed down by sweeping up of interstellar material of constant density with conservation of momentum[3,4], n equals 3 and the true age required by model (*a*) is then 110 years. The distance of 3,400 p.s.c., determined from the present velocity of expansion and transversal motion of the circular nebulosity, remains unchanged and stays in agreement with the observations of the 21 cm-absorption lines. The velocity of expansion of the flare, assumed to be the original velocity of all ejected matter, becomes 34,000 km/sec. A velocity of this order has not been observed in any supernova; it seems excessive and makes the one-shell model (*a*) unattractive.

The two-shell model (*b*) leads to a more acceptable interpretation. It admits constant velocity of expansion of the circular shell. The age of 256 years remains valid, the velocity of expansion of the slow shell is 7,400 km/sec and the velocity of expansion of the fast shell becomes 14,500 km/sec, comparable to the velocity of expansion of 12,000 km/sec observed in the supernova in *NGC* 4303 (1961) (ref. 5).

This supernova is an unusual object. It is clearly not a supernova of type I. It has some similarities to supernova

of type II, but differs so much from typical supernovae of that type that Zwicky[5] suggests that it is a supernova of a new type III. Its early spectrum is continuous and blue, as are the spectra of type II supernovae, but it remains in this phase for several weeks, much longer than supernovae of type II. The decline of the light from the maximum is much slower than in supernovae of type II, and the levelling-off of the light curve, shown by most supernovae of type II, occurs much later and after a much greater decline. The line spectrum that eventually emerges is similar to that of supernovae of type II, except for the much larger velocity of ejection. The slow initial changes of light and spectrum suggest that the ejected mass is much larger than that ejected by supernovae of type II. No information is available on the detailed structure of the emission lines; it is thus not possible to judge whether more than one shell was ejected. In some supernovae of type II, multiple absorption lines show the presence of two or three shells.

The information now available on the structure of the source and of the nebulosity seems to be consistent with the interpretation that the two-shell model (*b*) is acceptable and that Cassiopeia *A* is the remnant of an unusual supernova similar to the supernova in *NGC* 4303 (1961) rather than the remnant of a typical supernova of type II.

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COSMOLOGY

Why is the Sky Dark at Night?

Bondi[1] has pointed out that with certain very general assumptions, notably the cosmological principle, it can be inferred from the observed darkness of the night sky that the universe is either young or expanding. Harrison[2] has discussed the same question from a somewhat different point of view. He considered evolving world models populated by stars of fixed luminosity and finite age and concluded that "the night sky is dark simply because the stars are so widely separated from each other . . .; . . . the radiation level is affected only in a comparatively slight way by whether the universe is expanding or contracting . . . ". Although Harrison's argument is mathematically correct, his models are somewhat unrealistic. The present communication shows that the darkness of the night sky actually has a number of distinct causes, among which the one given by Harrison may not be especially important.

It can be shown that the density of radiation in a uniform and uniformly expanding universe of zero spatial curvature is given in the grey-body approximation (absorption coefficient independent of frequency) by the formula

$$E_0 = \int_{t_i}^{t_0} [S(t)/S_0]^4 \exp\left[-\int_t^{t_0} \frac{du}{\tau(u)}\right] j(t)dt + E_i\left(\frac{S_i}{S_0}\right)^4 \qquad (1)$$

where E_0 represents the mean energy density of radiation, $S(t)$ is the cosmic scale factor, $j(t)$ is the volume emission coefficient (integrated over frequency) and τ is the inverse absorption probability per unit time, assumed to be independent of frequency. The suffixes 0 and i refer respectively to the present epoch and to an arbitrary 'initial' epoch. The integral in equation (1) has the following interpretation. In the absence of absorption, the density of radiation emitted during the time interval $(t, t + dt)$ would have been reduced at time t_0 by the factor $(S/S_0)^4$, owing to dilution resulting from the cosmic expansion and to the redshift. The exponential factor represents the probability that a photon emitted at time t will survive until time t_0. The second term on the right side of (1) comes from the initial radiation field.

In a static universe, S, j, and τ are all constant, and we have:

$$E_0^* \equiv E_0 - E_i = j_0\tau(1 - e^{-T/\tau}),\ T = t_0 - t_i \qquad (2)$$

When $T \ll \tau$, $E_0 = jT$. When $T \gg \tau$, $E_0 = j\tau$, which is Olbers's result, since in a universe populated by stars, $\tau = (n\sigma c)^{-1}$, where n is the number density of stars and σ is the geometrical cross-section of a star; thus in this case $E_0 = E_s$, the radiant energy density at the surface of a star.

In a steady-state universe $t_i = -\infty$, $S_i = 0$, j and τ are constants. $S(t) = \exp(t/T)$, and we may set $t_0 = 0$. We then have:

$$E_0 = j_0\left[\frac{4}{T} + \frac{1}{\tau}\right]^{-1} \qquad (3)$$

If $T \ll \tau$, $E_0 = 1/4\ jT$. If $T \gg \tau$, we again recover Olbers's result, $E_0 = j\tau$.

Finally, in an evolving universe, $t_i = 0$, $S \sim t^{2/3}$ as $t \to 0$, and $E_iS_i^4 \to$ constant as $t_i \to 0$. In the model discussed by Harrison[2], $j \sim n \sim S^{-3}$ and $\tau \sim n^{-1} \sim S^3$. Since S is an increasing function of t, it is clear that under these assumptions the major contribution to the integral in (1) comes from values of t not very different from t_0. Thus, as Harrison emphasized, this case is qualitatively similar to the static case. But the assumption that the ratio of luminosity to mass is constant in an evolving universe is highly artificial. Both the lifetime and the mass-to-luminosity ratio of a star decrease rapidly with increasing mass. As we go backward in time, we should accordingly expect to find an ever-increasing proportion of high-luminosity stars and hence an ever-increasing value of the quantity (jS^3). If this quantity were to increase faster with decreasing t than $S^{-1}(t)$, the qualitative similarity between the evolving case and the static case would be destroyed.

For sufficiently small values of t, a nearly uniform distribution of fully or partially ionized gas must prevail. The dominant contribution to the emission coefficient j will then be from free–free emission, so that $j \sim n^2 \sim S^6$. This emission has been studied in detail by Miss Kaufman[3]. At radio wave-lengths it contributes much more to the background sky brightness than does stellar radiation. It can easily be found that the contribution to the background radiation density from free–free emission is given in the grey-body approximation by:

$$E_0\ (\text{free–free}) \doteq \Gamma\left(\tfrac{1}{3}\right)(S_1/S_0)^4 j_1\tau_1 \qquad (4)$$

where the suffix 1 refers to the epoch at which the characteristic absorption time τ is just equal to the age of the universe:

$$\tau_1 \equiv \tau(t_1) = t_1 \qquad (5)$$

Formula (4) shows that the brightness of the night sky is finite for the reason given by Olbers: the radiating material is opaque, whence the factor $j_1\tau_1$. The night sky is dark because of the expansion, which is responsible for the factor $(S_1/S_0)^4$.

To sum up: the intensity of the background radiation field is limited by quite distinct physical effects in the three cosmologies considered here. In a static universe it is limited (under present conditions) by the finite lifetime of the stars. In a steady-state universe the darkness of the night sky is a direct consequence of the cosmic expansion. In an evolving universe, the situation is more complex. The stellar contribution to the background radiation field is limited in part by the finite lifetimes of stars and in part by the cosmic expansion. At radio wave-lengths, free–free emission at an early stage in the cosmic expansion may be the most important contributor.

The intensity of this radiation is limited partly by opacity and partly by the cosmic expansion. In addition, a vestigial radiation field, represented by the second term on the right side of (1), may be present. Arguments in favour of such a field have appeared in the recent literature.

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GEOPHYSICS

Collision-induced Absorption in the Earth's Atmosphere

MOST of the absorption which occurs in the atmosphere in the infra-red region is due to the minor constituents such as water-vapour, carbon dioxide and ozone. The major constituents oxygen and nitrogen possess no dipole moment so that their vibrational and rotational energy states cannot be excited directly by absorption of radiation. However, dipole moments are induced during collision processes; transitions due to these can be seen in very long absorbing paths such as are present in the atmosphere. This report shows that absorption in the solar spectrum near 4·2μ is due to collision-induced absorption by nitrogen, and presents a short discussion of the importance of the rotational collision-induced bands of oxygen and nitrogen in the radiation budget of the atmosphere.

Williamson and Houghton[1] considered collision-induced absorption in the atmosphere and showed that, providing temperature variation of the absorption coefficient can be neglected, the transmission τ of a slant path at zenith angle θ extending from the top of the atmosphere down to the level where the pressure is p atmospheres (1 atmosphere = 10^6 dyne cm^{-2}) is:

$$\tau = \exp\left(\frac{-kcp^2H \sec\theta}{2}\right)$$

where k is the absorption coefficient expressed in cm^{-1} (atm.)$^{-2}$, c is the volume fraction of the absorbing gas present in the atmosphere (0·8 for nitrogen), and H is the atmospheric scale height ($\simeq$ 8 km). The absorption due to collision-induced processes is not only dependent on the amount of absorber in the path, but also on the frequency of collisions; the p^2 term in the exponent arises from these two factors.

The fundamental vibration frequency of nitrogen lies at 2,331 cm^{-1} so that the centre of the band is completely obscured by the ν_3 band of carbon dioxide centred at 2,349 cm^{-1}. The carbon dioxide band terminates on its short wave-length side in the band head at 2,397 cm^{-1} and it is therefore possible that, if the nitrogen band is sufficiently broad, absorption due to it will be seen at a higher frequency than 2,397 cm^{-1}. The solar observations summarized by Farmer and Todd[2] and the horizontal path measurements of Berry, Farmer and Lloyd[3] show that there is some absorption there which has not been accounted for. In order to test whether in fact this is the case, some measurements on synthetic nitrogen and carbon dioxide atmospheres have been carried out in the laboratory using a 15 m multitraversal sample cell in which path lengths of up to 1 km can be obtained.

Fig. 1 shows a laboratory spectrum for 720 atm. cm of nitrogen containing a small amount of carbon dioxide (0·5 atm. cm). The spectrum was taken at a total pressure of 1 atm. The S-branch of the nitrogen collision-induced band can be clearly seen on the high-frequency side of the carbon dioxide band. From a series of measurements made on nitrogen at pressures up to 2 atm. the transmittance τ of a path of length l was shown to be of the form:

$$\tau = \exp(-kp^2l)$$

which is consistent with the 'pressure squared' behaviour for collision-induced absorption. The absorption coefficients determined from these measurements are in good agreement with the values obtained recently by Gush[4] and by Reddy and Cho[5] at higher pressures. Using the coefficients obtained in this way, spectra were computed for the conditions of observations which have been made in the Earth's atmosphere. Fig. 2 shows solar spectra taken from sea-level (curve A) and from 5·2 km altitude

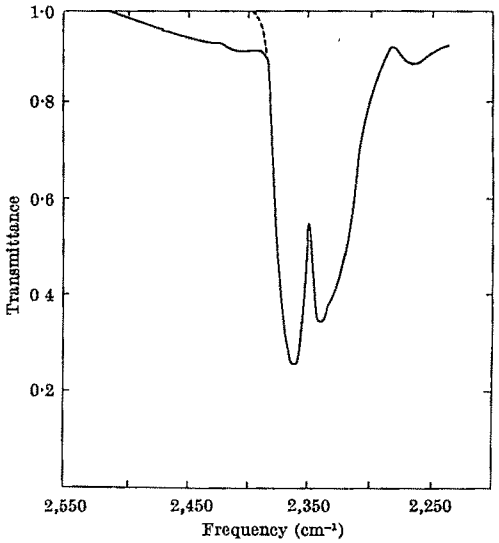


Fig. 1. Laboratory spectrum of nitrogen with carbon dioxide impurity. Nitrogen, 720 atm. cm; carbon dioxide, 0·5 atm. cm; total pressure 1 atm.

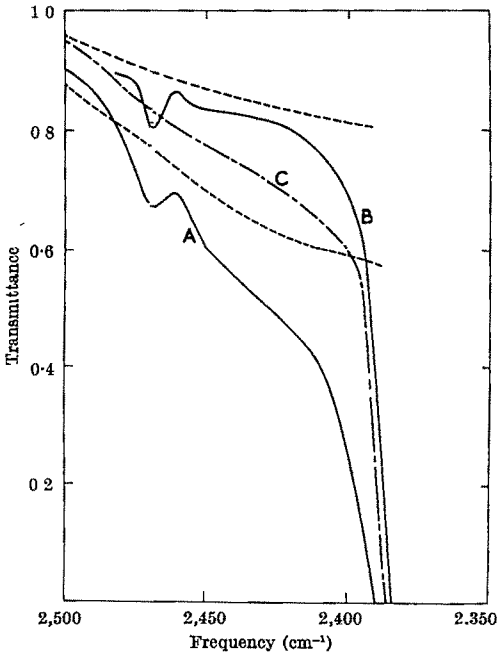


Fig. 2. Solar and horizontal path spectra on the high-frequency side of the ν_3-carbon dioxide band. A, Solar spectrum from sea-level, $\theta = 46°$; B, solar spectrum from 5·2 km altitude, $\theta = 11°$; C, 8·4 km horizontal path at 5·2 km altitude

(curve B) ($p \sim 0{\cdot}5$), together with a horizontal path spectrum at 5·2-km altitude (curve C) measured over a path of 8 km length (that is, a path containing the same number of molecules as a zenith path from the same base altitude). It can be seen that there is a significant difference in absorption from 2,400 to 2,500 cm^{-1} when all the nitrogen present in the atmospheric path is at the base altitude pressure (comparing curves A and C). Furthermore, the increase in the distortion of the shape of the solar spectrum produced by the nitrogen, as the base altitude is lowered, is also evident (comparing curves A and B). The dotted lines indicate the computed contribution to absorption by nitrogen under the conditions of the two solar spectra.

The other collision-induced bands which occur in the atmosphere are the fundamental vibrational band of oxygen at 1,556 cm^{-1}, and the rotational bands of oxygen and nitrogen which extend downwards in frequency from about 300 cm^{-1} with their centres at about 100 cm^{-1}. The vibrational band of oxygen was observed by Crawford, Welsh and Locke[6]. It is completely obscured by the ν_2 band of water-vapour. It contributes very slightly to radiative exchange in the upper troposphere (see ref. 1).

The rotational bands have been observed by a number of workers, most recently by Bosomworth and Gush[7]. At the wave-length of the atmospheric window at 29 cm^{-1} (see ref. 8), they account for 22 per cent absorption in a vertical path of atmosphere from sea-level; this is almost entirely due to nitrogen. Otherwise, so far as the lower atmosphere is concerned, they are completely obscured by the rotational band of water-vapour. If the water-vapour content higher in the troposphere is very low, these rotational bands can have a small effect on the radiative budget, adding to the radiative cooling there by up to 0·1° C day^{-1} at 500 mbars and 0·03° C day^{-1} at 200 mbars.

We thank Mr. T. M. McCormick and Mr. J. E. A. Selby (E.M.I. Electronics, Ltd.) for assistance with the measurements and computations described here.

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Correlation of Dust and Ozone in the Stratosphere

The vertical distribution of dust and ozone was measured on a series of five balloon flights over Minneapolis, Minnesota. The ozone detector was of the chemiluminescent variety, while the dust detector was a modified version of the one described previously by Rosen[1]. Both detectors were flown on the same balloon but were electrically and mechanically separated.

The results show that above the dust maximum (5 km above the tropopause) there is apparently no appreciable correlation of dust and ozone; that is, the ozone profile can have considerable structure, but the dust profile is always relatively smooth. A thin dust layer of significant concentration was never observed in this region. Pittock[2] has described a persistent dip on the ozone concentration at 21 km and suggested that it was due to the destruction of ozone by a thin volcanic dust cloud. Such an explanation seems rather unlikely in view of experimental evidence.

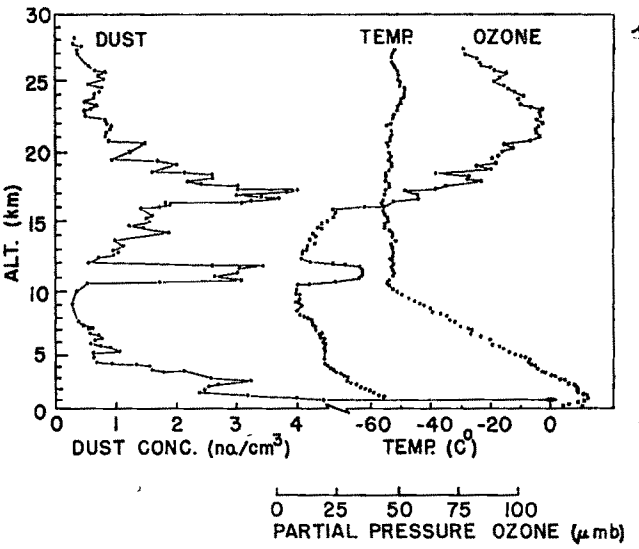


Fig. 1. The dust and ozone distribution over Minneapolis, Minnesota, on December 22, 1965. The dust concentration refers to particles larger than 0·25μ in diameter

The region between the dust maximum and the tropopause is characterized by layers rich in both dust and ozone. An example of this phenomenon is shown in Fig. 1. These layers were named ozone rivers by Kroening and Ney[3] before it was known that they also contain dust. The life of these rivers is probably much too short for the dust to have any noticeable effect on the ozone concentration.

I thank John Kroening for use of his ozone data before publication. This work was supported by the Office of Naval Research.

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PHYSICS

Airborne Observations of Natural Radioactivity

Under the above title Warburton, Fookes and Watt[1] have described some measurements performed in June 1959 in Australia with a scintillation detector at altitudes up to 3·6 km. The day-to-day variations found by the authors "were surprisingly large and were found to occur mainly in the atmosphere between the ground and temperature inversion layers". They concluded that it was probably mainly radon and its decay products which were detected and attributed their γ-counting to the γ-rays from lead-214 and bismuth-214, estimating that a record of 8 c/s corresponds to 10^{-17} c./cm^3 of radon of a uniform concentration.

I should like to direct attention to the following two points: (1) the reaction of the probe by Warburton *et al.* on cosmic rays; (2) the results of the direct determination of the radon content of free atmosphere at different altitudes made as early as 1928 by Wigand and Wenk[2]. In fact the curves produced by Warburton *et al.* for the count rate versus altitude have an almost identical course from 2·5 km upwards which agrees fairly well with the assumption that at these heights the probe used registers mainly the cosmic rays. This conclusion is corroborated by the

results of Wigand and Wenk who found the following range of values in a series of airplane measurements:

Altitude (km)	Radon content (10^{-16} c./cm^3)
0·35–1·5	4·28–28
2·0 –2·3	0–24

The regular course of the curves reproduced by Warburton *et al.*[1] from 2·5 km upwards is in agreement with Wigand and Wenk's finding that practically no radon exists above 2·5 km. With regard to their short life-time, it is unlikely that lead-214 and bismuth-214 could ascend from lower altitudes to those explored by Warburton *et al.* and be detected by scintillation counter.

The large variations in counting at lower altitudes, and mainly under the temperature inversion layers, agree with the findings of various authors before the Second World War, that at such atmospheric conditions the radon content varies erratically and sometimes presents exceptionally high values near the inversion layers[3].

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On the Combination of an Electron Spin Resonance Spectrometer and a Multichannel Analyser

In a recent communication, Kent and Mallard[1] pointed out that the versatility of an electron spin resonance-spectrometer can be considerably improved by combining it with an analogue digital converter and a commercial multichannel analyser as they are commonly used in nuclear spectroscopy. We have used a similar piece of equipment, and have already presented a preliminary report[2]. The basic technique was introduced by Klein and Barton[3]. It should be noted that any real improvement is based on the fact that the noise seen in electron spin resonance-spectra is not 'white noise'. That is to say that the probability for a certain frequency to occur in the noise-spectrum is not equal for all frequencies, but is enhanced for lower values[3]. It is therefore clear that no higher sensitivity can be achieved if the time constant of the amplifier and the recording time are enhanced above a certain limit being about 100 sec for the time-constant[4]. The sensitivity is restricted, for example, by instabilities of the whole equipment. This applies to relatively long times of measurement. If the recording time is short, a sampling procedure as described by Kent and Mallard[1] is not expected to bring any improvement if the time-constant is correctly adjusted. Thus the subdivision of one measurement into ten single ones appears to offer no advantage and the same result may be obtained by increasing the apparatus time constant or by repeating ten times the recording of the whole spectrum at a higher speed. The basic law of communication theory, namely, that any improvement in signal-to-noise ratio requires an increase in time of measurement, cannot be overcome by any of these methods.

We have used an '*AEG*'-electron spin resonance-spectrometer (12-cm magnet) in combination with a self-designed analogue-digital converter and a commercial 'Intertechnique'-400-channel analyser. In order to ensure correct superposition of the spectra, the start of each measuring cycle is triggered by a signal from a nuclear magnetic resonance magnetic-field meter, shaped by a special circuit, to the flow-time input of the analyser. Thus it was possible to superpose more than 1,000 single spectra yielding an improvement in signal-to-noise ratio by a factor of more than 30.

In preliminary experiments we were able to detect 10^{-7} M Mn^{2+} in aqueous solution. A more detailed account of our device and the measurements carried out will be published elsewhere.

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We agree in principle with the conclusion of Kiefer and Neubacher on the multi-sampling method. However, this was not the main point of our communication—this sort of work has been introduced before by Klein and Barton; our interest lay mainly in the other versatile features that we described. We have, however, some comment to make, following this note by Kiefer and Neubacher. They say that the same result (as the multi-sampling method) is obtained by (i) increasing the apparatus time constant, or by (ii) repeating 10 times the recording of the whole spectrum at a higher speed (with single samples per channel, and, we presume, with a shorter time constant).

We assume by (i) that they also mean that the duration of the sweep should be increased as the time constant has already been selected as optimum for the spectrum involved and any increase in the time constant, without a proportionate increase in sweep period, would result in distortion of the spectrum. The advantage of this would then appear to be obscure since to achieve the same result one would need to take a longer time.

Statement (ii) appears to be merely a reiteration of our statement that N sweeps of a spectrum, with 10 samples of signal and noise per channel per sweep, represent $10N$ faster sweeps. We admit our error in failing to add the important point that we meant 'faster sweeps'. It has been our experience that, whenever the sensitivity of instruments has been in dispute, the contenders frequently fall into the trap of failing to state all the conditions under which the sensitivity was measured, such as time constant, sweep speed, and so on. It would appear that we also have fallen into this trap on this occasion.

We found on our machine that, as we stated in our original communication, interference from 50 c/s ripple coherent with the analyser channel periods tended to obscure information so that, if we performed $10N$ fast sweeps, the 50 c/s ripple would be accentuated $10N$ times, whereas by performing N slower sweeps with 10 multi-samples, this does not occur. We would, however, hasten to add that the multi-sampling method has no advantage over single-sampling methods when enhancement of signal-to-noise is related to the result of just one sweep performed in the same time at the same time constant. We hope that we have now made this point clearer.

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Asymmetric Nuclear Fission and the Liquid Drop Model

In a previous article[1] we directed attention to the resemblance between curves representing the division of electrically charged liquid drops and mass yield curves for nuclear fission.

More recently, we have become aware of fission yield data which further illustrate this resemblance. The two sets of curves in Fig. 1 are a photograph of original drawings. We reproduce, on the left, the fission yield obtained by Gibson[2] for fission of plutonium-239 induced by bombardment with deuterons of various energies, and, on the right, Fig. 5 of our previous paper[1]. In the latter we had plotted the fractional energy change, E_f, versus the fractional volume change, V_f, for the division of a surface-

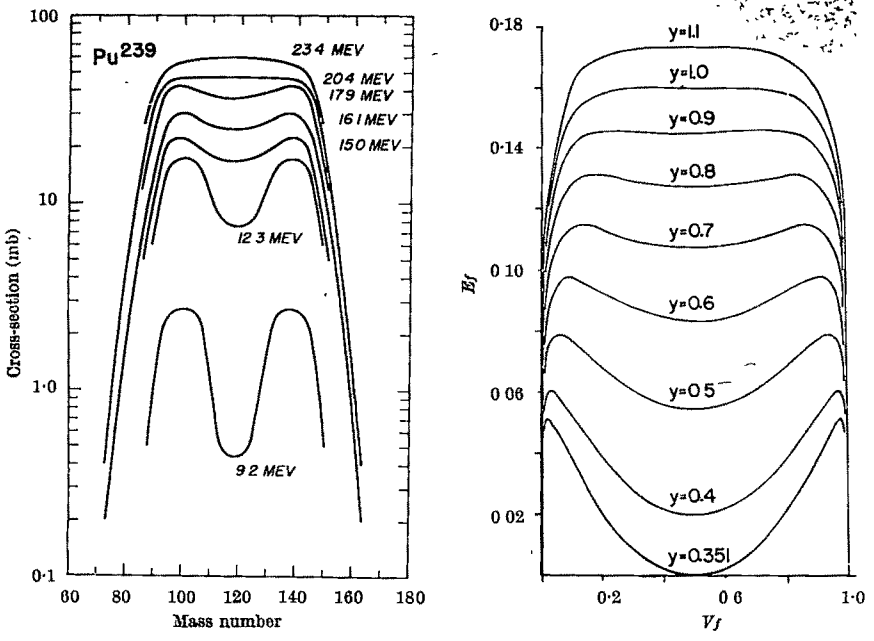


Fig. 1. On the left, fission yield for fission of plutonium-239 induced by bombardment with deuterons of various energies, on the right, the fractional energy change, E_f, plotted against the fractional volume change, V_f, for the division of a surface-charged drop

charged drop. A V_f value of 0·5 corresponds to symmetric division. The parameter y is defined as:

$$y = Q^2/16\pi R^3\gamma$$

where Q is the charge on the drop, R the radius and γ the surface tension.

It is remarkable that curves based on energy considerations in the division of a surface-charged drop resemble the experimental fission yield curves so closely in shape and form for 'y' values between 0·7–1·1, and deuteron energies between 15·0–23·4 MeV, respectively. In making this comparison we assume a relation between the ordinates (energy and cross-section) and between the abscissae (drop volume and mass number).

The traditional liquid drop model considers the nucleus as a drop of incompressible fluid with a spherical shape at equilibrium and with a uniform and constant volume charge. The process of fission is thought of as a competition between the long-range Coulomb forces throughout the volume of the drop and surface forces representing the effect of non-saturation of the short-range attractive forces at the nuclear boundary. Quantum mechanical effects, such as tunnelling and shell structure, are not a part of this model.

It has become customary in the liquid drop model to introduce a fissionability parameter x which is defined as:

$$x = 3(Ze)^2/40\pi R^3\theta$$

where Ze is the nuclear charge and θ the nuclear surface tension. Thus the parameter x for the volume-charged drop is analogous to the parameter y, already noted, for the surface-charged drop.

Central to the following argument is the difference in behaviour between a volume-charged drop of fixed-charge density and a surface-charged drop for which instantaneous charge redistribution is allowed.

A simple approach to binary division is made by considering the final Coulomb and surface energy of the system. It has been shown[1,3] that for a volume-charged drop, independent of the value of x, the final energy will be a minimum when the drops formed are of equal radius.

As the minimum final energy of the system predicts symmetry, attempts have been made to explain the asymmetry of nuclear fission by a computation of the energies of the saddle shapes which are intermediates in the fissioning process. The usual method is to represent small deformations of the drop by an expansion of its radius, R, in terms of Legendre polynomials. Frankel and Metropolis[4] computed symmetrical deformations on the 'Eniac', with expansion of R up to and including the tenth Legendre polynomials. The effect of the introduction of asymmetric terms in their calculation was that the energy increased, and they concluded that for $x \geqslant 0{\cdot}65$ the saddle configuration is a purely symmetric deformation. More recently, Cohen and Swiatecki[5] found the saddle shapes stable against asymmetry down to $x = 0{\cdot}394$.

It appears, therefore, unlikely that the saddle shape energies will provide an explanation of asymmetry on the basis of the present volume-charged drop model. However, from the point of view of our argument, the consideration of the saddle shapes as such has an additional significance.

Suppose there is for some value of 'x' an asymmetric shape at the saddle point. Such a shape must be the precursor for the asymmetry of the fragments. Implicit in this supposition is the necessary conclusion that in its final state the system is not at the energy minimum, the reason being that for a volume-charged drop, as already pointed out, drops of equal radius correspond to the energy minimum for a binary division.

We return now to the division of a surface-charged drop. In this case a pair of asymmetric droplets[1] represents the lowest final energy state for any value of $y < 1$. Furthermore, the experimental evidence (Fig. 2, ref. 1) shows that the degree of asymmetry of the saddle shapes is approximately the same as that of the final droplet pair. In this instance, it is not a necessary conclusion that the system cannot attain the lowest final energy state.

At the point of separation during the division of a surface-charged drop, the two droplets are connected by a thin neck (Fig. 3, ref. 1), and are at the same electrical potential (ref. 1). On the other hand, the division of a volume-charged drop leads to the formation of two droplets which have the same charge to mass ratio whatever their relative size. Hence, for volume charge, only droplets of the same size have the same potential.

It is suggested that a liquid drop model of the nucleus in which charge redistribution is admitted will account for the asymmetry of nuclear fission as such. Furthermore, this model predicts the change of asymmetry as a function of the energy of the bombarding particle. The degree and mechanism of charge redistribution are matters for further investigation.

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Superiority of Atomic Mass per Nucleon as a Parameter for Nuclides rather than the Conventional Packing Fraction

The results of the calculations undertaken by Prof. Durnford[1] are very interesting, and enable him to claim "uniqueness of the mass-surface relative to iron-56".

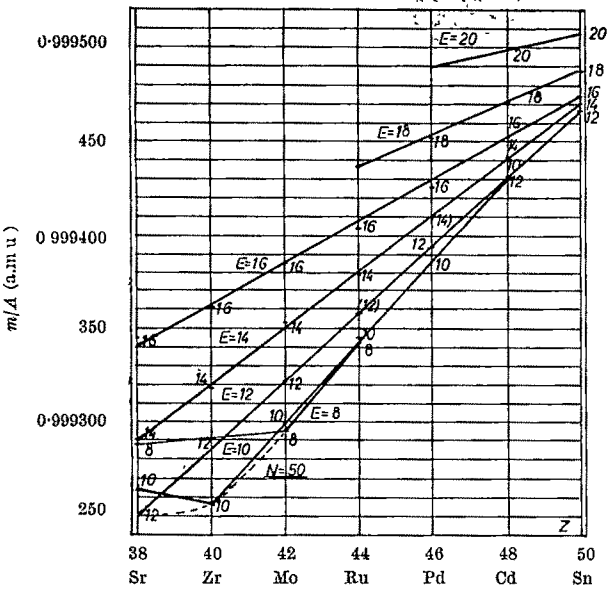


Fig. 1. $Z=38 \cdots N=50 \cdots Z=50$ Excess number $E=N-Z=A-2Z$

Actually, what Durnford has brought to light is the ambiguity of the packing fraction as a parameter. This ambiguity can be avoided simply by using the atomic mass per nucleon (m/A) as the parameter for nuclides instead of the packing fraction, thereby freeing the calculations from any dependence on particular standards of atomic masses. Incidentally, $^{56}_{26}$Fe competes with $^{62}_{28}$Ni for the minimum mean nucleon mass value[2].

The superiority of the mean nucleon mass as a parameter is especially emphasized by plotting (m/A) for a nuclide against its atomic number Z and denoting each point by its neutron excess number $E = N - Z = A - 2Z$ (ref. 3). Points of equal E then form straight or 'isoexcess' lines, which break off and start again with new slopes at magic numbers in N and Z. The magic numbers occurring here are first of all those discussed fully by Mayer and Jensen[4], and a set of as many again, which could be called sub-magic numbers, and which are easily fitted into the Mayer-Jensen shell structure.

The plotted points fall on the isoexcess lines with an accuracy of within 2×10^{-6} atomic mass units per nucleon —this being far more accurate than that of any mass formula so far available[5].

Since the aforementioned system of atomic masses was introduced[3], it has been used to correct and predict the atomic masses of a number of nuclides to the mentioned accuracy.

A sample plot of isoexcess lines appears in Fig. 1.

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Electric Breakdown in Non-virgin Gases

ALTHOUGH the mechanism of the electric breakdown of atomic and molecular gases at low pressure is well understood[1], most investigations have been confined to gases in the virgin state—that is, when the atoms or molecules are in potential energy states corresponding to the ambient temperature and pressure. However, the collision processes involved are substantially altered if the gas contains species with large potential energy, such as excited atoms or molecules at room temperature.

Recent observations on nitrogen show that the breakdown potential is reduced by $\leqslant 10$ per cent when a small number of excited molecules of nitrogen (N_2*) is added to the virgin gas at pressures of about 1–10 mm mercury. To avoid contamination, a long glass tube 4 cm in diameter is filled with spectroscopically pure nitrogen and the breakdown potential at 15 Mc/s is measured with external electrodes at one end of the tube while at the other end, at a distance of up to 60 cm, N_2* with a long free-life (order 1 sec) is produced by an independent auxiliary discharge. By a series of experiments[2], other factors which could be responsible for the reduction in breakdown potential have been excluded such as impurities, pick-up, diffusing electric charges, light and thermal changes. The reduction observed depends on the strength of the auxiliary discharge, its distance from the region measured, and on the pressure. A similar effect has been observed in exploratory experiments in hydrogen.

The presence of active molecular species carrying potential energy of several eV has been demonstrated by a new detection method: N_2* interacting with a metal oxide can reduce its order by one. For example, the white-yellowish trioxide of molybdenum is changed to the lead-grey dioxide. This and other metal oxides, such as vanadium pentoxide and tungsten trioxide, were exposed to N_2* diffusing from the auxiliary discharge. The time was recorded after which a clearly observable colour change occurred when the powdered oxides are placed at different distances from the auxiliary discharge. These experiments confirm that potential energy carriers diffuse relatively slowly down the tube. From the chemical energy balance and by assuming that the colour change corresponds to a certain thickness of the de-oxidized layer, a lower value of the concentration of excited particles can be estimated. This is compared with the concentration necessary to account for the reduction in breakdown potential by pre-excitation. The de-activation of N_2* by spontaneous radiation is strongly forbidden and that by collision with nitrogen molecules is a rare process[3]. Since N_2* is produced in a relatively weak discharge, nitrogen atoms can be taken to be absent[4].

It is concluded that the reduction in breakdown field is due to the presence of a distribution of vibrationally excited molecules in the ground state or in a higher electronic state[5]. These molecules possess virtually lower ionization potentials than nitrogen in the ground state, possibly with larger ionization cross-sections. Also in nitrogen a very small fraction of the electrons have energies above ionization energy and the population of excited molecules produced before breakdown is small, whereas in a pre-excited gas their population is considerably increased so that a large fraction of electrons previously unable to ionize can now contribute to ionization by collision.

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CHEMISTRY

Effect of Arsenic Pressure on Dislocation Densities in Melt-grown Gallium Arsenide

In the course of an investigation into the growth of gallium arsenide crystals in a horizontal Bridgman apparatus, it was found that the dislocation density at the centre of the cross-section of ingots increased with increasing arsenic vapour pressure in the ampoule during growth. The crystals were grown by moving the charge contained in a sand-blasted silica boat through a temperature gradient of about 10° C cm^{-1} at a rate of 0·5 cm h^{-1} (ref. 1). No seed crystal was used. Using the data of Honig[2], the arsenic pressure was estimated from the temperature of a thermocouple at the coolest point of the sealed growth ampoule where the excess arsenic condensed. Dislocations were detected by etching *A* faces of (111) oriented slices with a mixture of two volumes of water, one volume of nitric acid and one volume of hydrochloric acid after grinding with 15μ alumina and polishing with a mixture of two volumes of water, three volumes of nitric acid and one volume of hydrofluoric acid.

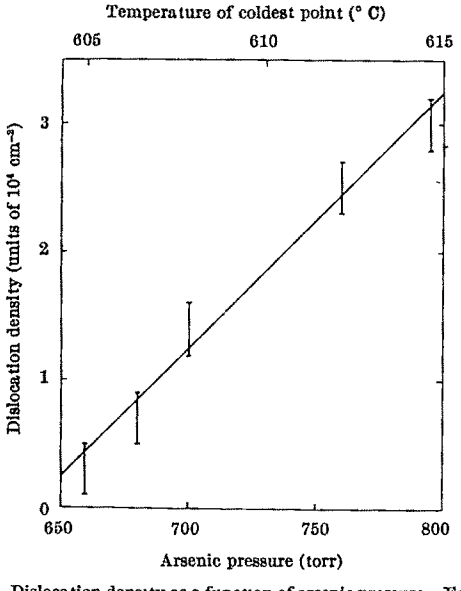


Fig. 1. Dislocation density as a function of arsenic pressure. Each point is the mean of five observations

The results obtained are shown in Fig. 1 and suggest that vacancy condensation to form dislocation loops[3,4] is probably one mechanism responsible for the introduction of dislocations into melt grown gallium arsenide. Work on the phase diagram of the system[5–8] suggests that material with maximum melting point is in equilibrium with about 720-torr pressure of arsenic. In accord with this, when pressures less than 650 torr were used, traces of constitutional supercooling were seen, suggesting the presence of excess gallium in the melt. At pressures greater than 800 torr, blow holes formed, as would be expected if the melt contained excess arsenic. The monotonic increase of dislocation density is difficult to account for in terms of strains due to residual constitutional supercooling. However, if a vacancy mechanism is responsible, the use of the Schottky product shows that minimum total concentration of vacancies would occur at the stoichiometric composition. Since the dislocation densities shown in Fig. 1 increase monotonically with pressure, it seems either that gallium vacancies alone are important in the formation of dislocations or that the compound is arsenic rich near its melting point so that gallium vacancies predominate, as is known to be the case at 1,000° C where the solid extends from 49·998 to at least 50·009 atom per cent arsenic[9].

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Chemical Nature of Free Retine, an Anti-tumour Agent

The wide distribution of natural anti-tumour agents has been shown by Szent-Gyorgyi *et al.*[1] and many others. There is, notably, such a compound in human urine, which has been called retine and has been previously isolated by Hegyeli *et al.*[2]. This compound has a molecular weight of approximately 430 (ref. 3) and induces a significant regression of Krebs-2 carcinoma in Swiss albino mice. Treatment of retine with hot acetic acid releases a compound which is responsible for the anti-tumour activity[4].

This small molecule, which will be called 'free retine' in this report, is soluble in organic solvents. The ultra-violet spectrum is characterized by a sharp absorption peak at approximately 275 mμ and by its absence of a *B* band. This feature makes it possible to rule out certain structures for free retine. For example, a diazine structure is not permitted, because the corresponding $n \rightarrow \pi^*$ transition generally occurs in the region 300–400 mμ[5]. The infra-red spectrum of free retine in chloroform solution is characterized by a strong band at 1,710 cm^{-1}, suggestive of a carbonyl group. It was thus tentatively assumed that this carbonyl group is a keto group, and a chloroform solution of free retine was treated at low *p*H by 2,4-dinitrophenyl-hydrazine (as suggested by Mr. John Sousa (U.S. Army Natick Laboratories)). Triclinic crystals (point-group I) were thus formed, the crystallographical description of which does not appear to have been published.

An infra-red spectrum of the derivative was recorded (micro-pellets in potassium bromide); it had the general aspect of the spectrum of 2,4-dinitrophenylhydrazine, although it differed significantly. A new band (*X*–*Y* stretch) appeared at 3,362 cm^{-1} and a very broad band at and below 1,645 cm^{-1} was suggestive of a non-ketonic carbonyl group.

The 2,4-dinitrophenylhydrazine derivative was dissolved in deuterated dimethyl sulphoxide and a nuclear magnetic resonance spectrum was recorded. In addition to the peaks of H_3, H_5 and H_6 of the substituent phenyl group, there also appeared an NH peak and a peak corresponding to two protons.

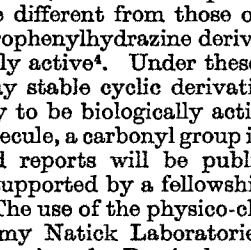
On the other hand, when a chloroformic solution of free retine is shaken with water, no biological activity[4] or any corresponding ultra-violet peak can be detected in the aqueous phase, whatever the *p*H. This rules out the possibility of a conventional ketone and of a keto-enol tautomerism because, at high *p*H, some of the material would dissolve in the water phase. The same is true of a quinone.

These findings were checked by spectroscopy, with the following results: (*a*) The band at 3,362 cm^{-1} which might have been due to a bonded OH does not appear with the 2,4-dinitrophenylhydrazine derivative of tetrahydroxy-1,4-quinone. (There is only a diffuse band centred on 3,420 cm^{-1}.) (*b*) The broad band at and below 1,645 cm^{-1} is not due to an amide group, as it does not appear with

the 2,4-dinitrophenylhydrazone of *o*-acetamide-acetophenone. (*c*) The broad band is not due to a C=N stretch of the addition compound of the reagent with a quinone, as it does not appear with the derivative of tetrahydroxy-1,4-quinone. (*d*) The derivative is not of the hydrazide type as, in that case, two different NH peaks would be distinguished by nuclear magnetic resonance.

In these conditions, the only remaining possibility is that the broad band is due to some other type of oxo-group. Free retine is, however, very volatile: if a chloroform solution of free retine is left at the air and room temperature, or if inert gases are bubbled through such a solution, all the activity disappears.

Therefore, the only compound with which free retine can be identified is carbon suboxide, for: (*a*) The two carbonyl groups are very reactive, as is always the case with ketenes. (*b*) It is a volatile compound (b. pt. 7° C (ref. 6)). (*c*) It is not soluble in water, but decomposes with formation of malonic acid. (*d*) It reacts with hydrazines[7]. With 2,4-dinitrophenylhydrazine it forms 1-(2,4-dinitrophenyl)-3,5-dioxopyrrazolidine:



a compound which obviously satisfies the spectroscopic (infra-red and nuclear magnetic resonance) observations.

It is of interest to recall that carbon suboxide undergoes photolytic reactions, reacts with proteins and that its toxic effects are different from those of malonic acid[8].

The 2,4-dinitrophenylhydrazine derivative of free retine is not biologically active[4]. Under these circumstances, it appears that any stable cyclic derivative of carbon suboxide is unlikely to be biologically active. It seems that in the retine molecule, a carbonyl group is readily available.

More detailed reports will be published later. This work has been supported by a fellowship of the Commonwealth Fund. The use of the physico-chemical equipment of the U.S. Army Natick Laboratories was invaluable, and samples were given by Dr. Andrew Hegyeli (Institute for Muscle Research).

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Structure of a *bis*Glycylglycinate-cobalt(III) Complex

COBALT complexes of glycylglycine are of considerable importance in connexion with the activation of glycylglycine dipeptidase by cobalt ions[1]. Following early work by Smith[2], Gilbert, Otey and Price[3] studied the oxidation of cobaltous glycylglycine complexes and isolated a red complex to which the formula $[(GGH)_2CoO_2Co(GGH)_2]$ was assigned (the neutral glycylglycine molecule is abbreviated as GGH_2). Later studies[4] showed that an intermediate brown complex was formed at high *p*H which was converted to the red complex on lowering the *p*H. A polarographic study[5] found no evidence for a peroxy group in the red complex, it being suggested that the complex contained hydroxy groups as ligands.

We have, in attempting to prepare the red 'irreversible oxygen carrying' complex of Gilbert *et al.*, isolated a number of cobalt complexes with the unsubstituted glycylglycine and with its mono-anion $(GGH)^-$ and di-anion $(GG)^{2-}$. A single crystal X-ray diffraction analysis of the structure of $NH_4.[Co(GG)_2].2H_2O$ has now been completed, the stereochemistry of the complex anion being shown in Fig. 1. Corresponding to a present reliability index of 0·10, the average estimated standard deviations of the bond lengths are Co—N and Co—O 0·02 Å and C—N, C—O and C—C 0·03 Å.

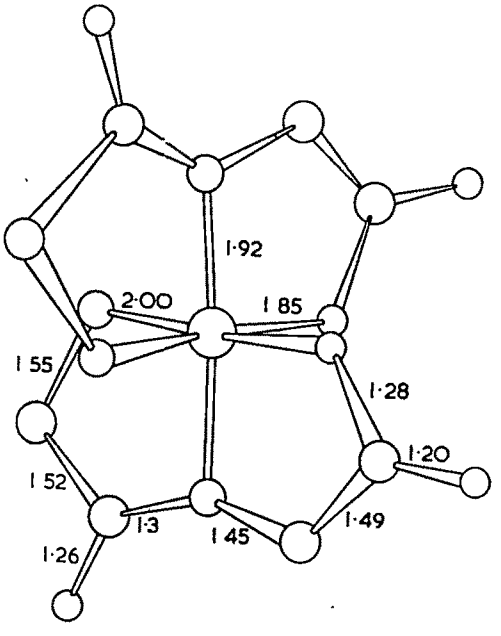


Fig. 1

The complex anion contains two terdentate glycylglycinate ligands arranged so that the co-ordination of the Co(III) ion is a distorted octahedron which has precise C_2 symmetry as a requirement of the crystallographic space group. The bond lengths in the co-ordinated ligands, which are not precisely planar, are very similar to those reported for copper(II) monoglycylglycine trihydrate[6] and β-glycylglycine[7]. As in the copper complex[6], the difference in the metal-nitrogen bond lengths, M—NH_2 and M—N, is significant.

The complex anions are extensively hydrogen bonded throughout the crystal to neighbouring ammonium ions and water molecules. Four N—H···O bonds of length 2·79 Å (twice) and 2·85 Å (twice) connect the ammonium ion with the unco-ordinated oxygens of the glycylglycinate ligand. The unco-ordinated carboxyl oxygen atom is also hydrogen bonded to a water molecule (O···O = 2·77 Å) which, in turn, is bonded to the co-ordinated oxygen (O···O = 2·80 Å) and to the terminal nitrogen atom (O···N = 3·06 Å).

Crystal structure analyses of several of the other complexes isolated are at present in progress. It seems possible that the mode of attachment of the ligand to the metal ion depends critically on the intermolecular hydrogen bonding. As yet, we have no evidence that the cobalt-glycylglycine system is different from that of the well-known cobaltammine complexes so far as the reaction with oxygen is concerned. The oxygen carrying complex appears to be the brown intermediate of Tanford *et al.*[4] which decomposes to a monomeric Co(III) complex.

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Reactivity of Aromatic Compounds towards Hydrogen Atoms

The reactivity of aromatic compounds toward hydrated electrons and hydroxyl radicals has been the subject of various recent investigations[1,2]. The effects of substituents on the reactivities of benzene and benzoate ion were correlated with the substituent constant (σ) of Hammett's equation. It has been shown that the mechanism of attack of hydroxyl radicals on aromatic compounds is analogous to electrophilic aromatic substitution[2]. On the other hand, the reactions of hydrated electrons followed a pattern analogous to nucleophilic substitution. The reactivity of aromatic compounds toward hydrogen atoms was investigated in order to study the nature of the PhX + H reaction.

The specific rate constants of substituted benzenes with hydrogen atoms were determined by competition kinetics, using isopropanol as the competing compound. De-aerated solutions containing 5×10^{-2} M isopropanol, 1×10^{-3} M acetone (as e^-_{aq} scavenger in order to avoid the e^-_{aq} + PhX reactions) and different concentrations of PhX at *p*H 8–9, were irradiated in a cobalt-60 γ-source (4×10^{17} eV g^{-1} min^{-1}). The doses of irradiation were $8–40 \times 10^{17}$ eV g^{-1}. The yield of hydrogen was determined by gas chromatography (on a column of 'molecular sieves' 5 Å). Each experiment was repeated at least eight times and the coefficient of variation was ± 10 per cent.

Under the experimental conditions the hydrated electrons react with acetone, whereas the hydroxyl radicals react with isopropanol. Hydrogen atoms may react with isopropanol to form hydrogen, or with PhX to form PhXH radicals, which do not yield hydrogen[3]. The experimental yield of hydrogen is therefore the sum of the 'molecular' yield G_{H_2} and of G(H + isopropanol). G_{H_2} was determined under similar conditions using a solution of 1×10^{-3} M potassium bromide. The sum of G_H and G_{H_2} the total yield of hydrogen atoms, was determined using a solution of 5×10^{-2} M isopropanol + 1×10^{-3} M acetone. Using these values and the known value of the specific rate constant for the reaction of hydrogen atoms with isopropanol, $k_{H + isopropanol} = 5{\cdot}0 \times 10^7$ M^{-1} sec^{-1} (ref. 4), the rate constant of the reaction H + PhX was calculated:

$$k_{H + PhX} = \frac{k_{H + isopropanol} \cdot [G_H - G(H + isopropanol)]\,[isopropanol]}{G(H + isopropanol)[PhX]}$$

The results are summarized in Table 1. The order of reactivity is $PhSO_3^-$ < PhCN < $PhNHCOCH_3$ < $PhCH_2COO^-$ < $PhCOO^-$ < $PhCOCH_3$ < $PhNO_2$ < $PhNH_2$ < PhOH. This order is in fair agreement with the order of reactivity of these compounds toward electrophilic aromatic substitution, with one exception: the reactivity of nitrobenzene is much higher than would be expected from the general behaviour of aromatic substitution. This is most probably due to a direct attack by hydrogen atoms on the NO_2 group, as may be inferred from their fast reaction rates with NO_2^- ion[5,6] and $C(NO_2)_4$ (ref. 7).

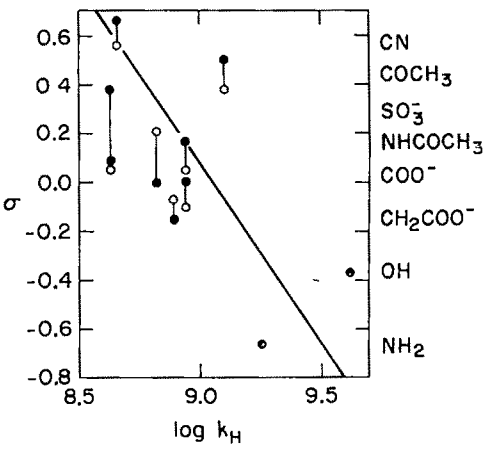


Fig. 1 Plot of log $k_{H + PhX}$ against σ

Table 1. Reactivity of Aromatic Compounds towards Hydrogen Atoms

Compound	Rate constant (M^{-1} sec^{-1})
$C_6H_5SO_3Na$	$4{\cdot}30 \times 10^8$
C_6H_5CN	$4{\cdot}53 \times 10^8$
$C_6H_5NHCOCH_3$	$6{\cdot}70 \times 10^8$
$C_6H_5CH_2COONa$	$7{\cdot}70 \times 10^8$
C_6H_5COONa	$8{\cdot}70 \times 10^8$
$C_6H_5COCH_3$	$1{\cdot}26 \times 10^9$
$C_6H_5NO_2$	$1{\cdot}66 \times 10^9$
$C_6H_5NH_2$	$1{\cdot}76 \times 10^9$
C_6H_5OH	$4{\cdot}20 \times 10^9$

A quantitative treatment of the results is shown in Fig. 1, where log $k_{H + PhX}$ is plotted against Hammett's σ values (taken from refs. 8 and 9). Both σ_m and σ_p values were taken for substituents having a deactivating effect, but only the σ_p values were taken for the other substituents. (The attack by hydrogen atoms on aromatic compounds is electrophilic in nature, thus their attack on the *meta* position of aromatic compounds carrying *ortho–para* directing substituents can be neglected.) A σ value similar to that of CH_2CH_3 and $CH_2CH_2COO^-$ groups was taken for the CH_2COO^- group. Neglecting the value for nitrobenzene, which is unexpectedly high, as explained before, a straight line could be drawn with a slope = − 0·7, which demonstrates the electrophilic nature of the attack of hydrogen atoms on aromatic compounds. The absolute value of ρ = − 0·7 is higher than that observed for the reaction of hydroxyl radicals (ρ = − 0·41) (ref. 2). This is in accordance with the relatively lower reactivity of hydrogen atoms, which makes them more selective.

The electrophilic behaviour of hydrogen atoms in the H + PhX reaction is not surprising, since a higher electron density on the aromatic ring promotes the formation of the new C—H bond and stabilizes the PhXH radical formed.

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BIOCHEMISTRY

Activity of Lysosomal Enzymes in White Muscle Disease

DEFICIENCY of selenium and vitamin E in the diet of late-gestation ewes is known to cause white muscle disease (WMD) in lambs[1,2]. It is generally characterized by degenerative changes in skeletal and cardiac muscles. Since the myopathy is prenatally influenced, the development of the disease presumably has a direct relationship with the deprivation of protectors essential for the prevention of nutritional muscular dystrophy.

Lysosomes, a group of sub-cellular particles rich in a variety of acid hydrolases, have already been shown to occur in the dystrophic tissues of vitamin E-deficient rabbits[3] and chicks[4] and in the genetic dystrophy of mice and chickens[5]. Since white muscle disease is a degenerative disease of nutritional origin, I set out to investigate its lysosomal activity so as to make possible a comparison between the biochemical lesions of this disease and those observed in the dystrophies of other species. This report concerns the activities of five typical lysosomal enzymes in the muscle tissues of normal and dystrophic white muscle disease lambs.

Lambs, 4–6 weeks old and showing distinct symptoms of leg weakness were killed and the dystrophic tissues from leg muscles were collected and immediately frozen. Equivalent tissue samples were also collected from normal lambs of similar age. For chemical analysis, a 10 per cent tissue homogenate was made in ice-cold, de-ionized water by mincing the tissue in a Servall 'Omnimixer' for 1 min.

The activity of individual lysosomal enzymes was determined by several assays as follows. Acid phosphatase, β-glucuronidase and cathepsin were determined by the methods of Gianetto and de Duve[6]. β-Galactosidase was assayed by the method of Sellinger *et al.*[7]. Aryl sulphatase was assayed by the method of Roy[8] with 2-hydroxy-5-nitrophenyl sulphate as substrate. The specific activities of the enzymes are expressed in mμmoles substrate hydrolysed/mg nitrogen/min.

Table 1. LYSOSOMAL ENZYMES IN LEG MUSCLE OF NORMAL AND DYSTROPHIC WMD LAMBS

Enzyme	Specific activity* Control	Dystrophic WMD	Increase in lysosomal activity over control
Aryl sulphatase	0·16	5·63	35 ×
β-Glucuronidase	3·01	15·18	5 ×
β-Galactosidase	0·64	1·80	3 ×
Cathepsin	0·14	0·44	3 ×
Acid phosphatase	4·64	9·98	2 ×

* Expressed as mμmoles of substrate hydrolysed per mg nitrogen per min. Values represent average of two lambs in each group.

The specific activities of five typical lysosomal enzymes in muscle tissues of normal and dystrophic white muscle disease lambs are presented in Table 1. The activity of individual lysosomal enzymes in dystrophic tissues was greater than in the controls, and the increases were as follows: aryl sulphatase, 35-fold; β-glucuronidase, 5-fold; β-galactosidase and cathepsin, 3-fold; acid phosphatase, 2-fold. These ratios are similar in magnitude to those observed in dystrophic muscle tissues of vitamin E, selenium and sulphur amino-acid-deficient chicks[4] and vitamin E-deficient rabbits[3]. Increased 5-nucleotidase activity in white muscle disease of lambs[5] may also be associated with the lysosomal complex.

It appears from all these observations that the phenomenon of increased lysosomal activity is directly associated with degradative changes occurring in tissues undergoing dystrophy. A recent time-sequence study[10] of the appearance of nutritional muscular dystrophy in chicks indicated that initiation of the primary damage is usually caused by the deficiency of the tissue protectors and that the activities of the lysosomal enzymes increase as a result of the infiltration of macrophages, the carriers of lysosomes. Although lysosomes may not be the primary initiators of the tissue damage, the greatest part of the hydrolytic breakdown in a tissue undergoing dystrophy is the result of large increases in free and total activities of various lysosomal hydrolases. Recent studies showed that isolated lysosomes are fully capable of digesting most cellular constituents[11]. The triggering mechanism responsible for the involvement of lysosomes in muscular dystrophy still remains obscure.

I thank Dr. D. E. Hogue for providing sheep tissues and Dr. M. L. Scott for providing research facilities.

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Abnormal Isoenzyme Patterns in Human Myopathies

PROTEINS may exist in different molecular forms possessing the same enzymatic activity and which have been called isoenzymes[1] or isozymes[2]. Lactate dehydrogenase has been extensively studied and appears to consist of five isoenzymes, each of which is a tetrameric association of two sub-units, *M* and *H*. It has been shown that the synthesis of either sub-unit may be altered in response to hormonal, environmental or neural changes[3]. Evidence has also been presented which suggests that the synthesis of the two sub-units is controlled by two separate genes[4].

In normal adult human skeletal muscle, the slowest isoenzyme, LDH-5, predominates[5–7]. (By convention[1], the isoenzyme having the highest mobility towards the anode is numbered 1.) In normal foetal skeletal muscle, LDH-5 possesses little or no activity[8,9]. Recently, Emery reported that LDH-5 is also absent in skeletal muscle from victims of Duchenne muscular dystrophy[8], but this gross aberration has not been found in all cases of Duchenne dystrophy[10].

The isoenzyme patterns of NADP-linked isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) as well as lactate dehydrogenase (LDH) were investigated electrophoretically in human myopathies to see if any alterations were specific for one disease.

Biopsy samples were removed from the gastrocnemius muscle, cooled in ice and processed immediately. The specimens were dropped in 0·25 M sucrose and blotted to remove blood. A 100-mg sample of tissue was then homogenized briefly in a small glass tissue grinder. Then 0·2 ml. of distilled water were added and the homogenization was continued. The whole procedure was performed in an ice-bath and the homogenization was discontinued frequently to prevent the accumulation of heat. The time occupied in homogenization was 2 min. The homogenate was transferred to a micro-centrifuge tube and was frozen and thawed once, after which it was centrifuged at 20,000*g* and 0° C for 15 min. The resultant supernatant

liquid was applied directly to the gel so as to localize MDH and ICDH. A portion of the supernatant was diluted with four volumes of distilled water and applied to the gel for LDH localization. Vertical starch-gel electrophoresis was used with the *p*H 7·0 phosphate–citrate buffer system of Fine and Costello[12]. A potential gradient of 4 V/cm was applied to the gel for 18 h at 4° C. The enzymes were made visible by the methods of Fine and Costello[12].

In all the cases tested, MDH was normal. There were no alterations among the six isoenzymes of MDH which are found in normal human muscle[13]. This agrees with the preliminary findings of Pearson[10] for the Duchenne cases.

In two cases of Duchenne dystrophy, both LDH and ICDH patterns were normal. In two other cases of Duchenne, LDH-5 was absent. One of these cases also exhibited an absence of ICDH-2. The other sample provided insufficient material to allow for ICDH detection. (In normal skeletal muscle there are three isoenzymes of ICDH, ref. 13.) In three other cases of the same disease, there was a decrease in the activity of LDH-5 and an increase in activity of the rapid isoenzymes. Two of these three cases also lacked ICDH-2.

In two cases of Werdnig–Hoffmann muscular atrophy, LDH-5 was extremely weak and LDH-1 was more active than LDH-5. From one sample there was again sufficient supernatant to show that ICDH-2 was absent. In one case of possible amyotonia congenita, LDH-5 was very weak and ICDH-2 was absent. The gels for this case are shown in Fig. 1. In one case of possible myotonia congenita, both LDH and ICDH were normal.

It has already been proved[13] that ICDH-2 is the most labile isoenzyme of ICDH. Exposure of normal skeletal muscle autopsy material to room temperature for several hours brings about a decreased activity of ICDH-2. However, exposure of liver samples to room temperature results in a complete loss of the same isoenzyme. Hence the loss of the liver isoenzyme may possibly be explained on the basis of an attack by proteolytic enzymes which are present in the liver.

Similarly, one can postulate the presence of an abnormal amount of catheptic activity in certain pathological muscles to account for the absence of ICDH-2. Indeed, there have been reports of increased catheptic activity in human Duchenne muscular dystrophy[14], hereditary mouse muscular dystrophy[15,16] and in experimental denervation[17]. Denervation also brings about a loss in LDH-5 in rabbit muscle[3]. The alterations in isoenzyme patterns may, on the other hand, reflect a delayed maturation of the enzymes in skeletal muscle[18].

From the results of this brief survey, it is evident that alterations in the isoenzyme patterns of LDH and ICDH occur in several myopathic conditions. Further investigation is required to determine whether or not these findings are of possible diagnostic or aetiological value.

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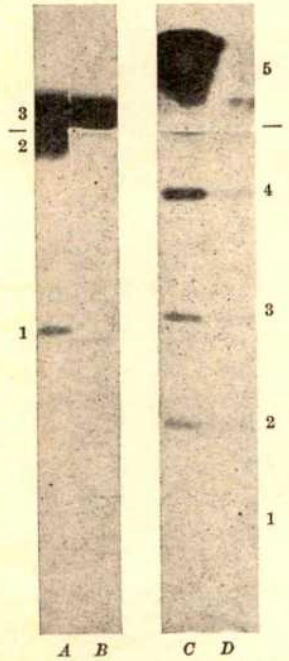


Fig. 1. Isoenzymes of ICDH in normal muscle (*A*) and in amyotonia congenita (*B*) and isoenzymes of LDH in normal muscle (*C*) and in amyotonia congenita (*D*). The anode is at the bottom of the picture and the origin is indicated by the strokes

Limitations on the Use of the Concept of Quantum Efficiency in Rhodopsin Bleaching

The purpose of this communication is to discuss the extent to which the concept of quantum efficiency can be applied to the bleaching of rhodopsin. Quantum efficiency of bleaching, γ, is defined by:

$$\gamma = \left(\frac{\text{No. of molecules bleached}}{\text{No. of quanta absorbed}}\right)$$

By making use of this definition and by careful consideration of both theoretical and experimental contingencies, Dartnall, Goodeve and Lythgoe[1] concluded that $\gamma = 1{\cdot}0$ (or not much less) when fairly dim, visible light was used for bleaching. This conclusion remained virtually unquestioned until Hagins[2] reported the observation that, no matter how high its intensity was made, a short (less than about 1 msec) flash would not bleach more than about 50 per cent of the rhodopsin present. Because of this result, the idea was unfortunately introduced that γ at high intensities had a limiting value of 0·5.

At first, these two results appeared to contradict each other. How can $\gamma = 1{\cdot}0$ when the upper limit on bleaching is 50 per cent? Hubbard and Kropf[3] were among the first to suggest that the two results were not mutually exclusive. They were investigating the quantum efficiencies with which various species involved in bleaching are interconverted by light. One of these conclusions was that the quantum efficiency of bleaching is not constant but depends on both intensity and temperature.

I investigated the effect of one of these parameters, intensity, on the extent of bleaching with short duration flashes[4]. It was found that, while $\gamma = 1{\cdot}0$ for low intensities, there was a systematic decline in the efficiency of bleaching as the intensity was increased. The maximum extent of bleaching in those experiments probably was about 50 per cent. Care was taken, at that time, not to confuse the maximum extent of bleaching with the upper limit of γ. Failure to clearly distinguish between these two has caused[2], and is at present causing[5], confusion.

The high-intensity limit on γ is zero; it is not 0·5. This is readily shown with two examples. (1) In low temperature experiments, where bleaching is arrested at one stage or another, long-term irradiation results in only a 50 per cent bleach on warming, even though each molecule may have absorbed many quanta. In the limit as irradiation time goes to infinity, each molecule absorbs an infinite number of quanta and still only 50 per cent of them bleach. Hence, in this case, $\gamma = 0{\cdot}5 \times$ (No. of molecules present)$/\infty = 0{\cdot}0$. (2) The same situation as in (1) obtains if, instead of using low temperatures to prevent actual bleaching during the irradiation time, one uses very short duration flashes. (This is discussed thoroughly in refs. 3 and 4). In this case, if a short flash of (hypothetically) infinite intensity is given, no more than 50 per cent bleaching occurs even though an infinite number of quanta were absorbed. Again, $\gamma = 0{\cdot}0$.

Experimentally, of course, γ never reaches a zero value. Theoretically, however, the foregoing arguments show that it can take on any value between 1 and zero. This fact, it seems, seriously limits the usefulness of the concept of quantum efficiency as applied to bleaching. In short, without an independent measure of the number of quanta actually absorbed (say, by actinometry), only the use of very low intensities makes it possible to invoke a particular value of γ (namely, 1·0) in order to explain experimental results.

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Detection of Cholinesterase-inhibiting Insecticide Chemicals and Pharmaceutical Alkaloids on Thin-layer Chromatograms

This communication reports a sensitive and rapid method for detecting cholinesterase (ChE) inhibitors, such as certain phosphate and carbamate insecticides and pharmaceutical alkaloids utilizing a direct assay on thin-layer chromatography plates coated with cellulose and developed in a reverse phase solvent system.

Recently, Bunyan[1] described a sensitive indirect detection method for organophosphorus insecticides based on ChE inhibition on thin-layer chromatography plates coated with silica gel *H*. Spots of inhibitory compounds were transferred by contact from the developed thin-layer chromatography plates to 3-mm Whatman paper which was pre-treated with human plasma as the source of ChE. Subsequent treatment of the paper with acetylcholine (ACh) and indicator gave spots corresponding to inhibitors. Attempts to detect inhibitors directly on thin-layer chromatography plates were unsuccessful. Crosby *et al.*[2] reported a similar thin-layer chromatography procedure for detection of a number of carbamate insecticides using plates coated with aluminium oxide *G*; after development the chromatograms were sprayed with the plasma-indicator solution and spots corresponding to inhibitors were shown on a filter paper sheet soaked in ACh solution by placing the latter in contact with the thin-layer chromatography plate.

In the present investigation, thin-layer chromatography plates, 20 × 20 cm, coated with 1·0 mm '*MN*-cellulose powder 300 *G*' (Macherey, Nagel and Co., Düren, Germany) were used. The adsorbent slurry of 86 g cellulose and 435 ml. water (ratio 1 : 5) was adjusted to *p*H 11 with aqueous dilute sodium hydroxide solution, applied to the plates with a Brinkman–Desaga applicator and air-dried overnight. The dried plates were coated by dipping with a stationary phase consisting of a 5 per cent v/v solution of Dow Corning 'Silicone 555' fluid in acetone and the carrier solvent was evaporated. Acetone, methanol or water solutions of analytical grade insecticide chemicals and pharmaceutical alkaloids were spotted in 2-μl. volumes 2·0 cm above the lower edge of the plates. Development was accomplished with a mixture of water, absolute ethanol and chloroform (56 : 42 : 2). This reverse phase solvent system was similar to that used by Hopkins[3] for paper chromatograms. Cook's method[4] as modified by Getz and Friedman[5] for detection of ChE-inhibiting spots on paper chromatograms was adapted to thin-layer chromatography plates. The plates were sprayed with human blood plasma (undiluted, pooled, outdated) as the ChE source. After incubation for 0·5 h at room temperature, the plates were dried and liberally sprayed with a freshly prepared substrate indicator solution consisting of 1 part of 0·6 per cent bromthymol blue in 0·1 normal sodium hydroxide and 15 parts of 1 per cent ACh chloride in water. Inhibiting substances appeared as intense blue spots against a yellow background. Maximum definition of the spots usually resulted when the thin-layer chromatography plates were lightly resprayed several times with the substrate indicator solution. These spots faded within 30 min to several hours after development; however, by sacrificing some sensitivity, it was possible to redevelop partially faded spots after a longer interval by respraying the plates with substrate indicator solution. Attempts to detect spots by this direct enzyme inhibition method on thin-layer chromatography plates coated with silica gel and 'Florisil' were unsuccessful. The insecticides and alkaloids included in this investigation are listed in Table 1 with their respective R_P values and limits of detection as distinct blue spots on a yellow background. The procedure proved to be highly sensitive for detection of various phosphate and phosphorothiolate esters where in several instances ChE-inhibition was evident for amounts in the nanogram range or less. The streaking associated with 'Zectran' was probably due to a number of inhibitory compounds. Crosby *et al.*[2] reported that an analytical sample of 'Zectran' contained two major ChE inhibitors and one minor ChE inhibitor.

The ChE inhibition detection procedure was also very sensitive for a number of pharmaceutical alkaloids (kindly presented by Dr. Werner Deckers, C. H. Boehringer Sohn, Ingelheim am Rhein, West Germany). The alkaloids for which the greatest sensitivity was noted are given in Table 1. Several additional alkaloids were detected at a 20-μg level: ajmaline, arecoline hydrobromide, atropine methylbromide, emetine hydrochloride, hyoscyamine hydrobromide, and yohimbine hydrochloride. The following alkaloids gave no detectable spots with 20 μg: atropine sulphate, scopolamine methylbromide and rescinnamine. Solanine and its aglucon derivative, solanidine (both obtained from K and K Labs., Plainview, N.Y.), gave two inhibition zones on the thin-layer chromatography plates. By using a similar procedure on paper chromatograms, solanidine is resolved into at least five inhibitory spots and solanine into a single spot[6].

The described procedure appears to be an improvement over previous methods for rapid qualitative determination of trace amounts of ChE-inhibiting chemicals. It may be

Table 1. Thin-layer Chromatography of Insecticide Chemicals and Pharmaceutical Alkaloids active as Cholinesterase Inhibitors

Chemical name*	R_F value Organophosphates	Sensitivity limit (μg)
O,O-dimethyl S-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethylphosphorothiolate	0·95	0·01
S-[(*p*-chlorophenylthio)methyl] O,O-diethylphosphorothiolate	0·34	0·01
O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) phosphate	0·87	< 0·001
S-[1,2-*bis*(ethoxycarbonyl)ethyl] O,O-dimethylphosphorothiolate	0·95	0·001
Diethyl *p*-nitrophenylphosphate	0·82	< 0·001
O,O-diethyl S-(ethylthio) methylphosphorothiolate	0·66	0·001
O,O-diethyl S-(ethylsulphonyl) methylphosphorothiolate	0·98	0·001
	N-Methyl carbamates	
2-Chloro-4,5-xylylmethylcarbamate	0·77	< 0·2
2-*Iso*propoxyphenylmethylcarbamate	0·94	0·2
1-Naphthylmethylcarbamate	0·93	0·2
4-Dimethylamino-3-cresylmethylcarbamate	0·94	0·2
4-(Methylthio)-3,5-xylylmethylcarbamate	0·72	0·2
3-*Iso*propylphenylmethylcarbamate	0·79	< 0·2
4-Dimethylamino-3,5-xylylmethylcarbamate	0·56–1·0	< 0·2
	Alkaloids	
Eserine salicylate	0·80–1·0	0·2
Ephedrine hydrochloride	1·0	0·2
Homatropine hydrobromide	1·0	2·0
Lobeline hydrochloride	0·6	< 20·0
Pilocarpine nitrate	1·0	0·2
Reserpine	1·0	0·2
Solanine	0·5–0·75	< 0·2
	0·8–1·0	< 0·2
Solanidine	0·5–0·75	< 0·2
	0·8–1·0	< 0·2

* The common and trade names of several phosphate and carbamate insecticides are listed in *Bull. Entomol. Soc. Amer.*, **9**, No. 3, 189 (1963).

applicable to research on pesticide chemicals and pharmaceutical alkaloids and the detection of these agents in various biological substances and preparations.

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A Factor inhibiting the Adenosine Triphosphatase Activity and the Superprecipitation of Actomyosin

Ebashi[1] has produced evidence for a protein factor in the presence of which the magnesium-activated ATPase[2] and the superprecipitation[1] of actomyosin are inhibited by low concentrations of ethylene *bis*(NN′diacetyl β amino ethyl) ether (EGTA). To observe the effect of this factor the actomyosin was prepared from separately purified actin and myosin, so-called 'synthetic' actomyosin. We have obtained similar effects with an actomyosin preparation extracted as the complex from muscle and treated by a procedure described elsewhere[3]. This preparation is referred to in this communication as desensitized actomyosin.

During the course of investigations[3] on the EGTA-sensitizing factor, it was noted that in some circumstances an apparently different preparation was obtained which inhibited the magnesium-activated ATPase of actomyosin in the absence of EGTA. There was also some evidence that the amount of the inhibitory factor increased as EGTA-sensitizing factor preparations aged. Active inhibitor preparations were consistently prepared from rabbit skeletal muscle by the following procedure. A crude concentrated myofibril suspension (prepared essentially as described by Perry and Zydowo[4]) was extracted with an equal volume of a solution containing 1·2 M potassium chloride, 0·08 M sodium bicarbonate and 0·02 M sodium carbonate, the whole extract allowed to stand for 15 h at 0° C and then dialysed extensively against 40 mM potassium chloride, 6·7 mM phosphate buffer, *p*H 7·2. After the precipitate had been centrifuged down, the supernatant was filtered and freeze-dried. The factor was further purified from the freeze-dried protein by precipitation at 0° C by the addition of ammonium sulphate to 30 per cent saturation. When this preparation was added to 'desensitized' actomyosin marked inhibition of the magnesium ATPase occurred (see Fig. 1). Inhibition of the magnesium activation of the ATPase of 'synthetic' actomyosin was also obtained, but 'natural' actomyosin containing EGTA-sensitizing factor and myofibrils were less sensitive to the inhibitor. Little inhibition was obtained with desensitized actomyosin when calcium was the activating divalent cation (Fig. 1). The factor also inhibited the superprecipitation of desensitized actomyosin (Fig. 2).

The fact that the inhibitor was precipitated by ammonium sulphate, destroyed by trypsin and heat, and that it lost activity on prolonged storage at 0° C suggests that it is protein in nature. Studies on the turbid supernatant obtained after myofibrils were removed from muscle homogenates in 0·025 potassium chloride, 0·039 borate, *p*H 7·0, showed that inhibitory factor could be prepared neither from the reticular-granular fraction sedimented after 1 h at 100,000*g* nor from the soluble sarcoplasm. This suggests that the inhibitory factor is localized in the

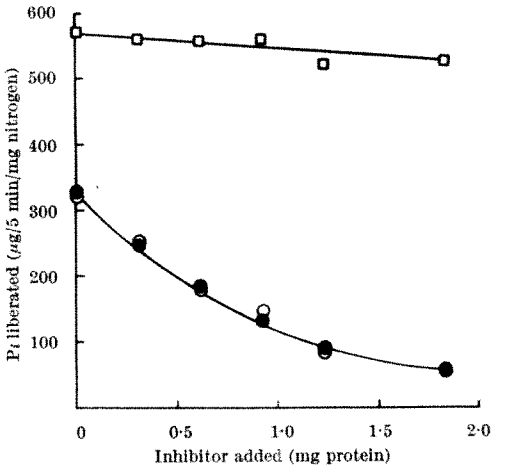


Fig. 1. Effect of inhibitor on the calcium and magnesium activated ATPase of desensitized actomyosin. The assay was carried out by incubating for 5 min at 25° C in 2 ml. containing 25 mM *tris*-HCl, *p*H 7·6, 2·5 mM *tris*-ATP and the following additions: ○, 2·5 mM magnesium chloride and 1·24 mg actomyosin; ●, 2·5 mM magnesium chloride, 1·24 mg actomyosin and 1 mM EGTA; □, 2·5 mM calcium chloride and 0·83 mg actomyosin

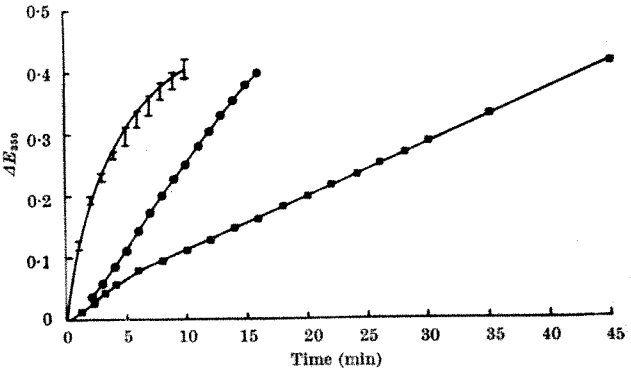


Fig. 2. Superprecipitation of desensitized actomyosin preparations in the presence of inhibitory factor. Superprecipitation followed by measurement of E_{350} at 25° C. Conditions: 25 mM *tris*-HCl, *p*H 7·6, 2·5 mM magnesium chloride 35 mM potassium chloride and 1·24 mg actomyosin; reaction started by addition of *tris*-ATP to 2·5 mM in a total vol. of 3 ml. at zero time. ——, Control, no further addition; ●, 0·61 mg inhibitor preparation added; ■, 1·83 mg inhibitor preparation added

myofibrils or associated with some component not readily removed from them by repeated washing and centrifugation.

The most active inhibitor preparations usually contained less than 1 per cent of tropomyosin when assayed by the method described by Perry and Corsi[5]. This fact coupled with its solubility characteristics indicated that the factor was neither 'natural' nor Bailey[6]-type tropomyosin[7]. Preparations which inhibited the magnesium-activated ATPase of desensitized actomyosin could not be obtained from actin by the procedure outlined here. The inhibitory factor does not seem to be identical with α-actinin which has the opposite effect on the superprecipitation of actomyosin gels[8].

The precise role of this inhibitor is not yet clear, but in so far as it inhibits the ATPase activity and superprecipitation of actomyosin it has the characteristics previously ascribed to relaxing factors. The best preparations obtained so far are not homogeneous but contain little or no EGTA-sensitizing factor activity (Fig. 1). Nevertheless it is possible that the inhibitor is a modified form of the EGTA-sensitizing factor, and investigations are now in progress to decide whether such a relationship exists.

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Higher Glucose Affinity of Hexokinase in Sheep Brain than in Rat Brain

The brain in sheep, as in non-ruminant mammals' appears dependent on glucose as the substrate for oxidation[1], yet the blood glucose concentration of adult sheep is only about 2·5 mM (50 mg/100 ml.) compared with about 5 mM (100 mg/100 ml.) in rats, dogs, men and pigs. If the availability of a substrate is less in one species than in another, and a tissue were to utilize the substrate at comparable rates in both species, then one might look for an inter-species difference in enzyme affinity for this substrate. So we have estimated the apparent *Km* for glucose of hexokinase in brain, also liver, muscle and adiposa, of sheep and rats.

The rats were killed by decapitation and the sheep by electrocution. Our procedures for the preparation of homogenate supernatants and for estimating glucokinase activity by NADPH generation in the presence of added ATP, Mg^{++} and glucose-6-phosphate dehydrogenase have been described previously[2], except that a single glucose concentration of 1 mM was used before, while a series, usually of twelve points between 0·002 and 150 mM, was used in the present investigations. Some supernatants were used immediately, and others were dialysed for 20 h. Ammonium sulphate fractions were prepared from brain and liver of three sheep and three rats; in these experiments the series of glucose concentrations was limited to four or five points. Double reciprocal plots were made (for example, Fig. 1), and the apparent *Km* was calculated as the reciprocal of $-1/S$ when $1/V = 0$.

In liver, muscle and adiposa, the apparent *Km* of hexokinase for glucose was about the same in the sheep as in the rats (Table 1). Our rat data agree with those of previous authors[3]. A second hexokinase of rat liver, often called 'glucokinase', which has a *Km* of about 11 mM (Table 1), was absent from sheep liver. This was previously reported by Ballard and Oliver[4]. In sheep brain, the apparent *Km* of hexokinase for glucose was about half that of rat brain (Table 1): this difference was statistically significant. This difference should persist through purification procedures if it lies in the enzyme proteins, but not if it is due to accessory factors which may affect the apparent *Km*. A two-fold increase in specific activity was achieved by ammonium sulphate fractionation (Table 2): the rat brain glucokinase activity was maximal in the 30–50 per cent fraction, while the sheep brain glucokinase activity was maximal in the 50–75 per cent fraction (Table 2). Moreover, the apparent *Km* was about the same in the dialysed supernatants and corresponding ammonium sulphate fractions (Fig. 1). These two observations suggest that, compared with rat brain, the hexokinase of sheep brain is a larger protein with a greater affinity for glucose.

Any physiological significance of the data presupposes that the availability of glucose at the site of glucokinase activity inside cells is at least in part dependent on the

Table 1. Apparent *Km* of Hexokinases from Sheep and Rat Tissues*

		Mean (mM)	Maximum (mM)	Minimum (mM)	No. of observations
Liver	Rat	0·012	0·017	0·006	5
	Sheep	0·012	0·017	0·008	5
	Rat	11·0	12·5	9·5	4
Muscle	Rat	0·093	0·143	0·060	6
	Sheep	0·090	0·140	0·035	5
Fat	Rat	0·089	0·113	0·075	4
	Sheep	0·078	0·091	0·068	4
Brain	Rat	0·054†	0·086	0·040	7
	Sheep	0·020†	0·033	0·011	8

* Each *Km* was determined from the double reciprocal plot of rates of the reaction varying with glucose concentrations from 0·002 mM to 150 mM. Other final concentrations in the reaction mixture were 50 mM glycylglycine at *p*H 7·6, 8 mM magnesium chloride, 3·0 mM adenosine triphosphate (except in the blanks), 0·25 mM NADP, 2 mM EDTA, and each cell contained at least 0·02 Kornberg units of glucose-6-phosphate dehydrogenase activity.
† $t = 4{\cdot}536$, $P < 0{\cdot}001$.

Table 2. Glucokinase Activities of Ammonium Sulphate Precipitated Fractions of Rat and Sheep Brain

Fraction	Activity (units/g protein)* Rat	Sheep
100,000*g* supernatant	37·2	19·0
0–30 per cent ammonium sulphate	27·0	7·5
30–50 per cent ammonium sulphate	71·8	16·4
50–75 per cent ammonium sulphate	35·6	40·5

* 1×10^{-6} moles of NADPH generated/min/g biuret protein under the reaction conditions described in Table 1, except that only 1 mM glucose was used.

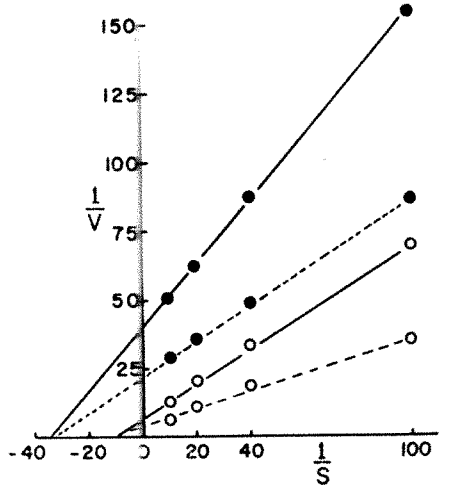


Fig. 1. Double reciprocal plots of the rate of glucokinase activity, *V*, against glucose concentration, *S*, show that the apparent *Km* for glucose (the reciprocal of $-1/S$) of a homogenate supernatant from sheep brain (plotted as ●) differs from that of rat brain (○), and that these values are unchanged by a two-fold purification. The hexokinase was concentrated in the 30–50 per cent ammonium sulphate precipitated fraction of rat brain (dashed line) but in the 50–75 per cent fraction of sheep brain (dotted line)

blood glucose concentration, which is much lower in adult sheep than in most monogastric mammals. It is a striking coincidence that the hexokinases of tissues which can utilize substrates additional to glucose, that is liver, muscle and adiposa, have the same glucose affinities in rats and sheep, while the tissue which is dependent on glucose as the substrate for oxidation, that is the brain, has a hexokinase with a higher affinity for glucose in the sheep than in the rat. The high affinity of sheep brain hexokinase for glucose offers a biochemical explanation of how sheep brain functions normally for years at a blood glucose concentration of 50 mg/100 ml. which would lead to hypoglycaemic manifestations in most mammalian species.

An exaggeration of this situation is found during insulin-induced hypoglycaemia. Men and rats exhibit tremors, convulsions and coma within 1–4 h when the blood glucose concentration falls to less than 50 mg/100 ml. (refs. 5–7). In contrast, sheep exhibit no ill effects in association with blood glucose-levels about 10 mg/100 ml. for 15 h or more[8–10]. This 'insulin resistance' has been attributed to the release of endogenous epinephrine, which is thought to cause cerebral vasodilatation and so sustain cerebral perfusion with glucose despite the hypoglycaemia[9,10]. It seems likely that the high affinity of its hexokinase for glucose would also contribute to the ability of the sheep brain to resist the ill effects of severe hypoglycaemia.

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Encephalitogenic Peptides from Spinal Cord

In a recent symposium on experimental allergic encephalomyelitis there was considerable discussion on the size of encephalitogenic factors from brain and spinal cord[1–4]. We have fractionated extracts from bovine spinal cord by gel-filtration with phenol–acetic acid–water. This solvent system was introduced by Synge and colleagues[5–7] for the fractionation of peptides on 'Sephadex *G*-75'. It is a strongly dissociating solvent which prevents adsorption of peptides on 'Sephadex', and gel-filtration in this solvent can be used to estimate the molecular size of peptides as there is an approximately linear relationship between the relative elution volume and the log of molecular weight[8,9]. This communication describes a typical fractionation of basic polypeptides which were found to produce experimental allergic encephalomyelitis in guinea-pigs.

For the fractionation shown in Fig. 1, 'Sephadex *G*-75' (crushed type, fine) (Pharmacia, Uppsala) was allowed to swell for 2 days in phenol–acetic acid–water, 1 : 1 : 1 (w/v/v). 'Sephadex *G*-75' swells less in this solvent than in water, so that a settled bed of 9·9 ml./g was obtained. An acetone-dried powder of bovine spinal cord was extracted at 4° C with 0·01 N hydrochloric acid (100 g/l.) for 2·5 h. The filtered extract was lyophilized to yield 3·24 g (0·7 per cent of fresh cord). The re-dissolved extract was dialysed against water in acetylated cellulose tubing (18/32 'Visking')[10] for 30 h with four changes of diffusate. The retentate was lyophilized and any acidic material present was removed by passing it through diethylaminoethyl 'Sephadex *A*-50' in pyridine, 1 per cent: collidine, 1 per cent, titrated with acetic acid to *p*H 7·5. The basic fraction from the column was collected and represented 0·12 per cent of fresh cord. A portion of this material (100 mg) was fractionated on a 3 g column of 'Sephadex *G*-75' with phenol–acetic acid–water as solvent. The fractions were examined by paper chromatography in *n*-butanol–acetic acid–water (4 : 1 : 5) and by electrophoresis in acrylamide gel[11] with formic acid–acetic acid–water (1 : 4 : 45 by volume) containing 4 M urea, as buffer. The elution weights of the fractions with encephalitogenic activity, 1*B* and 1*C* (Fig. 1), were between that of glucagon and bacitracin which should indicate a molecular weight between 3,500 and 1,500. Paper chromatography showed that fractions 1*D* and 1*E* were composed mainly of free amino-acids. Gel-electrophoresis showed in the starting material eight cationic bands (Fig. 1). The slowest bands were found in fraction 1*A*. Encephalitogenic activity was found to be associated with fractions which contained the electrophoretic bands '*g*' and '*f*'. Table 1 shows the recovery of dry matter and the encephalitogenic activity of the fractions. A portion of fraction 1*C* was rechromatographed on the same column. The fractions equivalent to 1*B* were found to be encephalitogenic at a dose of 5 μg.

In other gel-filtration experiments carried out on material prepared from bovine spinal cord by extraction with acidified potassium chloride followed by precipitation with saturated ammonium sulphate, and previously referred to as 'basic protein fraction'[1–4,12], it was found that about half this fraction could be recovered from a 'Sephadex *G*-75' column with phenol–acetic acid–water as solvent in a zone equivalent to a molecular weight of 1,500–2,000. Maximum encephalitogenic activity, as judged by the number of animals showing clinical and histological symptoms and by the day of onset of the disease, was consistently found to be associated with the fractions showing bands with electrophoretic mobility

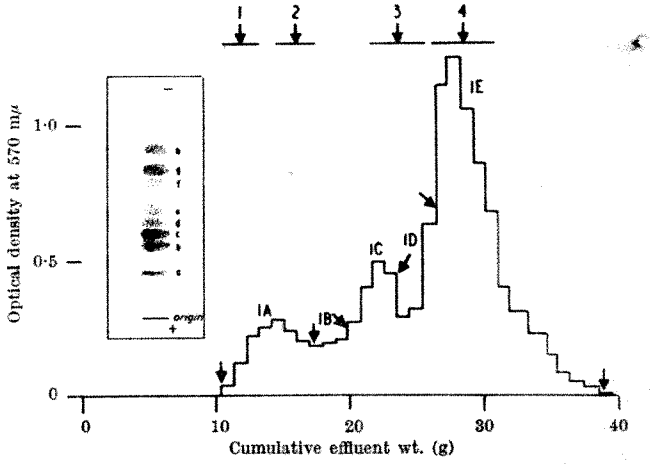


Fig. 1. Separation of basic fraction (100 mg) from bovine spinal cord on a 3 g column of 'Sephadex *G*-75' (crushed type) with phenol–acetic acid–water (1 : 1 : 1 w/v/v) as solvent. Fractions pooled for lyophilization are shown between the arrows. Arrows at the top of the graph show the peaks of reference compounds, bars indicate the limits of these zones. (1) Cytochrome *c*, molecular weight 12,400; (2) glucagon, 3,485; (3) bacitracins, 1,400–1,500; (4) free amino-acids. Inset shows acrylamide gel-electrophoresis of starting material, in formic acid–acetic acid–water (1 : 4 : 45 by volume) *p*H 2·0 containing 4 M urea

Table 1. Separation of Basic Fraction from Spinal Cord by Gel-filtration

Fraction	Dry matter recovered	Dose used (μg)	Encephalitogenic activity* Incidence (clinically positive)	Day of onset Range	Day of onset Mean	Histology positive
1A	20·4	50	0/5	—	—	1/4
1B	9·6	100	3/5	11–17	13·7	3/5
1C	21·6	100	3/5	11–13	11·7	4/5
1D	11·3	—	—	—	—	—
1E	37·8	100	0/5	—	—	0/5

* Encephalitogenic activity was determined, in guinea-pigs, by a single intradermal injection (0·1 ml.) of the test material, at the dose shown, dissolved in sterile saline and emulsified with an equal volume of 'complete Freund's adjuvants' composed of 'Bayol F', 85 parts, and 'Ariacel A', 15 parts, with added heat-killed *Mycobacterium tuberculosis* to the final amount of 0·25 mg bacillary powder per animal (see ref. 12).

Table 2. Amino-acid Composition of an Encephalitogenic Fraction from Bovine Spinal Cord

Amino-acid	Moles/100 moles amino-acid	Amino-acid	Moles/100 moles amino-acid
Lysine	9·3	Half cystine	0·0
Histidine	4·7	Valine	1·6
Arginine	6·7	Methionine	0·4
Aspartic acid	5·3	Isoleucine	1·7
Threonine	5·0	Tyrosine	2·7
Serine	10·5	Phenylalanine	3·6
Glutamic acid	7·3	Unknown 1	0·2
Proline	8·3	Unknown 2	0·3
Glycine	18·1	Ammonia	6·1
Alanine	8·1		

Sample was hydrolysed with 6 N hydrochloric acid for 22 h at 110° C. Amino-acids were determined on a Spinco model 120*B* amino-acid analyser by Dr. C. M. Mauritzen. Serine and threonine have been corrected for 10 per cent destruction. Unknown 1 was just before arginine and unknown 2 was just before aspartic acid.

equivalent to bands '*g*' and '*f*' and with an elution weight equivalent to that of a peptide with a molecular weight of about 3,000.

In Table 2 is given the amino-acid composition of an encephalitogenic fraction obtained from the 'basic protein fraction'. The amino-acid composition is unusual in its high content of glycine, serine and basic amino-acids. This amino-acid composition is similar to those of the preparations analysed by Kies[14] and by Caspary and Field[3] and, apart from these materials, the only group of proteins of remotely similar composition is a recently reported histone-like protein isolated by Hnilica[15] from thymus gland and named by him a 'lipohistone'. Our fraction obtained from the 'basic protein fraction' was eluted from a column in a position equivalent to the tail of the chymotryptic haemo-peptide (molecular weight 3,439) from a digest of cytochrome *c* (ref. 13). High-voltage gel-electrophoresis showed that it was composed of at least three peptides of cationic mobility similar to bands '*e*', '*f*' and '*g*' in Fig. 1. The minimum effective dose of this material was 2 μg per guinea-pig. The mild and rapid procedure used to obtain fractions 1*B* and 1*C* suggests that the encephalitogenic peptide(s) occurs naturally in spinal cord and that it is (or they are) not derived from proteins during the fractionation. In this latter connexion, it is to be said that phenol–acetic acid–water was found by Bagdasarian *et al.*[6] to produce no significant hydrolysis of peptides at room temperature and that we ourselves found that there was no liberation of peptides or amino-acids from cytochrome *c* kept in this solvent for 5 months at 4° C. It is probable, from the work of Kies and others[16–20], that they are loosely bound in some way to the lipid components of myelin. Evidence is accumulating that the encephalitogenic factor from spinal cord is a polypeptide which is free to diffuse through 'Cellophane'[1–4,21,22]. Recently, Nakao and Roboz-Einstein[23] briefly reported that there were two encephalitogenic factors in extracts of spinal cord which, from gel-filtration experiments on 'Bio-Gel', were found to be smaller than cytochrome *c*. Lumsden *et al.*[20] previously investigated diffusible polypeptides obtained by mild treatment of 'basic protein' with alkali. Their most highly purified product differed from the polypeptides described in this communication in that its R_F value was 0·5 rather than 0·00–0·01 in butanol–acetic acid–water. It is probable that the encephalitogenic 'far cathodic protein' isolated by Roboz-Einstein, Robertson, D Caprio and Moore[24] is equivalent to our fractions, which show the faster-moving cationic bands '*e*', '*f*', '*g*' and '*h*' of Fig. 1 but, from preliminary results on the ultracentrifuge, these last-mentioned authors concluded that their material had a molecular weight of 18,000–20,000. We now believe that this apparently much higher molecular weight must be due to peptide aggregation, as occurs with histones. Kibler[25] has observed that his encephalitogenic preparation, which was similar to that of Kies[2], is dissociated by raising the ionic strength.

Finally, although gel-filtration in phenol–acetic acid–water is useful in assessing the approximate molecular weight of the encephalitogenic factor(s), it is not so convenient for large-scale isolation of the peptides. Preliminary experiments on the polyacrylamide gel, 'Bio-Gel *P*-6', show that it can be used to prepare, from the acid potassium chloride extract, the fractions containing cationic bands equivalent to '*e*', '*f*', '*g*' and '*h*' in Fig. 1. 'Bio-Gel *P*-6' is claimed to have an exclusion limit of 4,600 mol. wt. Using this material, with formic acid–acetic acid–water (1 : 4 : 45 by volume) as solvent, bands '*f*' and '*g*' were eluted from a column with about the same elution volume as was required for the *B* chain of insulin (molecular weight 3,500).

In summary, a fraction composed of basic polypeptides of unusual amino-acid composition has been prepared by gel-filtration from extracts of defatted spinal cord. From the gel-filtration experiments using phenol–acetic acid–water as solvent it is suggested that the molecular weight of the peptide fraction, which can produce experimental allergic encephalomyelitis, is about 3,000.

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PHYSIOLOGY

Active Transport of Iron by Intestine: Effect of Erythropoiesis stimulated by Phenylhydrazine

Intestinal absorption of radio-iron in intact rats was increased during the enhanced erythropoiesis which followed haemolysis induced by phenylhydrazine[1]. Similarly, erythropoiesis stimulated by acetylphenylhydrazine in dogs also increased iron absorption *in vivo*[2]. This report describes experiments designed to study the effects of increased erythropoiesis in the rat on iron transport across everted duodenal gut sacs *in vitro*. The *in vitro* technique has defined an active transport mechanism for iron absorption[3,4] which varies adaptively with dietary iron and pregnancy[5] and, as will be shown, the cellular mechanism is also involved in the response to increased erythropoiesis.

Male, albino, Sherman strain rats, 80–140 g, were injected subcutaneously with 100 mg/kg body-weight of phenylhydrazine-HCl (recrystallized from boiling water) dissolved in 0·9 per cent sodium chloride, and controls received saline alone. Seven days later rats were killed, the proximal 10 cm of small intestine excised and two consecutive everted gut sacs prepared[4], each 5 cm long. Mean wet weights of the proximal and distal sacs were approximately 770 and 500 mg, respectively. Sacs were incubated in Warburg vessels under oxygen, at 37° C, for 2·5 h in the ambient medium previously described[4], containing 0·1 mM freshly prepared ferrous sulphate, 0·8 mM sodium ascorbate, and sufficient $^{59}FeSO_4$ (Abbott Laboratories, Oak Ridge) to give about 10,000 c.p.m./ml. in a well-type scintillation counter. After incubation sacs were drained, and iron-59 and iron in the mucosal and serosal media were estimated as previously described[4]. Heparinized capillary tubes were used for haematocrit determinations.

Following phenylhydrazine injection the initial mean haematocrit, 42 per cent, decreased to 29 per cent at 3 days and returned to the initial control value at 7 days. Everted gut sacs were then prepared from thirty-one control and forty phenylhydrazine-treated rats and tested *in vitro* (Table 1). Phenylhydrazine treatment increased net transfer of iron (estimated chemically) to the serosal surface by 19·7 per cent ($P < 0·05$) and 54·7 per cent ($P < 0·01$), respectively, in the proximal and adjacent distal segments. Final iron-59 concentration ratios serosal/mucosal were increased correspondingly by 24·4 per cent ($P < 0·02$) and 54·8 per cent ($P < 0·001$) in the proximal and distal segments, respectively. Gut sacs prepared from intestine more than 10 cm distal from the pylorus showed no clear effect of prior phenylhydrazine treatment.

Table 1. Effect of Prior Phenylhydrazine on Iron Transport by Everted Duodenal Gut Sacs *in vitro*

Group	No.	Net Fe transfer to serosal medium mμmoles/gut sac		Iron-59 concentration ratio serosal/mucosal	
		Proximal sac*	Distal sac	Proximal sac	Distal sac
Control	31	31·4 ± 10·5†	12·8 ± 6·6	4·5 ± 1·4	2·0 ± 1·1
Phenylhydrazine	40	37·6 ± 12·0	19·8 ± 10·3	5·6 ± 2·1	3·1 ± 1·4
P value		< 0·05	< 0·01	< 0·02	< 0·001

* Two gut sacs were prepared from the proximal 10 cm of rat small intestine and results are shown for the more proximal and adjacent distal sac.
† Mean ± *S.D.*

The results indicate that the mucosal mechanism for active transport of iron is responsible, in part at least, for enhanced absorption observed after phenylhydrazine treatment. Treated rats were not anaemic at the time of the experiment, and the stimulus for increased iron transport was apparently increased erythropoiesis following haemolysis[1]. Response of the iron transfer mechanism observed *in vitro* was limited to the proximal 10 cm of small intestine and distinctly less in magnitude than the more than two-fold increase observed in intact rats[1]. Thus factors other than the mucosal mechanism studied here may participate in the response to increased erythropoiesis *in vivo*. Increased iron transport observed in the present experiments also differs from that following a low iron diet[5]. Dietary restriction yielded much greater increases in transport *in vitro*, and the most proximal segment showed the greatest effect.

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Increased Active Transport of Glucose through the Intestine during Pregnancy

A well-known aspect of the physiological adaptation to pregnancy is the increase in ingestion of food as a result of stimulation of the lateral nuclei of the hypothalamus.

From a teleological point of view, it might be thought that an increase in the absorptive capacity of the intestine would be associated with this increase in ingestion. It is, in fact, known that iron is absorbed more rapidly during pregnancy, in proportion to the development of the haematopoietic organs of the foetus[1], but few other investigations of intestinal absorption in pregnancy have been carried out.

We have therefore investigated the effect of pregnancy on the absorption of *d*-glucose—one of the most actively transported substances. Other experiments dealing with the absorption of glycine are in progress. The Sols y Ponz technique of successive absorptions *in vivo* was used, as in previous investigations[2].

Table 1. Influence of Pregnancy on the Absorption Rate of *d*-Glucose through the Intestine of Rats *in vivo*

No. of rats	Glucose (mmole)	Intestinal* absorption (μmole/cm)
Not pregnant		
8	300	43·3 ± 0·8
9	150	20·0 ± 0·05
12	75	13·0 ± 0·02
7	20	5·0 ± 0·09
Pregnant		
9	300	52·1 ± 0·5
10	150	26·1 ± 0·7
10	75	16·8 ± 0·2
7	20	7·0 ± 0·4

* Time of absorption, 30 min

Thirty-six white Wistar rats, weighing from 180 to 200 g and 12–15 days pregnant, and a control group of thirty-six rats of similar characteristics but not pregnant, were perfused with solutions of 300, 150, 75 and 20 mM glucose at a pressure of 12 cm of water for 30 min. In each animal, four successive absorptions were carried out.

In Table 1 the average values for both types of experiments are indicated with their corresponding standard errors. The results show a very significant increase ($P < 0·01$) in the absorption of glucose in the pregnant rats over that in the controls. This increase is observed with all the concentrations of glucose, amounting to 21 per cent for 300 mM, and 40 per cent for 20 mM.

Similar results have been found for glycine[3]. The observed increase in the absorption rate is another example

of the modification of the active transport of glucose through the intestine in different functional states, such as diabetes[4], fasting[5] and hyperthyroidism[6], and these results suggest that in studying the maintenance of homoeostasis of certain body constituents it is necessary to take into account this activity of the intestinal mucosa, in addition to increased ingestion and other variations in the activity of the digestive system.

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PHARMACOLOGY

Antagonism of Colistin-sulphonamide Synergism by *para*-Aminobenzoic Acid

Russell[1] reported an *in vitro* synergism between colistin methane sulphonate and sulphonamide drugs using a number of *Proteus* isolates. He found that the combinations were bactericidal at therapeutic levels, that the ratios of the drugs were not critical, and that the effect was present over a wide range of hydrogen ion concentration. The observations were extended to polymyxin *B* sulphate and sulphonamides with similar results.

Since colistin alters the permeability of certain bacteria[2], the possibility was considered that the synergism may be mediated by an alteration of the cytoplasmic membrane to a degree insufficient to cause bacteriostasis in itself, but sufficient to permit the passage of sulphonamides into the cell. If such were the case, the actual inhibition of growth would be a result of a block in folic acid formation and would be overcome by *p*-aminobenzoic acid[3].

To test this, *Proteus vulgaris* and *P. mirabilis* were inoculated into culture tubes containing 'Bactotryptic' soy broth (Difco), with and without *p*-aminobenzoic acid (PABA), and with colistimethate sodium and certain sulphonamide drugs, singly and in combination. Growth was assessed by means of the optical density of each culture after incubation for eleven hours at 37° C. Results were expressed as the percentage of inhibition as compared with appropriate control cultures without drugs.

Table 1 shows the results of one experiment in which sulphanilamide was used; Table 2 shows a similar pattern when the sulphonamides used were extracted with water from sensitivity disks (Baltimore Biological Laboratories) each of which contained 1·0 mg total of a mixture of sulphadiazine, sulphamethazine, and sulphamerazine (triple sulpha). The *Proteus* isolates had a low order of sensitivity to sulphonamides alone and to colistimethate sodium alone. The combination of the two drugs resulted in much greater inhibition, and this enhanced activity was largely overcome by PABA. Attempts to antagonize the synergism with folic acid were not successful; the extent to which this may be related to the inability of the organisms to incorporate or utilize pre-formed folic acid has not been determined.

Table 1. Synergism between Colistimethate Sodium and Sulphanilamide and its Reversal by PABA

	P. vulgaris Inhibition (per cent)		*P. mirabilis* Inhibition (per cent)	
	No PABA	With PABA	No PABA	With PABA
Sulphanilamide	7	0	25	2
Colistimethate sodium	10	2	8	15
Combined	71	7	86	15

Sulphanilamide, 400 μg/ml.; colistimethate sodium, 50 μg/ml.; PABA, 0·01 per cent.

Table 2. Synergism between Colistimethate Sodium and Triple Sulpha and its Reversal by PABA

	P. vulgaris Inhibition (per cent)		*P. mirabilis* Inhibition (per cent)	
	No PABA	With PABA	No PABA	With PABA
Triple sulpha	13	0	5	0
Colistimethate sodium	14	7	10	3
Combined	61	10	72	4

Concentrations same as in Table 1.

The present results tentatively suggest that the sulphonamide-colistin synergism is mediated through an initial alteration by colistin of the permeability of the cytoplasmic membrane of *Proteus* species in such a way as to permit entry of the sulphonamide into the cell. The sulphonamide then retards cell replication by interference with folic acid synthesis.

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HISTOLOGY

Branched Cells in the Epidermis of the Sheep

Branched cells positive for alkaline phosphatase, and which are darkened by osmium iodide, have been found in the epidermis of certain African primates[1–3]. Other dendritic cells have been revealed by gold impregnation and methylene blue in the non-pigmented epidermis of guinea-pigs, rabbits and man[4], and by gold impregnation and electron microscopy in the epidermis of man[5].

Histochemical preparations for acetylcholinesterase have revealed numerous large branched cells in the epidermis and upper external sheaths of the follicles of several breeds of sheep (Merino, Southdown, Suffolk, English Leicester and Border Leicester). By contrast, such acetylcholinesterase-positive branched cells were not revealed in other species investigated at the same time (bandicoot *Perameles nasuta*, possum *Trichosurus vulpecula*, laboratory rabbit, laboratory rat, and ox). Neither the techniques for butyrylcholinesterase nor for alkaline phosphatase revealed these branched cells in sheep skin. Moreover, they did not stain with osmium iodide or gold chloride and did not produce a positive 3,4-dihydroxyphenylalanine (DOPA) reaction.

The regions of the sheep examined included several producing hair and several producing wool. Samples were also taken from four regions (lower lip, between nares, nipple and vulva) devoid of follicles. In addition, samples were taken from the placenta of a full-term sheep foetus with plaques of wool follicles on the amnion[6]. Acetylcholinesterase-positive branched cells were found on all of the regions where there were follicles, but they were not found in the non-follicular regions. Even on the amnion the branched cells were confined to the plaques having follicles.

The processes of the branched cells (Fig. 1) extend throughout the epidermis, some even to the cornified layer, but the perikarya are evenly spaced (about 50μ

apart) and are located in the basal layer near the dermo-epidermal junction. Although the population densities of melanotic melanocytes in the epidermis and follicles vary greatly between sheep, the number of branched cells per unit area was essentially the same in all sheep.

The population density of these branched cells was not modified by contact with solid carbon dioxide for up to 30 sec duration in any of the sheep examined, nor by exposure to ultra-violet light, except in a Merino with a very sparse population of melanotic melanocytes. In this animal there was a reduction of the acetylcholinesterase-positive cells after a moderate exposure to ultra-violet radiation and an almost complete loss of the cells from the epidermis, but not from the upper external sheaths of the follicles, following a more severe ultra-violet burn. In the other animals so treated, a dark-skinned Merino and a Southdown, both with numerous melanotic melanocytes, there was tanning but not burning, and the number of positive branched cells per unit area was not changed. From these limited observations it appears that the basal layer, including the perikarya of branched cells, is protected from ultra-violet radiation by the presence of melanin in the melanocytes.

The precise nature and function of these cells are not known. Even though these branched cells in the sheep are acetylcholinesterase-positive and do not react for alkaline phosphatase or with osmium iodide, they seem to be generally similar in morphology and pattern of distribution to the branched cells of some African primates[1-3]. They also appear similar in morphology to the epidermal dendritic cells which have been revealed in other animals[4,5]. The similar distribution of branched cells in epidermal regions with melanocytes and in epidermal regions without melanocytes indicates that branched cells in sheep are not effete melanocytes. It is conceivable that these branched cells are derived from stem cells which can mature and differentiate either into the Langerhans type[5,7] or in the direction of a melanotic melanocyte.



Fig. 1. Acetylcholinesterase-positive branched cells in the basal layer of the epidermis of the mid-lateral region of the trunk of an adult Merino ewe. Frozen section (40μ thick) cut parallel to the skin surface

Alternatively, it may be that at the dermo-epidermal junction there are stem cells for melanocytes and separate stem cells for branched cells, both derived from the neural crest. Branched cells may well have a supportive role, or perhaps a trophic function as indicated by their regular pattern (Fig. 1) and close proximity to all epidermal cells below the stratum corneum.

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HISTOCHEMISTRY

Particles in Leucocytes of Normal Humans Beings after Negative Staining in Electron Microscopy

The negative staining technique for the study of cells and cellular components by electron microscopy has been described by Almeida and Howatson[1]. A similar method has been applied to study the morphology of the Moloney leukaemia virus in megakaryocytes in experimentally infected mice[2]. Recently, virus-like particles have been described in the plasma of many leukaemic humans examined by a negative-staining technique[3-6]. This report describes the application of a similar technique to leucocytes of normal human beings.

Leucocytes of normal adult humans were separated from heparinized fresh blood (0·5 mg heparin to 1 cm³ of blood) from five healthy individuals. The erythrocytes were allowed to settle at room temperature for 1 h. The plasma, rich in leucocytes, was drawn off, centrifuged 15 min at 10,000*g*, washed with 0·05 M citrate buffer *p*H 6·8, recentrifuged, and resuspended in the citrate buffer. (Some agglutination occurred.) The cells were frozen at −65° C, thawed, and mixed with 2 per cent phosphotungstic acid (PTA) (*p*H 7·0) to a final concentration of 1 per cent PTA. They were examined with an EMU-3 microscope.

Particles were seen within leucocytes and emerging from them (Figs. 1 and 2). The specific types of leucocytes could not be identified because of distortion during the procedure. The particles varied in shape and included toroidal, tailed, reticulated and stranded forms. The toroidal particles were relatively numerous and ranged in diameter from 90 to 350 mμ. Their nature is not definitely known, but we suspect that they consist of nuclear material because of their central location when seen in intact cells.

Because many of the smaller particles from normal individuals bear a striking resemblance to particles described by Dalton[2] in leukaemic mice, it appears that the negative-staining technique must be used with caution in identifying viruses within cells or in preparations made from cells. This is in agreement with Almeida's conclusion that preparations of the blood of normal people reveal many particles in the same size range as virus particles. These may be mistaken for virus particles unless other criteria are considered regarding the morphology of the

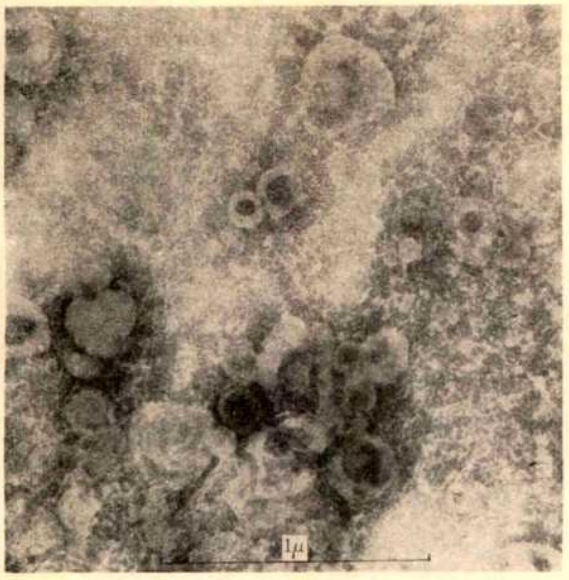


Fig. 1

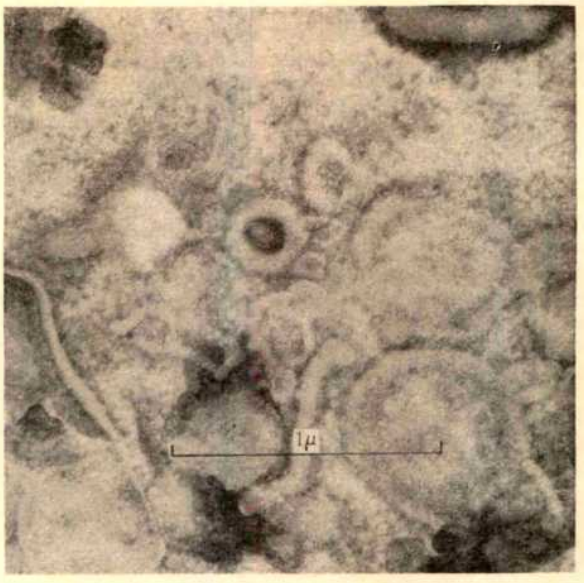


Fig. 2

Figs. 1 and 2. Particles emerging from a leucocyte of a normal human being after heparinized cells were frozen, thawed and mixed with PTA. Toroidal forms are common. Other particles in evidence include reticulated, stranded, encapsulated and tailed forms

particles known to be associated with negatively stained materials.

We therefore consider that morphological criteria by the PTA technique are inadequate when searching for new human leukaemogenic viruses in tissue, since normal human cell components have a strikingly similar morphology to well-known murine leukaemia viruses by the PTA technique.

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IMMUNOLOGY

Failure to transfer Sensitivity to Skin Homografts by Means of 'Immune' Lymphoid Cells in Diffusion Chambers

ALTHOUGH there has been a considerable number of reports dealing with the susceptibility of dissociated cellular homografts of many tissues, notably those derived from the lymphohaematopoietic system, to humoral isoantibodies[1], the majority of attempts to demonstrate that humoral factors play any significant part in the destruction of skin and other solid tissue homografts have failed[2]. Indeed, most of the available evidence sustains the premise that a 'cellular' immunity is involved and that the demise of a homograft is causally related to its characteristic infiltration by mononuclear cells.

What might be construed as a serious challenge to this thesis are the reports of Najarian and Feldman of the transferability of sensitivity to skin homografts in mice and guinea-pigs by means of viable suspensions of 'immune' lymphoid cells, enclosed within cell-impenetrable 'Millipore' diffusion chambers, implanted either intraperitoneally or subcutaneously into isologous hosts[3,4]. Similar findings have been reported in rabbits[5]. Our preliminary, small-scale attempts to confirm these observations, using both immunologically tolerant and normal mice as hosts, were unsuccessful[6]. However, we felt that the far-reaching implications of Najarian and Feldman's observations[3,4] concerning the mechanism of homograft destruction justified a more extensive investigation, and this communication reports our findings.

As before[6], every attempt was made to follow Najarian and Feldman's protocols as closely as possible. Adult mice of both sexes, derived from domestically maintained sub-lines of strains *A* and *CBA*, were used throughout, and both the possible donor/recipient combinations were tested. The median survival times (± standard error) of skin homografts exchanged between members of these strains, as determined from a histological appraisal of biopsied specimens removed at regularly spaced intervals, are[7]:

$CBA \rightarrow A$	$10{\cdot}2 \pm 0{\cdot}3$ days
$A \rightarrow CBA$	$11{\cdot}0 \pm 0{\cdot}3$ days

Sensitization of donors of immune lymphoid cells was achieved by grafting them bilaterally with skin homografts followed, 2 days later, by an intraperitoneal inoculation of a suspension of 10 million homologous spleen cells in Hanks's balanced salt solution. Nine days after exposure to the skin homografts these animals were killed and their spleens, axillary, brachial, cervical and mesenteric nodes excised, pooled and dissociated in Hanks's solution containing polyvinylpyrrolidone (final concentration 0·35 per cent v/v) to produce a cell suspension. On the basis of trypan blue dye exclusion tests, not less than 90 per cent of the cells were viable.

0·12-ml. portions of this suspension, containing about 200 million cells, were introduced into a series of sterile 'Millipore' chambers (pore size 100 mμ) which were then sealed. Two of these were then inserted into the peritoneal cavity of each of the isogenic host mice.

In some experiments host mice were challenged with skin homografts from the alien donor strain immediately after receipt of the diffusion chambers; in others, test grafting was delayed for 2 days. Care was taken to avoid areas of 'active' skin as donor sites for the test grafts. Instead of suturing the grafts in place, as did Najarian and Feldman, we used our standard procedure of keeping the grafts in position on their beds—afforded by the intact panniculus carnosus with its overlying vasculature—under light pressure by means of dressings around the thorax[8].

In some experiments, the survival times of the test grafts were assessed visually, scoring daily the degree of epithelial survival from the sixth day onwards. In others, the grafts were removed on the sixth post-operative day for histological appraisal of the percentage of epithelial survival.

Diffusion chambers containing normal instead of immune lymphoid cell suspensions were implanted into some groups of mice, and these acted as our controls. Our criterion of transfer of immunity was any evidence of accelerated destruction of test grafts borne by recipients of immune cells.

Table 1. Comparison of the Fate of Test Skin Homografts on Mice bearing Diffusion Chambers containing Isologous Lymphoid Cells from (*a*) Normal and (*b*) Specifically Sensitized Mice

Strain combination	Immunological status of transferred cells	No. of hosts tested	Appraisal of test skin homografts: Survival times (days)	Or percentage of epithelial survival in 6-day biopsy specimens
CBA→*A*	Normal	12	9, 4×10, 4×11, 2×12, 13 (MST = 10·2 ± 1·12)	—
	Sensitized	20	9, 2×10, 7×11, 5×12, 13, 14, 3×15 (MST = 11·5 ± 1·16)	—
	Normal	19	—	13×100, 3×95, 75, 2×50
	Sensitized	20	—	6×100, 7×95, 5×75, 2×50
A→*CBA*	Sensitized	9	9, 7×10, 12 (MST = 10·2 ± 1·06)	—
	Normal	13	—	11×100, 95, 75
	Sensitized	17	—	12×100, 2×95, 75, 2×25

Table 1 summarizes the various tests carried out and their findings. Since the results of delaying test grafting for 2 days were similar to those obtained when test grafts were applied at the time of insertion of the diffusion chambers, the data have been pooled. With neither donor/recipient strain combination was there any evidence that sensitivity had been transferred. The median survival times of *CBA* grafts on *A* strain recipients bearing chambers containing normal or immune lymphoid cells were 10·2 ± 1·12 and 11·5 ± 1·16 days, respectively. The *CBA* recipients of sequestered immune node cells rejected their *A* skin test grafts within about 10 days (median survival time 10·2 ± 1·06 days).

Histological scores of the degree of epithelial survival in the 6-day biopsy specimens were in complete accord with the determinations of survival time. At the sixth post-operative day, 90 per cent of the grafts exhibited high degrees of survival of a hyperplastic epidermis characteristic of first-set skin homografts. With the *CBA* → *A* combination, several test grafts on the recipients of sensitized node cells survived a few days longer than on the controls. A likely explanation of this slight prolongation is that it is due to the immunological enhancing activity of antibody released by the sequestered immune node cells[9].

In view of the close similarity of design between the present experiments and those of Najarian and Feldman, it is difficult to account for the discrepancy in the results obtained. The essential differences between the two procedures were: (*a*) bilateral grafting of the donors of the immune lymphoid cells rather than giving them a single mid-dorsal graft, and (*b*) securing the test grafts in place by means of dressings, rather than suturing.

That the lymphoid cell suspensions introduced into our chambers were viable was attested by the results of the dye exclusion tests, and also by the fact that a small number of cells prepared by this procedure and injected intraperitoneally into tolerant mice were highly effective in procuring the destruction of hitherto long-tolerated grafts[6].

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Inhibitory Effect of Carcinostatic Agents on Antitumour Activity of Immune Lymphoid Cells

It has been widely demonstrated that *in vivo* application of carcinostatic agents inhibits the host immune response, namely, inhibition of antibody production by antimetabolites[1–4], alkylating agents[1,5] and antibiotics[6,7], and prolongation of homograft survival by a nitrogen mustard derivative[8]. Investigations of the mode of action of these agents on the immune function of lymphoid cells sensitized with homologous cells would provide interesting information on the mechanism of action of the lymphoid cells in transplantation immunity, since some of these agents have been known to act on the specific sites of the cellular functions.

We have recently demonstrated that the peritoneal fluid cells of Donryu rats[9] immune to homologous tumour, Yoshida sarcoma, showed a marked antitumour action both in transfer system and in diffusion chamber[10]. The experiment described here shows the effects of several carcinostatic agents on the cellular immunity of the peritoneal fluid cells sensitized with Yoshida sarcoma and on the growth of the tumour cells.

Peritoneal fluid cells were collected, following intraperitoneal injection of 5 ml. heparin–Hanks's solution in a rat, from male Donryu rats which had been repeatedly immunized with Yoshida sarcoma by a method previously reported[10]. The pooled fluids, containing 10^7 cells/ml., were incubated for 30 min at 37° C both with and without the carcinostatic agent. The cells were sedimented and washed twice with fresh Hanks's solution. The washed cells were then suspended in Hanks's solution with a concentration of 10^7 peritoneal fluid cells/ml. In all experiments in which the viability of these cells was examined by the eosin stain test, more than 90 per cent of them were demonstrated to be viable at this time. The suspension was mixed with an equal volume of suspension containing 10^5 Yoshida sarcoma cells/ml., and the mixture was kept at 25° C for 10 min. Two ml. of the mixture was intraperitoneally injected into a recipient Donryu rat, and the tumour development was observed. When the antitumour function of the immune peritoneal cells diminished, the recipient animals died due to tumour growth.

Table 1. Effects of Carcinostatic Agents on the Anti-Yoshida Sarcoma Function of Immune Peritoneal Fluid Cells and on Yoshida Sarcoma Cells

Series 1

Agent	Cells for inoculation	Concentration of agent for treatment of cells						
		No. of recipient rats dying with tumour/animals tested						
Nitrogen mustard		0	0·0016	0·016	0·16	1·6	16 μg/ml.	Control
	YS + treated PFC	0/10		0/10	3/10	10/10	10/10	10/10
	Treated YS	3/3	6/6	2/6	0/6			
Mitomycin *C*		0	0·2	2	20	100	μg/ml.	
	YS + treated PFC	1/9	0/5	0/9	3/9	4/4		10/10
	Treated YS		3/3	1/3				
Actinomycin *D*		0	0·06	0·6	6	μg/ml.		
	YS + treated PFC	0/5	1/5	0/5	4/5			5/5
	Treated YS		3/3	2/3	0/3			
Puromycin		0	0·48	4·8	48	μg/ml.		
	YS + treated PFC	0/5	1/5	1/5	1/5			5/5
	Treated YS		3/3	3/3	3/3			
6-Mercaptopurine		0	150	1,500		μg/ml.		
	YS + treated PFC	0/5	1/5	1/5				5/5
	Treated YS		3/3	3/3				

Series 2

Actinomycin *D*		0	0·3	0 65	1 3	2 6 μg/ml.	Control	
	YS + treated PFC	0/5	0/5	0/5	5/5	4/5	5/5	
	Treated YS	3/3	3/3	3/3	1/3	0/3		
Mitomycin *C*		0	4	8	16 5	33	66 μg/ml.	Control
	YS + treated PFC	0/5	0/5	0/5	2/5	3/5	5/5	5/5
		0·2	0·4	0·8	1·65	3 3	6·6 μg/ml.	
	Treated YS	3/3	3/3	3/3	2/3	1/3	0/3	

Underlining indicates that the first group was significantly affected by an agent, and the concentration of the agent was designated as the minimum effective concentration to the treated cells. Each animal in the first and second groups was given 10^7 cells of treated PFC (peritoneal fluid cells from the immune rats) mixed with 10^5 YS (Yoshida sarcoma cells), and 10^7 cells of treated YS, respectively. Control animals were transplanted with 10^5 YS cells alone intraperitoneally.

The anti-Yoshida sarcoma activity of an agent was tested in the similar way to that already described. Suspensions containing 10^7 tumour cells/ml. were incubated with the agent in Hanks's solution and the twice-washed 10^7 cells were injected intraperitoneally into a rat.

It can be seen from Table 1 that in Series 1 exposure to nitrogen mustard, mitomycin *C*, or actinomycin *D* at certain concentrations abolished both the antitumour function of the sensitized peritoneal fluid cells and the growth of Yoshida sarcoma cells; on the other hand, puromycin and 6-mercaptopurine did not act on these cells even at higher concentrations.

The antibiotics mitomycin *C* and actinomycin *D* are especially interesting, since it is known that they interact specifically with nucleic acids. Bloom *et al.*[11] reported that exposure to mitomycin *C* *in vitro* at rather high concentration abolished the capacity of cellular transfer of delayed-type hypersensitivity in sensitized lymphoid cells. Actinomycin *D* has been found to be an effective inhibitor of antibody formation in lymphoid cells[12–14], and this antibiotic acted differentially on the immunologically determined and competent cells[13]. The heterologous host–antigen systems were used in these *in vitro* experiments.

Thus a more detailed study of the action of actinomycin *D* and mitomycin *C* on the immune peritoneal fluid cells and on Yoshida sarcoma cells was undertaken. The results of Series 2 in Table 1 show that the minimum effective concentrations of actinomycin *D* to the sensitized peritoneal fluid cells and to Yoshida sarcoma cells were 1·3 μg and 1·3 μg/ml., respectively, while those of mitomycin *C* were 16·5–33 μg and 3·3 μg/ml. Thus, the ratios of minimum effective concentration to the immune peritoneal cells against that to tumour cells were 1 for actinomycin *D* and 5–10 for mitomycin *C*. In the case of nitrogen mustard, the ratio was approximately 10 (Table 1—Series 1). If these agents act in a similar manner on the sites of the cells which control growth and viability of the tumour cells (or antihomograft function of the lymphoid cells) the ratios may be equal among them. It is possible that the significant difference of the ratio indicates that the antitumour function of the sensitized peritoneal fluid cells is specially inhibited by short-term exposure to actinomycin *D*.

It has been known for some time that actinomycin *D* inhibits DNA-directed RNA synthesis[15,16], and, moreover, that when the RNA synthesis is inhibited the immune cells lose their immunological function[11]. It is thus possible that the antitumour function of the sensitized peritoneal fluid cells in the present experiment are related to an RNA-dependent function of the cells. However, whether the duration of the viability of the cells before and after exposure to the carcinostatic agents is equal, and whether the non-specific affinity to the cells is different among these agents, require further investigation.

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PATHOLOGY

Increased Localization and Incorporation of Iododeoxyuridine into Tumour DNA *in vivo* when coupled to Polycation

There is some evidence that tumour cell surfaces are in a hyperanionic state which could be associated with lowered intercellular adhesion and contact inhibition of mitosis[1,2]. If cells of tumour tissues have a higher anionic surface charge or the hyperanionic state lasts for a greater portion of the intermitotic cycle than other growing cells of the body, it should be possible to accumulate cations in favour of the tumour cell surface. By coupling cytotoxic drugs to these cations it might be further possible to attain lethal toxicity in tumour cells before destroying other vital tissues.

This communication describes the incorporation of the cytotoxic and radiosensitizing drug 5-iododeoxyuridine (IUdR) into the DNA of various tissues of the C_3H strain of mice bearing spontaneous mammary tumours after prolonged injection of either the free drug or its water-soluble complex with polycation. The polycation polyethylene imine (PE) (molecular weight $3–5 \times 10^3$) was complexed with IUdR or its derivative 5-iododeoxycytidine monophosphate (IdCMP) which provides a stronger salt link by way of its phosphate group to the basic nitrogen atom of PE. IdCMP, which is readily deaminated by mammalian tissues to yield iododeoxyuridine monophosphate, was prepared by direct iodination of IdCMP (ref. 3). Complex formation was achieved by simple mixing in aqueous solution and integrity of the product was confirmed by elution from 'Sephadex *G*-25' as a single component.

The first aim of these experiments was to trace the relative incorporation of IUdR into tumour DNA and DNA of other tissues known to incorporate more IUdR than tumour, namely, spleen and intestinal mucosa[4]. Leucopenia results from IUdR administration at doses subtoxic to tumour tissue even though bone marrow DNA contains less IUdR than tumour DNA, so white blood cell counts were included in these experiments.

The second aim was to discover conditions which would produce sufficient IUdR replacement of tumour DNA thymidine to render it lethally radiosensitive. It has been shown that 7–9 per cent replacement of DNA thymidine by IUdR in human cells *in vitro* produces a radiosensitization factor of 1·6 (ref. 5), but toxic doses of IUdR given systemically *in vivo* result in only 1–2 per cent replacement of tumour DNA thymidine[6]. If 5-fluorodeoxyuridine (FUdR) is given simultaneously to inhibit thymidine synthesis, IUdR incorporation in DNA of dividing cells can be increased at the expense of greater toxicity—chiefly leucopenia. In these experiments FUdR or its derivative 5-fluorodeoxycytidine monophosphate (FdCMP) was coupled along with IUdR or IdCMP to PE such that the iodine : fluorine ratios were 2·5 : 1 or 10 : 1. The tumour-localizing compound[7], 1 : 4-naphthaquinol-*bis*disodium phosphate ('Synkayvite', Roche), was also coupled to the drug-polycation complex in the expectation that it might favour accumulation on the tumour cell surface even further. Drugs and their complexes were injected subcutaneously for 5 days, after which blood was sampled from a tail vein for the white cell count. The animals were then killed and the intestines removed into ice-cold 0·14 M sodium chloride; 0·02 M to versene at *p*H 8·0. Spleen and tumour tissue free of necrotic debris were next removed and placed on dry ice. Intestinal contents were lightly squeezed out, the lumen opened and epithelium rapidly scraped off into 0·02 M sodium chloride containing 0·02 M versene at *p*H 8·0 and 1 per cent sodium lauryl sulphate. Tumour and spleen were thawed out and homogenized in the same medium. DNA was then prepared from these homogenates in a fibrous deproteinized form by the method of Kay *et al.*[8], which excludes all sources of iodine other than incorporated IUdR. After accurate assay by the diphenylamine reaction, 100–200 μg samples of DNA were then ashed with sodium bicarbonate and iodide content measured by the arsenious acid/ceric sulphate titration method described by Szabo[9]. Although accurately reproducible, this method results in 20–25 per cent losses of iodine during ashing.

Table 1, line (*a*), confirms the leucopenic effect and greater incorporation of IUdR into spleen and mucosae DNA than tumour DNA when free nucleosides are used. Presence of PE restores the white cell count to normal ($3·5–4·5 \times 10^3$/mm^3 in these animals); increases overall IUdR incorporation and improves localization in favour of the tumour (line *b*). The incorporation and tumour localization of IUdR are even greater when PE-coupled nucleotides are used (line *d*).

Table 1. Incorporation of IUdR into DNA of Tumour, Spleen and Intestinal Mucosa after Administration of IUdR and FUdR in the Ratio 2·5 . 1

	μg iodine/mg DNA (1) Tumour	(2) Spleen	(3) Mucosa	Ratio I/II*	Ratio I/III*	White cell count/ mm^3 × 10^3
(*a*) Free nucleosides	0·67	0 82	1·92	0 82	0·35	1·6
(*b*) (*a*) with PE	1 98	1·52	3·55	1·30	0·55	5·9
(*c*) Free nucleotides	1·81	2·01	2·82	0·90	0 64	0 6
(*d*) (*c*) with PE	2·44	1·90	3·94	1·28	0·62	3·4

All results are the mean of three animals. Daily dosage divided into three 8-hourly injections subcutaneously, in 0·2 ml. water for 5 days. (*a*) IUdR 50 mg, FUdR 20 mg/kg/day; (*b*) as (*a*) coupled to 10 mg PE/kg/day; (*c*) IdCMP 60 mg, FdCMP 24 mg/kg/day; (*d*) as (*c*) coupled to 10 mg PE/kg/day.
* Ratios are calculated from the individual iodine assay data, then averaged.

In Table 2, data from several types of experiments differing slightly in their dosage schedule or drug vehicle have been pooled to evaluate the localizing effect of polycation and 'Synkayvite' on tumour DNA incorporation of IUdR. Animals, the results of which are shown in lines (*b*) and (*c*), have been paired under each of the slightly varying conditions. When 'Synkayvite' is coupled to the polycation-drug complex there is an increase of 28 per cent in the tumour : spleen ratio and 42 per cent in the tumour : mucosa ratio. The magnitude of standard deviation, however, renders these differences statistically insignificant. Further experiments under standardized conditions will be undertaken to confirm this effect when more FdCMP is available.

Table 2. Effect of Coupling 'Synkayvite' to Polycation Complex with IdCMP and FdCMP (Ratio 2·5 : 1) on the Localization of IdUR in Tumour DNA

	Tumour DNA iodine / Spleen DNA iodine	Tumour DNA iodine / Mucosa DNA iodine	White cell count/ mm^3 × 10^3
(*a*) Nucleosides only	0·86 ± 0·23 (12)	0 45 ± 0·19 (12)	1·01 ± 0·48 (12)
(*b*) Nucleotides with polycation	1·85 ± 1·26 (12)	0 69 ± 0 24 (12)	3·55 ± 0·81 (12)
(*c*) as (*b*) with 'Synkayvite'*	2·33 ± 1·46 (12)	0·98 ± 0·41 (12)	3 55 ± 1·02 (12)

(*a*) IUdR 50 mg, FUdR 20 mg/kg/day; (*b*) IdCMP 60 mg, FdCMP 24 mg coupled to 10 mg PE or 80 mg protamine free base/kg/day, (*c*) as for (*b*) coupled to 4 mg 'Synkayvite'/kg/day. Daily dose divided into either 3 × 8-hourly injections in 0 2 ml. water or 2 × 12-hourly injections in peanut oil emulsion (0·3 ml.) given subcutaneously for 5 days.
* In 3 animals 0 75 mg methotrexate/kg/day substituted for FdCMP.

Table 3 shows the results of using a 10 : 1 IUdR : FUdR ratio with and without PE and 'Synkayvite'. As judged by comparison of the white cell counts in line (*a*) of Tables 1 and 2, this increased ratio of IUdR to FUdR has a lower antimitotic effect, but incorporation of IUdR into tumour DNA is increased. The dosage schedule for line (*c*) Table 3 gives both the best localization and highest incorporation of IUdR into tumour DNA. The value of 3·2 μg iodine/mg DNA is equivalent to nearly 6·5 per cent thymidine replacement after compensating for iodine losses in the ashing step of the assay. By using PE-coupled nucleotides one can increase IUdR incorporation into tumour DNA over three-fold compared with free nucleosides. This could be due either to the protective effect of phosphate or polycation groups against enzymes which rapidly dehalogenate free IUdR, or to the pinocytotic transport of the intact complex into tumour cells such

Table 3. INCORPORATION OF IUdR INTO DNA OF TUMOUR, SPLEEN AND INTESTINAL MUCOSAE AFTER ADMINISTRATION OF IUdR AND FUdR IN THE RATIO 10 . 1

	'Synkayvite'	γ g Iodine/mg DNA			Ratio I/II	Ratio I/III	White cell count/ mm³ × 10³	No. animals
		I Tumour	II Spleen	III Mucosa				
(*a*) Nucleosides only	−	0 98	1·67	1·80	0·61	0·55	2·5	(2)
	+	1 21	1·32	1·28	0·92	0·94	2·4	(3)
(*b*) as (*a*) with PE	−	1·54	1·81	2 29	0·87	0·67	4·4	(3)
	+	2 25	1 86	3·00	1·50	0·75	4·5	(2)
(*c*) Nucleotides with PE	−	3 20	2·03	2 50	1·73	1·51	4·5	(3)
	+	2·80	1 96	2 18	1·30	1·58	3·8	(3)

(*a*) 70 mg IUdR, 7 mg FUdR/kg/day; (*b*) as (*a*) coupled to 10 mg PE/kg/day; (*c*) 80 mg IdCMP, 8 mg FdCMP, coupled to 10 mg PE/kg/ day. 'Synkayvite' where present at 4 mg/kg/day. Daily dose divided into 2 × 12-hourly subcutaneous injections in 0·3 ml. water for 5 days.

that IdCMP can enter directly into the DNA synthetic pathway. If this is true, then PE-coupled iododeoxycytidine triphosphate would be utilized even more efficiently since nucleoside triphosphates are the immediate substrates for DNA polymerase. This and other factors designed to increase the localization and incorporation of IUdR into tumour DNA *in vivo* still further are being investigated.

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RADIOBIOLOGY

On the Shape of the Dose-response Curve for HeLa Cells cultured *in vitro* and exposed to Gamma-radiation

PROGRESS in theoretical radiobiology depends very largely on the interpretation of dose-response curves; nowadays, interest is being focused on the reproductive survival of mammalian cells. Unfortunately research workers measure, not survival 'curves', but a limited number of discrete points. These data, even if free from artefacts which would render them meaningless, have such limited statistical significance that it is often possible to fit more than one mutually exclusive theoretical curve through them.

Much effort has been devoted to the development of the multi-target and multi-hit models of radiation damage at the cellular level[1–4]. If survival curves are plotted in the conventional way, that is, the fraction surviving on a logarithmic scale, versus dose on a linear scale, the multi-target model leads one to expect a dose-response curve which, after an initial shoulder, becomes straight; the multi-hit model would predict a curve which bends continuously and never straightens, although this curvature may be very small over the range of survival used in experimental investigations[3]. X-ray survival curves obtained *in vivo* by serial dilution assay of transplantable isologous murine tumours, after a small initial shoulder, are straight lines down to a surviving fraction of 10^{-7} (ref. 5). Survival curves reported by different workers using the Puck clonal technique would seem to conflict; some remain straight on a logarithmic-linear plot, while others bend. Before the shape of these experimentally determined curves is regarded as evidence for or against any theoretical model, it is of prime importance to ensure that all artefacts are removed from the experimental technique. Berry[5] has pointed out one such artefact which caused an apparent bending of his HeLa survival curve; failure to change medium at 7 days in plates containing a large number of cells resulted in spuriously low values of survival at high doses.

The present communication describes another artefact, which is a direct result of a basic technique in this type of *in vitro* cell survival assay. It is common practice to inoculate enough cells into irradiated plates so that approximately the same number of cells will survive to form colonies as will form on the control plates into which about 250 cells are seeded. As the dose is increased, therefore, the number of cells seeded must be rapidly increased, and it is not surprising that a point is reached where nutrient depletion and/or overcrowding would result in a spuriously low survival. When Puck and Marcus[6] first described the clonal technique they gave a warning that the low survival estimates would "necessitate such tremendous cell crowding as to require changes in the experimental technique". This advice has not always been heeded.

The following experiments were carried out to determine at what point overcrowding would seriously affect the surviving fraction. The cell line used was the HeLa $S\text{-}3_{0xf.}$ obtained from Dr. C. E. Ford at the Medical Research Council's Unit at Harwell, and recloned in this laboratory by Dr. R. J. Berry. The methods of culture followed closely those described by Puck and Marcus[6]; the medium used as a 1 : 1 mixture of *N*16 and saline *F*[7], supplemented with 15 per cent human serum. 60-mm Falcon plastic Petri dishes were used throughout. Plates to be exposed to 1,000 rads were seeded with 5×10^3 or 5×10^4 cells; plates to be exposed to 1,250 rads received 5×10^4 or 5×10^5 cells; plates to be exposed to 1,500 rads received 10^6, 5×10^5, 10^5 or 5×10^4 cells. In each experiment the medium was changed at 7 days following the advice of Berry[5]; it was found that this procedure did not affect the survival in plates containing 5×10^4 or less cells, but produced a marked effect in plates containing 5×10^5 or 10^6 cells. Surviving colonies (of more than 50 cells) were counted 14 days later, after staining and fixing. The results are plotted in Fig. 1. It is evident that changing from 5×10^3 to 5×10^4 cells per dish did not effect surviving fraction (as evidenced by the survival to 1,000 rads); however, increasing the number of cells per dish more than 5×10^4 resulted in a continuously decreasing surviving fraction (as indicated by the survival to 1,250 and 1,500 rads). Under these conditions, the survival curve shown in Fig. 2 was obtained. The number of 5×10^4 cells per plate was never exceeded; for doses more than 1,000 rads the number of plates per point was increased instead of the number of cells per plate in order that enough colonies could be counted to give a meaningful estimate of survival. Each point on the survival curve is the mean of between three and nine separate determinations.

It would seem, therefore, that after an initial shoulder, the survival curve for HeLa $S\text{-}3_{0xf.}$ cells does not depart

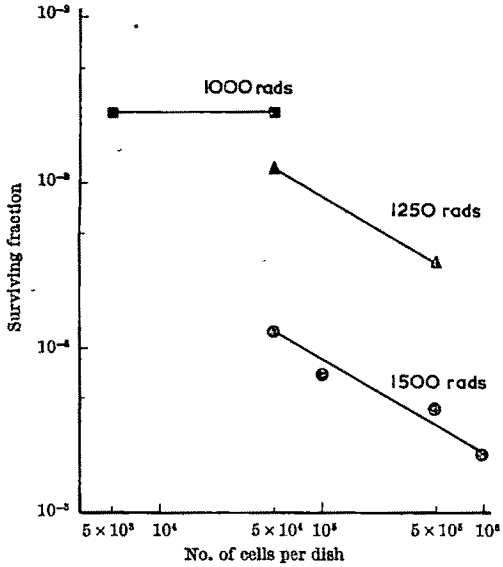


Fig. 1. Illustrating the decrease in surviving fraction that results from increasing the number of cells inoculated into each Petri dish

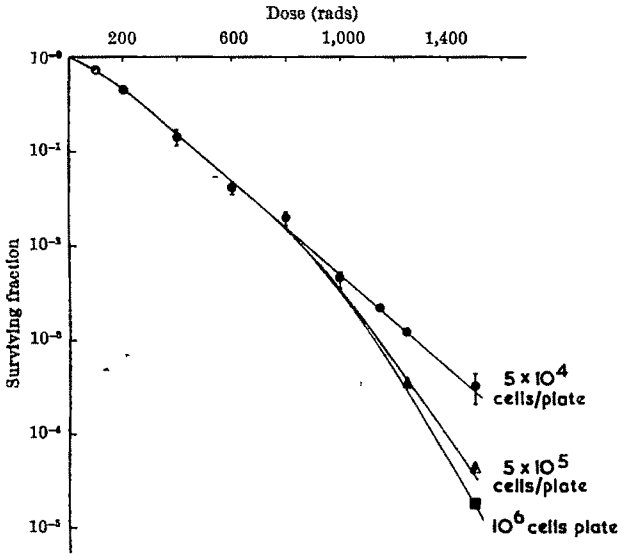


Fig. 2. Survival curve for HeLa *S*-3 *str.* cells exposed to *γ*-radiation from cobalt-60. After an initial shoulder, the curve remains straight provided that not more than 5×10^4 cells are inoculated per Petri dish. If larger numbers of cells are used, the curve continues to bend downwards

from a straight line at least down to a surviving fraction of 10^{-4}. Also plotted on Fig. 2 are the data obtained if the foregoing precaution is ignored and the number of cells per plate increased for higher doses; by this method a continuously bending curve is obtained which can be attributed to overcrowding at higher doses.

In summary, then, it must be demonstrated unequivocally that survival estimates are independent of the number of cells plated before attributing any significance to the shape of the survival curve.

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BIOLOGY

Site of Action of Ribonuclease during its Inhibition of Egg Cleavage

The mitotic division of sea urchin eggs is affected by ribonuclease (RNase). Ledoux and Metz[1] found that cleavage was blocked by $1{\cdot}5 \times 10^{-4}$ M RNase, and Runnström[2] has observed that cortical changes following fertilization were delayed by RNase at one-tenth of this concentration. These effects might be expected to result from enzymatic attack of the RNA present in all parts of the egg, including the mitotic apparatus[3] and egg surface[4]. To determine which parts of the egg are affected by RNase, the site of interaction of the fluorescein-labelled enzyme with living eggs and embryonic stages of *Arbacia punctulata* and *Lytechinus variegatus* has been investigated, using fluorescence microscopy. RNase '5 X' crystalline (Nutritional Biochemicals Corp., Cleveland, Ohio) was conjugated at *p*H 9·0 with fluorescein isothiocyanate (Sylvana)[5]. Direct fluorescent labelling has been found suitable for investigating the uptake of enzymes by cells[6], and enzymatic assay and electrophoresis of fluorescein-labelled RNase showed that the fluorescein was firmly bound without destroying RNase activity[7].

Effect of RNase on cleavage. When applied at $1{\cdot}5 \times 10^{-3}$ M either 60 min before or 3 min after fertilization, RNase blocked the cleavage of at least 90 per cent of *Lytechinus* and *Arbacia* eggs, and arrested the development of the remainder at the two- or four-cell stage. The concentration required was ten times that reported[1] for a different batch of RNase from the same commercial source. The block was reduced to 75 per cent when the *p*H of the applied enzyme was adjusted from 5·1 to 7·0, and disappeared when the treated eggs were washed. Table 1 shows the delay in development resulting from different periods of treatment with $1{\cdot}5 \times 10^{-3}$ M RNase. At lower concentrations (10^{-4} to 10^{-3} M) development was delayed and the embryonic cells showed some dissociation. However, cleavage was not blocked at these concentrations, even in eggs treated after removal of their jelly coats by acidified sea-water, *p*H 4·5, and most of the eggs eventually developed into morphologically normal embryos.

To determine whether cleavage inhibition might be a general property of basic proteins, the effect of protamine sulphate on cleavage has also been investigated. At $3{\cdot}0 \times 10^{-4}$ M, protamine sulphate blocked cleavage, while the division of 50 per cent of the eggs was blocked at half this concentration. The protamine sulphate block was not reduced by adjustment to *p*H 7·0, and was only partially reversed by washing, becoming permanent after 30 min application. Since protamine sulphate also proved effective in blocking egg cleavage, inhibition of cleavage by RNase may be due primarily to its properties as a basic protein.

Site of action. When applied to living fertilized eggs at cleavage-inhibiting concentrations, fluorescein-labelled RNase (*p*H 7·2) was adsorbed on to the fertilization membrane, passed into the perivitelline space and became associated with the hyaline layer. The amount bound to the fertilization membrane and hyaline layer could be reduced by repeated washing, the reduction being constant over the *p*H range 5·0–7·0. The resolution of the technique was insufficient to permit detection of any interaction with the plasma membrane or structures associated with it, but in no case was the labelled RNase detected within the egg. When applied to unfertilized eggs, there was no interaction other than a weak surface adsorption, even after the jelly coats had been removed. During development from the blastula to the pluteus stage, labelled RNase was taken up as cytoplasmic droplets, mainly by cells of the alimentary canal. De Terra[8] has found that ferritin also becomes incorporated at the later stages of embryonic development.

Table 1. Effect of Ribonuclease ($1·5 \times 10^{-5}$ M) on the Development of *Lytechinus* Eggs

Duration of RNase treatment before washing	Delay in first cleavage (min)	Eggs developing to gastrulae Number	Per cent
None (control)	0	473/500	95
Pre-treated for 60 min before fertilization	0	469/500	94
1–30 min after fertilization	30	428/500	86
1–45 min after fertilization	90	423/500	85
Continuous after fertilization (not washed)	no cleavage	0/500	0

When fluorescein-labelled haemoglobin ($3·0 \times 10^{-4}$ M, *p*H 7·2) was applied to fertilized eggs it was bound at the same sites, suggesting that the observed binding of RNase was non-enzymatic. Treatment with fluorescein isothiocyanate at the concentration applied as the protein label (0·03 mg/ml.) showed that only the fertilization membrane was stained by unbound dye, and that the fluorescence of the hyaline layer was not an artefact due to any traces of free fluorescein. Runnström[2] also found that RNase affected the hyaline layer and fertilization membrane. The increase in birefringence[2] of the fertilization membranes of treated eggs may be partly due to the adsorption of RNase that has been observed with the fluorescent-labelled enzyme.

The reversibility of cleavage inhibition by washing and the site of interaction of fluorescent-labelled RNase with the egg both point to a post-fertilization effect on the egg surface rather than to direct interaction with any intracellular organelles. Since cleavage was blocked by similar concentrations of protamine sulphate, RNase may inhibit cleavage non-enzymatically by interacting with the egg surface as a basic protein.

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Factors controlling Sexual Reproduction and Growth in *Pythium graminicola*

Recently, it was found that sterols induce the formation of oogonia in certain species of *Pythium*[1] and *Phytophthora*[2]. We have shown that the response of these organisms to sterols depends on the composition of the medium in which they are grown[3]. This communication shows that zinc and manganese as well as sterol are essential to oogonium formation in an isolate of *Pythium graminicola*; calcium seems to be required for maturation of oogonia. Zinc, manganese and sterol are also very stimulatory to the vegetative growth of this fungus.

The culture used in this study was *Pythium graminicola* Subr. isolated from soil in Hawaii. Liquid media were used throughout. Test substances, as listed in Tables 1 and 2, were added to a basal medium containing KH_2PO_4, 0·68 g; $Na_2HPO_4 \cdot 7H_2O$, 1·34 g; glucose, 15 g; KNO_3, 1·08 g; $MgSO_4 \cdot 7H_2O$, 0·25 g; distilled, de-ionized water to 1 l.

The completed media, having initial *p*H values of 6·7–6·8, were autoclaved for 15 min at 120° C, and then dispensed into Petri dishes in 18-ml. aliquots. For each medium, six plates were prepared and inoculated in the centre with agar-mycelium plugs 4 mm in diameter. After 6 or 7 days at 26° C, the mycelium from three plates was collected on separate, tared filter papers, washed, dried and weighed. After 20 or 21 days, the other three plates were examined for oogonia, and the mycelium weights were then obtained as before. In contrast to earlier investigations in which agar media were used[3], we found that oogonial formation in liquid media proceeded more slowly and appeared to be optimal only after 3 weeks of incubation. The total number of oogonia in ten fields (150×) were counted, beginning at the colony margin and at 3·5-mm intervals along a radius.

Table 1 shows the response of the fungus to iron, calcium, thiamine, cholesterol, and a trace element mixture when added singly and in all possible combinations to the basal growth medium. Both cholesterol and the trace element mixture of zinc, manganese, copper and molybdenum were required for oogonial formation. Calcium caused an increase in the number of oogonia and was essential for their maturation since, in its absence, antheridia and oospores were not observed. Thiamine was generally slightly inhibitory to oogonial formation.

The 7-day mycelial weights show that all test substances were important to vegetative growth, since the omission of any one from the complete medium led to greatly decreased growth. The order of importance of these substances was shown by factorial analysis to be trace element mixture > sterol > thiamine > calcium > iron. Numerous significant interactions appeared, all indicating additive effects on growth. The 20-day weight results were not interpreted because autolysis was evident in some cases, and suspected in others.

In order to determine the individual and interaction effects of the components of the trace element mixture, these elements were added singly and in all possible combinations to the basal medium fortified with cholesterol, thiamine, calcium and iron. Treatments and results are shown in Table 2. The media containing both zinc and manganese produced much greater oogonial counts

Table 1. Effects on Vegetative Growth and Oogonial Formation of the Addition to a Basal Medium of One Multiple and Four Single Additives, in all Possible Combinations

(Data listed are averages of triplicate determinations per treatment)

Basal medium plus:	Mycelium weight (mg) 7 days	20 days	No. of oogonia 20 days
X—no additive control	7	18	0
Fe—iron*	10	18	0
Ca—calcium†	14	24	0
B_1—thiamine‡	15	17	0
S—sterol§	17	31	0
Tr—trace elements‖	8	28	0
Ca, Fe	19	51	0
B_1, Fe	22	38	0
S, Fe	16	36	0
Tr, Fe	12	43	0
B_1, Ca	16	23	0
S, Ca	25	40	0
Tr, Ca	19	33	0
S, B_1	17	37	0
Tr, B_1	18	36	0
Tr, S	28	89	367–*I***
B_1, Ca, Fe	21	52	0
S, Ca, Fe	13	49	0
Tr, Ca, Fe	29	93	10–*I*
S, B_1, Fe	16	35	0
Tr, B_1, Fe	28	69	0
Tr, S, Fe	36	84	333–*I*
S, B_1, Ca	27	50	0
Tr, B_1, Ca	30	88	0
Tr, S, Ca	60	98	1,860–*M*
Tr, S, B_1	48	96	760–*I*
S, B_1, Ca, Fe	38	62	0
Tr, B_1, Ca, Fe	63	91	11Tr–*I*
Tr, S, B_1, Fe	81	85	400–*I*
Tr, S, Ca, Fe	56	94	2,800–*M*
Tr, S, B_1, Ca	43	107	1,200–*I*
Tr, S, B_1, Ca, Fe	121	92	2,267–*M*
H.S.D. levels¶ <0.5	9	9	486
<0.1	10	10	545

* 1·0 mg/l. $FeSO_4 \cdot 7H_2O$.
† 50 mg/l. $CaCl_2 \cdot 2H_2O$.
‡ 2·0 mg/l. thiamine hydrochloride.
§ 10 mg/l. cholesterol.
‖ 4·4 mg/l. $ZnSO_4 \cdot 7H_2O$, 0·07 mg/l $MnCl_2 \cdot 4H_2O$, 0·08 mg/l. $CuSO_4 \cdot 5H_2O$, and 0·05 mg/l. $NaMoO_4 \cdot 2H_2O$.
¶ Honestly significant test between any two means (method of Tukey (ref. 4)) at the 1 per cent and 5 per cent confidence-levels.
** *I*, Immature; *M*, mature

Table 2. Effects on Vegetative Growth and Oogonial Formation of the Addition in All Possible Combinations of Four Additives to a Fortified Basal Medium

(Data listed are averages of triplicate determinations per treatment)

Fortified basal medium* plus:	Mycelium weight (mg) 6 days	Mycelium weight (mg) 21 days	No. of oogonia 21 days
X—no additive control	73	77	313–*I*§
Mo—molybdenum†	59	78	9–*I*
Cu—copper†	55	80	0
Mn—manganese†	58	93	0
Zn—zinc†	58	64	0
Cu, Mo	67	83	19–*I*
Mn, Mo	70	93	713–*M*
Mn, Cu	56	90	0
Zn, Mo	65	61	0
Zn, Cu	66	60	0
Zn, Mn	104	86	3,947–*M*
Mn, Cu, Mo	52	90	0
Zn, Cu, Mo	61	60	0
Zn, Mn, Mo	107	86	2,990–*M*
Zn, Mn, Cu	106	85	3,570–*M*
Zn, Mn, Cu, Mo	104	87	3,483–*M*
H.S.D. levels‡ <05	13	6	717
<01	15	7	843

* Basal medium fortified with cholesterol, thiamine, calcium, and iron at concentrations listed in Table 1.
† Added at the concentrations and in the salt forms listed in Table 1.
‡ Honestly significant test between any two means (method of Tukey (ref. 4)) at the 1 per cent and 5 per cent confidence-levels.
§ *I*, Immature; *M*, mature.

and 6-day mycelial weights than any of the media not containing both of these metals. The control and the manganese–molybdenum treatment are the only media which produced an appreciable number of oogonia without having both zinc and manganese present. By factorial analysis, both the latter metals were found to produce highly significant main factor effects. For vegetative growth, zinc had the greatest stimulatory effect; on interaction the effects of zinc and manganese tended to be additive. Copper and molybdenum showed no significant main factor effects. However, copper interacted significantly with the other three metals to inhibit vegetative growth, although it showed no effect on oogonial formation. As for the previous experiment, the 21-day mycelial weight results were not interpreted because of extensive autolysis.

Fothergill and Hide[5], working with four other species of *Pythium*, found that calcium, iron, trace minerals and thiamine had no effect on growth (no sterol was present). We found that all these additives stimulated the growth of *P. graminicola*, whether tested with our basal medium or with the media of Fothergill and Hide. This difference is undoubtedly attributable to a difference in the requirements of the species and isolates employed. Similarly, we found that *Pythium vexans* and *P. arrhenomanes* produced oogonia on Czapek's medium plus sterol, in the absence of zinc, manganese and calcium[3]. In addition to this, Hendrix[2] found that sterols induced sexual reproduction in *Pythium periplocum* and *Phytophthora magasperma*, although his basic medium contained no zinc, manganese or calcium. It is apparent that some species of *Pythium* have much more exacting metal ion requirements for reproduction and growth than do other species.

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Protandrous Hermaphroditism in Decapod Prawns of the Families Hippolytidae and Campylonotidae

Protandrous hermaphroditism, or the changing from the male sex to the female during the growth of the same individual, is now well known in certain northern-hemisphere commercial prawns of the family Pandalidae, namely, species of the genera *Pandalus* (*P. borealis*[1], *P. danae*[1], *P. hypsinotus*[1], *P. kessleri*[2], *P. montagui*[3] and *P. platyceros*[1]) and *Pandalopsis* (*P. dispar*[1]). In the Hippolytidae, this phenomenon has been demonstrated in two European and Mediterranean species of *Lysmata*, namely, *L. seticaudata* (the first decapod in which protandry was recognized[4]) and *L. nilita*[5].

In 1959, Carlisle[6] summarized our knowledge of sex reversal in decapods, discussing the aforementioned groups and adding the burrowing axiid *Calocaris macandreae*. The latter, however, has since been shown[7] not to be a protandrous hermaphrodite, but a functional hermaphrodite throughout life. Recently I recognized protandry in the Australasian, archibenthal slope species *Campylonotus rathbunae* Schmitt and discussed the changes in secondary sexual characters involved[8]. A partially sympatric pandalid, now known as *Notopandalus magnoculus* (Bate), is not a protandrous hermaphrodite. Another species in the southern, monogeneric family Campylonotidae, the Magellanic *C. semistriatus* Bate, was also shown to be protandrous from a re-examination of the *Challenger* type material in the British Museum (Natural History).

Protandry can now be recorded in the Magellanic and Antarctic hippolytid *Chorismus antarcticus* (Pfeffer). A collection taken at Isla Navarino off the southern coast of Tierra del Fuego during the Royal Society Darwin Memorial Expedition to Southern Chile, 1958–59, consisted of males of carapace lengths 6·5–10 mm with a well-developed appendix masculina on the second pleopod, intersex specimens of 10–10·5 mm with a vestigial appendix masculina and females of 12–13 mm with no trace of this appendix.

It was also considered likely that the Indian Ocean and Australasian subantarctic hippolytid, *Nauticaris marionis* Bate, would prove to be protandrous. Preliminary examination of recent collections from Auckland and Campbell Islands in the New Zealand subantarctic, and from deep water off southern New Zealand, revealed that all the large specimens were female, mostly ovigerous, while the smaller specimens were largely male. Detailed examination, however, showed males with a well-developed appendix masculina of carapace lengths 4·5–7·5 mm, no recognizable intersex specimens and females of carapace lengths 4·5–11·5 mm. This species, then, does not show sex reversal, but mature females do reach a much larger size than males.

Protandrous hermaphroditism in decapod Crustacea appears to be restricted, so far as our knowledge goes, to two genera of northern boreal and temperate pandalids, two Mediterranean species of the hippolytid *Lysmata*, the antiboreal South American and circum-Antarctic hippolytid *Chorismus* and at least two species of the antiboreal South American and deep-water Australasian palaemonoid *Campylonotus*. The three families represented are not closely related systemically, each presently being placed in a different super-family[9], and besides the rather tenuous zoogeographic relationships (? analogous to bipolarity) no link, other than that of reproductive physiology, can be seen.

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The Recent Sea-star *Platasterias* and the Fossil Somasteroidea

When discussing Fell's theories on the phylogeny of sea-stars[1–3], Philip[4] omitted any comparison of living forms with somasteroids. This comparison, however, deserves a short comment.

Spencer's sub-class of Asterozoa, the Somasteroidea[5], has always puzzled me, and I wonder whether the skeletal elements in *Villebrunaster* interpreted as ambulacralia may not be the adambulacralia, while the ambulacralia are hidden from view. I consider Spencer's virgalia to be ventrolateral ossicles. Figs. 1*a* and *b* show the resemblance between the reconstruction of a somasteroid and the ventrolateral skeleton of a recent *Porania*. (An aboral skeleton of radiate ossicles as found in some somasteroids is also present in *Porania*.) Spencer[5] compared this recent sea-star with his *Villebrunaster*, but only on account of its mucus-ciliary method of feeding which he assumed had also applied to the fossil. I would add that the resemblance stated is not taken to imply any close taxonomic relationship.

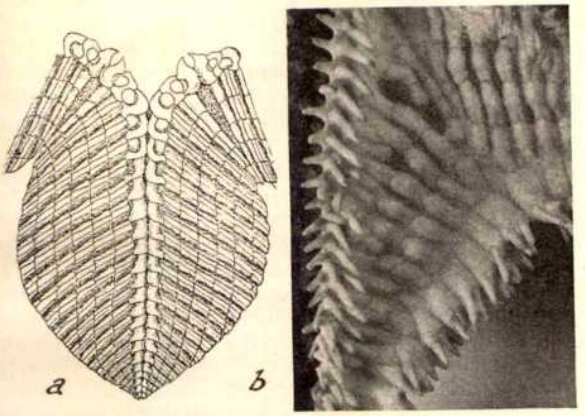


Fig. 1. *a*, *Villebrunaster* (after Spencer (ref. 5)); *b*, ventrolateral area of *Porania pulvillus* (O.F.M.)

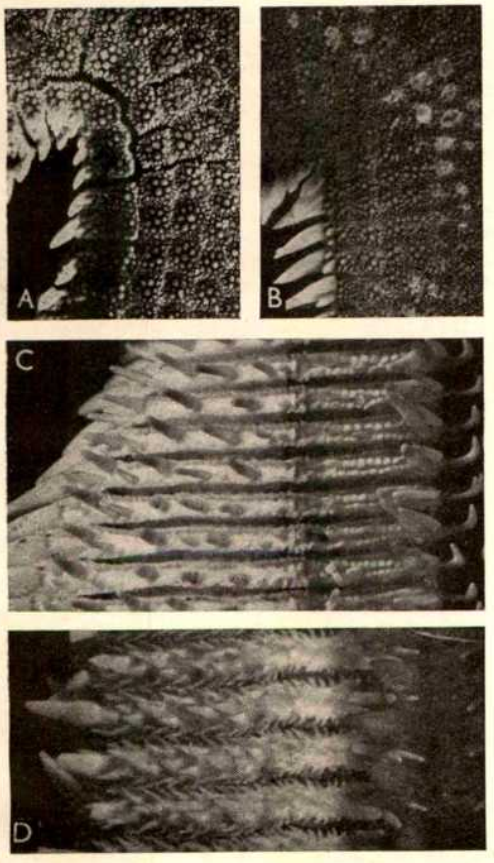


Fig. 2. Dorsal paxillae and ventrolateral skeleton of *Platasterias latiradiata* (*A*, *C*) and *Luidia* (*Petalaster*) *marginata* (*B*, *D*)

Fell[1–3] claims that *Platasterias latiradiata* Gray, 1871, is a living somasteroid. I consider this rare West-Mexican sea-star to be a somewhat aberrant *Luidia* of the sub-genus *Petalaster* (or of a sub-genus of its own). Fig. 2 illustrates the agreement in external characters between *Platasterias* and the East-Pacific *Luidia* (*Petalaster*) *marginata* Koehler. I assume the petaloid arms in *Platasterias* (brought about by a transverse elongation of the adambulacralia and inferomarginalia) to be a secondary adjustment to a life on a shifting sandy bottom (and perhaps a primarily ciliary method of feeding).

Fell[3] has put forward many interesting views on the phylogeny of sea-stars. I agree that the Luidiidae may be a very ancient group, but I fail to see how the Somasteroidea can indicate any closer affinity between asteroids and crinoids, even though *Villebrunaster* and *Chiniaster* may not be of so true an asteroid organization as I suspect.

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MICROBIOLOGY

Persistence of Fluoroacetate and Fluoroacetamide in Soil

Laboratory tests have shown that sodium fluoroacetate[1,2] and fluoroacetamide[3,4] can act as systemic insecticides, and the latter compound has been tested or used in the field for pest control (mainly against aphids) in several countries[5–7]. In Britain, however, following the Smarden incident, the use of these compounds as rodenticides has been severely restricted and their use as insecticides prohibited[8].

One question that arose in an acute form from the Smarden incident was the rate of decomposition of fluoroacetamide in the soil. It also arises when the materials reach the soil from rodenticides and insecticides. According to Horiuchi[9], bacteria in the soil break down fluoroacetamide; recently the organism responsible has been isolated[10].

This communication reports results of fuller tests, of the type already described[4], to determine the persistence in the soil of both sodium fluoroacetate and fluoroacetamide. The method used was to treat moist Kettering loam and local garden soil with 10 p.p.m. and 50 p.p.m. of the compounds, which were thoroughly incorporated. The soil samples were in 1 lb. screw-top jars and, in the case of the garden soil, half the batch was steam-sterilized after the chemicals had been added at 120 lb. pressure for 2 h. This enabled a comparison to be made between the rate of breakdown in unsterilized and sterilized soil. The jars were stored at 20° C and at intervals broad beans infested with *Aphis fabae* L. were planted in the soil samples and the fate of the aphid population on each plant was followed for 5 days. Similarly infested control plants in untreated soil were also kept.

The results obtained are shown in Table 1. It can be seen that 10 p.p.m. was not detectable in the Kettering loam by this means even immediately after addition. In garden soil toxicity could be detected and lasted for 2 weeks with sodium fluoroacetate or 3 weeks with fluoroacetamide. At 50 p.p.m. a lower level of toxicity was detected by this method in the ninth week but not in the eleventh week or later. In the sterilized garden soil, at

Table 1. Results of Biological Tests using *A. fabae* on Broad Beans to Test for Residues of Sodium Fluoroacetate and Fluoroacetamide in Two Soils

Dose applied to soil (p.p.m.)	Type of soil	Result of biological test for toxic residues after weeks: 0	1	2	3	5	9	11	12	17
		Sodium fluoroacetate								
10	Kettering loam	−	−	−	−					
	Garden soil	++	+−	−	−					
	Garden soil sterilized	++	+−	++	++	++	++	++	++	++
50	Kettering loam	++	+−	++	++	++	+	−	−	−
	Garden soil	++	+−	++	++	++	+	−	−	−
	Garden soil sterilized	++	+−	++	++	++	++	++	++	++
		Fluoroacetamide								
10	Kettering loam	−	−	−	−					
	Garden soil	++	+−	+	−	−	−			
	Garden soil sterilized	++	+−	++	++	++	++	++	++	++
50	Kettering loam	++	+−	++	++	++	+	−	−	−
	Garden soil	++	+−	++	++	++	+	−	−	−
	Garden soil sterilized	++	+−	++	++	++	++	++	++	++

++, Aphid population entirely eliminated in less than 5 days (high residues); +, aphid population reduced but not eliminated (some residue); −, no noticeable effect on aphid population (residue below level detectable).

both dosage-levels, toxicity was still evident at the end of seventeen weeks when the tests were concluded.

As an insecticide, in field tests, against aphids on beet, fluoroacetamide was used at 3 oz./acre in Britain[5]. However, it would probably be necessary to use 6 oz./acre to obtain satisfactory control of *Myzus persicae* (Sulz.). On the assumption that all this latter dose reached the upper 9–12 in. of the soil (which it would not) and remained there, this represents a concentration of 0·15 p.p.m. (taking the weight of top soil per acre to the depth indicated as 2,500,000 lb.[11]). At this concentration the compounds would not be detected by the biological method described here but, by inference, it seems safe to conclude that these small residues would also soon be destroyed.

Thus, although it may be desirable to prohibit the use of fluoroacetamide as an insecticide because of its high toxicity to mammals, the lack of a satisfactory antidote and the fact that its fate in mammals is not fully understood, there seem to be no grounds for condemning its use because of the fear that it might leave a persistent, harmful, residue in the soil. In fact, the evidence suggests that, when correctly applied as a systemic insecticide to the foliage of a field crop, any material reaching the soil would be destroyed within a few weeks, especially when the soil is warm. Of course, further tests under practical conditions and enquiries concerning the experience gained in Japan (where fluoroacetamide seems to have been fairly widely applied) would be essential before its use as an insecticide could again be contemplated.

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Staphylococcal Strains with Relation to Lysostaphin Sensitivity

The sensitivity of viable *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) and cell walls to the lytic action of lysostaphin has been described[1]. This communication reports experiments to determine the relative susceptibility of strains of staphylococci to lysostaph utilizing bacteriophage type and antibiotic sensitivity f strain differentiation.

It has been our experience that the rate of the decrea in turbidity of a staphylococcal suspension due to lyso staphin action varies with the different strains tested and that an extended incubation of bacterial cells with enzyme results in essentially equivalent numbers of survivors. Cropp and Harrison[2] have recently tested 252 clinical isolates of staphylococci and have compared their susceptibility to lysostaphin on the basis of the ratio of the reduction in turbidity of the test organism to that of a reference strain. Since differences in average bacterial cell size, quantity and quality of bacterial pigment, as well as released intracellular material, can all affect turbidimetric readings, experiments were designed to quantitate the number of bacterial survivors of different strains of staphylococci after a short incubation with a minima quantity of lysostaphin.

The strains of staphylococci were obtained from the Clinical Laboratory of the National Naval Medical Center, Bethesda, Maryland. Antibiotic sensitivity to penicillin (2 units), streptomycin (2 μg), tetracycline (5 μg), neomycin (30 μg), bacitracin (2 units), and kanamycin (5 μg was accomplished with 'Bacto-Sensitivity Disks' (Difco Laboratories, Detroit, Michigan) placed on agar overlays seeded with an 18-h culture of the strain under investigation.

Staphylococcal cells used in the lysostaphin susceptibility tests were from 18-h trypticase-soy broth (Baltimor Biological Laboratories, Baltimore) cultures resuspende in 0·05 M *tris*(hydroxymethyl)amino methane–hydrochloric acid buffer (*p*H 7·5) containing 0·15 M sodium chloride. Cell concentrations were adjusted to give an initial count of approximately 2×10^8 viable cells/ml. 0·2 ml. of lysostaphin (Mead Johnson Research Center Evansville, Indiana) was added to 6·0 ml. of the bacteria suspension to give a final enzyme concentration of 12 μ protein/ml. (ref. 3) (1·2 units/ml.). After 15 min a 37° C, serial dilutions of the suspension were made wit the first dilution tube containing trypsin (twice crystallized) at a concentration of 0·5 mg/ml. to stop further lysostaphin action[4]. Bacterial counts were made from pour plates after 18 h incubation. The results in Table 1 indicate that there is no definitive pattern of staphylococcal susceptibility to lysostaphin with either antibiotic sensitivity or bacteriophage type. In fact, it appears that the wide range of susceptibility to enzyme action over a short period of time is as correspondingly diverse as the bacteriophage type patterns of the different strains. Since the action of lysostaphin is directly on the bacterial cell wall mucopeptide[5] and the rate of killing cells per unit of time varies from strain to strain, it is believed that staphylococcal strain differences are directly related to cell wall composition in both the quantity and quality of the amino-acid composition of the peptide linkages. This

Table 1. Relationship between Bacteriophage Type, Antibiotic Sensitivity*, and Lysostaphin Susceptibility of Strains of Staphylococci†

Strain No.	Log drop in No. of survivors	Bacteriophage type	Antibiotic sensitivity: Penicillin	Streptomycin	Tetracycline
4073	2·21	3*C*, 71	−	+	+
3419	2·01	6, 53, 54, 73, 81	−	+	+
4201	1·85	6, 53, 54, 73, 75, 77, 81	−	+	+
4382	1·11	52, 80, 81	−	−	−
3539	1·01	53, 81	+	+	+
3529	1·14	Non-typable	+	+	+
3994	0·98	6, 53, 54, 73, 75, 77, 79, 81	−	+	+
4345	0·80	80, 81	−	−	−
3535	0·91	54, 73, 75	−	−	+
3621	0·88	3*A*, 3*C*, 6, 71, 81	+	+	+
4377	0·58	80, 81	−	−	+
4663	0·54	53, 54, 79, 80	−	−	+
3528	0·68	6, 42*E*, 54, 81	−	+	+
4098	0·65	Non-typable	−	−	+
4208	0·22	Non-typable	+	+	+
3538‡	0·09	——	+	+	−

* All strains were sensitive to neomycin, bacitracin and kanamycin.
† *S. aureus* strains were coagulase positive.
‡ *S. epidermidis*.

ould be in consort with the range of antigenicity due to ifferences of the surface mosaic and could account excluding strains that have been lysogenized) for bacteriophage susceptibility as being related to the detailed structure of the cell wall[6]. The results presented have been reproducible to ± 0·05 log, and it is conceivable that with a modification of the testing procedure to increase sensitivity and reproducibility, lysostaphin could be used to supplement bacteriophage typing and serotyping in epidemiological studies[7].

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Microbiological Oxidation of Cyclic Ketones

Ali Khan, Hall and Robinson[1] have reported that when a Gram-negative bacterium (probably *Pseudomonas* sp.) was grown in an aqueous suspension of *n*-octane plus mineral salts, dioic acids (mainly suberic acid) were recovered from the medium. The activity of this bacterium (culture kindly presented by Dr. A. N. Hall) and of ten other species of bacteria on cyclic ketones has been investigated. There appears to be no previous report of microbiological oxidation of cyclic ketones. In his limited survey, Baldacci[2] could not detect any microbial activity on *cyclo*hexanone.

Ten species of bacteria which had been previously reported to oxidize hydrocarbons[3–5] were obtained from the National Collection of Industrial Bacteria and, together with the bacterium of Ali Khan *et al.*, were cultured at 30° C in an aqueous medium containing 1 per cent w/v 2-heptyl*cyclo*pentanone, plus mineral salts (0·5 per cent ammonium nitrate, 0·1 per cent magnesium sulphate, 0·02 per cent calcium chloride, 0·01 per cent ferric nitrate, and 0·1 M phosphate buffer to *p*H 7·0). After 5 days, the culture solutions were exhaustively extracted with diethyl ether after acidification, and the extracts were subjected to gas-liquid chromatography. All showed varying amounts of residual heptyl*cyclo*pentanone. The culture solutions of *Pseudomonas oleovorans* 6576, *Ps. aeruginosa* 8295, *Ps. fluorescens* 8027, *Ps. methanica* 9133 and the bacterium of Ali Khan *et al.* also contained 0·2–0·5 mg/ml. of the lactone of 5-hydroxydodecanoic acid, the first-named giving the highest yield of lactone. The lactone was identified before and after conversion to the free acid and methylation, by comparison with authentic samples of the lactone and of 5-hydroxydodecanoyl methyl ester on gas-liquid chromatographic analysis. The ethereal extracts of the culture solutions of *Ps. aeruginosa* 950 and 5940, *Ps. rubescens* 8768, *Vibrio oleo* 8250 and *Arthrobacter simplex* 8929 contained only heptyl*cyclo*pentanone. A microbiological synthesis of such lactones has been reported by Tuynenburg Muys *et al.*[6]. These authors found that the microbiological reduction of racemic keto-acids could produce optically active lactones. Therefore, the 2-heptyl*cyclo*pentanone medium of *Ps. oleovorans* was examined after culture. It was found to be laevo-rotatory. As the cyclic ketone was racemic, this activity was ascribed to the lactone. Assuming all the lactone produced was optically active, $[\alpha]_D = -36{\cdot}6°$.

It was found that *Ps. oleovorans*, *Ps. fluorescens* and the bacterium of Ali Khan *et al.* would also oxidize 2-pentyl*cyclo*pentanone, giving the lactone of 5-hydroxydecanoic acid, and would oxidize *cyclo*pentanone to glutaric acid. The last-mentioned product was identified only after methylation.

Of the total ketone initially present, less than 5 per cent was recovered as partially oxidized product, and between 60 and 70 per cent of the cyclic ketone was recovered unmodified.

Thus two or perhaps three species of bacteria, of which one is already known to promote oxidation of an *n*-alkane to dioic acids, oxidize *cyclo*pentanone to glutaric acid, a dioic acid with the same number of carbon atoms. This process involves ring cleavage, which has not been demonstrated previously with these bacteria. When asymmetric molecules of alkyl-substituted *cyclo*pentanones are offered, cleavage occurs at the most labile bond in the ring, between the two substituted carbon atoms. However, in this case, the presence of the alkyl side-chain prevents more complete oxidation to give a dioic acid. The reaction goes no further than the production of a hydroxy acid, which then lactonizes. This is further evidence to add to that of Kester and Foster[7] that microbiological oxidation of alkyl compounds to give dioic acids proceeds by way of hydroxy acid intermediates.

I thank Mr. E. Taylor for performing the polarimetry examination.

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CYTOLOGY

Diffuse Centromeres in a Dicotyledonous Plant

A CYTOLOGICAL search for a sex chromosome mechanism in *Myristica fragrans*, the common nutmeg tree, has revealed some meiotic peculiarities. Mitotic metaphase counts in this admittedly unfavourable material usually gave a number of ≤4 small, more or less isodiametric elements. This is at variance with the results of Simmonds[1], who found 42 chromosomes.

At early first meiotic metaphase in a male-only flowering tree from Trinidad, I counted 22 strongly contracted chromosome bodies. These bivalents, in polar view as well as in side view, were often slightly sub-divided into two halves, thus in end view showing a quadripartite structure (Fig. 1). In late first metaphase, the configurations loosened up, mainly perpendicular to the metaphase plane, into what appeared to be four slightly interconnected chromatids. By releasing the connexions across the equatorial plane, the bivalents divided into two halves, each presumably consisting of two interconnected short chromatids, parallel to the equatorial plane. At second anaphase the chromosome bodies split into two short rods. Each attempt to localize centromeres with the help of meiotic behaviour failed. This suggested centric activity lacking localized character. Configurations really show a close similarity to those of *Luzula campestris*, as described by Brown[2].

As pointed out by Hughes-Schrader and Ris[3], induced fragments of chromosomes with such centric activity will perpetuate themselves through many cell generations. This is in contrast to the behaviour of acentric fragments of chromosomes with localized centromeres, which are

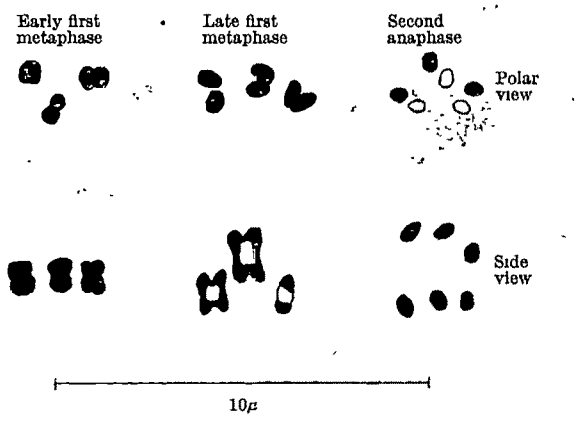


Fig. 1. Three of the 22 meiotic elements sketched with the aid of a camera lucida

rapidly lost at nuclear division. In order to verify the centromeric condition assumed, root tips irradiated with 1,000 r. were examined for fragments of chromosomes. At least one supernumerary fragment was recorded in half the metaphases counted. In preparations made at the day of irradiation and at the following day, no chromosome parts were found outside the metaphase plane of cells in full metaphase, nor any chromosome parts lagging in cells in anaphase or telophase. A second experiment (2,000 r.) did not reveal lagging fragments or micronuclei. Even 2 months after irradiation, several metaphases still showed as many as three supernumerary fragments.

These observations convince me of the presence of diffuse centromeres in the chromosomes of *Myristica fragrans*. As far as I know, this is the first report of diffuse centromeres in a dicotyledon. [Further details have been published in *Mededelingen Landbouwhogeschool, Wageningen*, 66, 1966, No. 1.]

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AGRICULTURE

Spread of Potato Virus *Y* curtailed by Oil

Aphids continue to spread stylet-borne viruses despite present-day insecticides[1]. The finding that aphids carrying potato virus *Y* seldom transmit it after probing a surface lightly coated with oil offers a novel way of bringing this spread under control[2]. Additional laboratory work has provided various reasons for believing that oil could be used to protect crops from stylet-borne viruses[3]. We have tried this in the field for 2 years now and the results are promising.

During both years we experimented with the spread of virus *Y* in Green Mountain potatoes. In 1963 the design was a 5 × 5 Latin square, in 1964 it was an 8 × 4 randomized block; each plot was four rows wide and 30 ft. long with 33 plants per row; the plots were separated longitudinally by 25 ft. of fallow ground, and laterally by strips of oats 10 ft. wide. Spread of virus *Y* was encouraged in two ways: (*a*) a series of plots were infested with viruliferous aphids, wingless adults of *Myzus persicae* (Sulz.); (*b*) in another series each plot had twelve implanted virus *Y* infected potatoes (hereafter referred to as infectors). These grew from infected tubers planted in positions 6, 17, and 28 of each row, an arrangement that meant each infector was preceded and followed in the row by a separate group of five healthy plants. In 1963 two series of plots were infested with viruliferous aphids, and two series had infector plants. One series of each pair had oil sprayed directly on the plants six times at weekly intervals, and its counterpart was left unsprayed. The fifth series was left untreated as a check for spread between plots and from outside the trial. The 1964 trial had three additional series, all of which had infector plants. These allowed the work to be enlarged to comparisons between: (*a*) oil applied as a direct spray and oil emulsified in water, (*b*) the first three applications of each kind of spray and six applications of each, all at weekly intervals. The three series that did not receive oil in 1964 were sprayed with water on the same days and in the same way as oil was applied.

Planting was done in the middle of May with commercial seed tubers certified to be less than 0·5 per cent infected with aphid-borne viruses. All plots were sprayed with calcium arsenate soon after plant emergence and thereafter as needed to control beetles, and with Bordeaux weekly from late June until mid-August to prevent blight. To determine spread of virus *Y*, we took tuber samples at the end of August and grew them indoors during the winter. Infected plants were diagnosed from visible symptoms, with occasional checks by serological precipitin tests. In 1963 we took individual tubers from: (*a*) every plant except the infectors in plots having these, (*b*) the 200 plants infested with viruliferous aphids, and (*c*) 100 plants in each of the five check plots. In 1964 we took one tuber from each plant in the middle two rows of every plot; and to make the plot samples comparable, we discarded the tubers from the 6th, 17th, and 28th plant in each row whether they were infectors or not. We recorded the position of each tuber in the row. Of a possible 3,820 tubers from both trials, 94 plants were missing in the field and 11 tubers either failed to grow or were lost.

The oil was supplied by Imperial Oil under the designation of '862*B*' (viscosity 90–105 S.U. sec at 100° F). There is no reason to believe that this oil has any unique properties in impeding aphid transmission of stylet-borne viruses, nor was it toxic to aphids at the levels used. In both years we began spraying with oil about a week after planting, and emergence exceeded 70 per cent in all plots. In 1963 oil was applied only as a direct spray. This was done with a portable petrol-powered suction-type sprayer (model 400010 made by Solo Kleinmotoren, Stuttgart, Germany). Not knowing how much oil young potatoes can tolerate, we made the first application in 1963 with 0·5 gal./acre, and then doubled the rate each week so as concurrent tests on potatoes outside the trial showed the plants could stand the higher amounts. The successive rates of application were 0·5, 1, 2, 4, 4, and 4 gal./acre on June 19, 26, July 4, 10, 17, and 24 respectively. In 1964 the first two applications of oil were at 2 gal./acre and the remainder at twice this rate; the application dates were June 19, 26, July 2, 9, 16, and 24. For the emulsion sprays we used the same oil but with an emulsifier supplied by Imperial Oil added. The emulsion was applied with a tractor-drawn sprayer operated at a pressure of 400 lb./in.^{2}, the delivery rate being 100 gal./acre.

For viruliferous aphids we used wingless adults from a laboratory culture of *M. persicae* reared on *Brassica napus* L. They were first kept from feeding for about an hour, and then allowed to probe briefly on virus *Y* infected tobacco plants before being placed on the upper leaves of the potatoes. The plants to be infested were first thinned to a single stalk to ensure that tubers taken later for disease readings would not be from uninfested stalks. The infestation was done in both years between the

fourth and fifth applications of oil. In each of the 10 plots infested in 1963, we tagged twenty plants at random and infested each with two aphids on July 10. In 1964 we infested every plant in the middle two rows of eight plots, first with two aphids per plant on July 9 and then with one more on July 15.

In 1963 oil reduced spread by 56 per cent in plots with infectors, and by 83 per cent in those infested with viruliferous aphids. In the untreated checks 9 per cent of plants became infected; this represents the natural spread between plots and from outside the trial. Because this spread would have occurred to all plots, it is probable that it also was reduced by oil; in the series infested with viruliferous aphids and sprayed with oil, only 3 per cent of plants became infected, which is significantly less than the 9 per cent in the checks.

In 1964 there was negligible spread between plots and from outside the trial. Although few infections occurred in the infested plots, here the effect of oil was the same as in the previous year. Most of the spread in 1964 was within plots with infectors, and oil reduced it even more than in 1963. Where oil was applied directly, three applications reduced spread by 71 per cent, whereas six did so by 88 per cent. There was little difference between the effectiveness of three and six applications of emulsion —the combined effect of these two treatments being to reduce spread by 78 per cent. Thus both the direct and emulsion sprays were effective.

In the four series that had infectors and were sprayed with oil in 1964, more than half the infections were to plants immediately adjacent to infectors (Fig. 2). If the percentages in Fig 2 are converted to numbers of independent infections[4], it can be shown that the overall effect of these four oil treatments was to reduce the number of infections by 87 per cent.

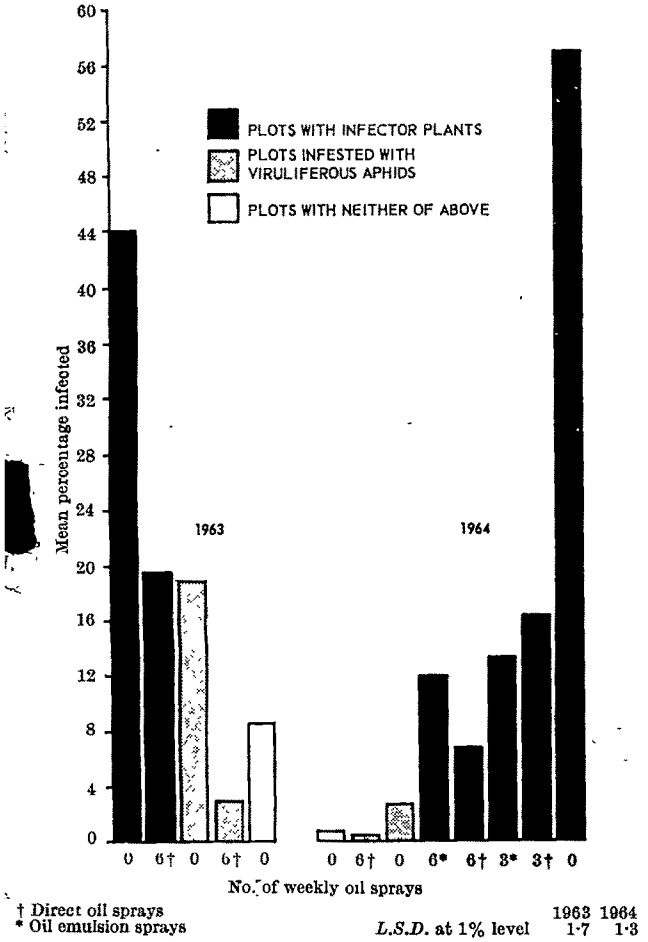


Fig. 1. Effect of oil on the spread of virus *Y* in Green Mountain potatoes during 1963 and 1964

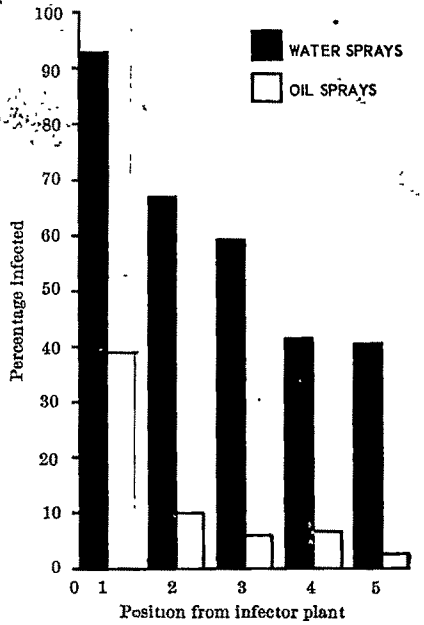


Fig. 2 Percentages of plants infected at each successive position from the infector plants in 1964—all four oil treatments were combined

Despite the rather high levels of oil applied in both years, the only visible damage to plants was some burning on occasional leaflets and petioles; direct oil sprays caused more of this than the emulsion. The oil did not reduce tuber yields in the first year; but six applications did so by about 15 per cent in 1964, although three did not affect yields.

Our results so far give us confidence that oil can be used to protect crops from virus *Y*, and there are good reasons for believing that this will also apply to other stylet-borne viruses.

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PSYCHOLOGY

Differential Effects of Group Density on Social Behaviour

In a study of the social behaviour of different groups of children, one of the aspects selected for specific investigation was the effect of group density. In view of the findings from animal studies, it was hypothesized that increasing group density would have adverse effects on the nature and frequency of social encounters, and that these effects would differ according to the personality of the subject. Furthermore, if earlier hypotheses regarding the physiological activation of autistic[1,2] and brain-damaged[3] children are correct, these two groups of children might be expected to manifest, in exaggerated form, the reactions of introverted or relatively inhibited individuals and extraverted individuals, respectively, in the normal population.

The subjects of this study were fifteen children who were in- or day-patients in the hospital: five were autistic[4], five had unequivocal evidence of fairly gross brain-

damage, and five were 'normal' (that is, two normal siblings of patients and three who had recovered from minor behaviour disturbance and were about to be discharged). The last-mentioned three children did not differ significantly on any of the measures from the two who were not patients, and there seemed no *a priori* reason for not grouping all five together as 'normals'. All subjects were between the ages of 3 and 8 years, and the groups were matched for age.

The situation used for the observations was a 27 ft. × 17·5 ft. playroom where the children customarily assembled for 'free play'. Three group sizes were used: (i) small ($n \leqslant 6$); (ii) medium ($n = 7$–11); (iii) large ($n \geqslant 12$). Other children in the hospital formed the rest of the group (only two or three of the subjects were present at any one session). Groups and densities were arranged in a 3 × 3 Latin-square design, four replicates of this design then being used. An observation period per subject was 15 min and for each subject under each condition the mean of three observation periods was obtained. Observations were recorded on: (*a*) check-lists (1 entry per 10 sec); (*b*) magnetic tape; (*c*) 8-mm ciné film. Two adults, usually nurses, were always present; in the larger groups three adults were often present.

The relative proportions of time spent in aggressive or destructive behaviour (that is, fighting, snatching or breaking toys, etc.) by the three groups of subjects under the different conditions are given in Table 1.

Table 2 shows the amounts of time that were spent in social encounters with other children or adults, and which did not involve any show of aggression.

With increasing density certain children appeared to seek the safety of the boundary (that is, within 3 ft. of the periphery of the room) more frequently (Table 3).

Table 1. Mean Percentage Time (±*S.D*) spent in Aggressive/Destructive Behaviour

	Small	Medium	Large
Normal	3·0±1·7	4·6±2 4	16·1±5·2
Brain-damaged	2·7±2·8	19·0±8·2	28·0±11·2
Autist	2·5±3·7	1·9±2·8	2·1±3·4

Table 2. Mean Percentage Time (±*S D.*) spent in Social Interactions

	Small	Medium	Large
Normal	63·0±17·3	49·3±16·2	39·1±11·0
Brain-damaged	23·0±4·0	43·0±15·6	31·0±14·7
Autist	15·0±5·3	6·5±6·7	14·2±11·8

Table 3. Mean Percentage Time (±*S.D.*) spent on the Boundary

	Small	Medium	Large
Normal	15·0±6·6	13·1±4·2	10·3±5·5
Brain-damaged	24 6±3·6	8·7±4·7	16 0±4·9
Autist	21·5±7·7	30 0±9·0	55·2±6·8

Analyses of variance were carried out on the data, the appropriate error term in these cases being the 'within cells' variance, since $n > 1$. Subsequently Scheffe's test for multiple comparisons of means was applied.

For aggressive/destructive behaviour, both the main effects between groups and between densities were significant ($F = 6·8$, d.f. 2/36, $P < 0·01$, and $F = 8·3$, d.f. 2/36, $P < 0·01$, respectively), as well as the groups × densities interaction ($F = 4·2$, d.f. 4/36, $P < 0·01$). The brain-damaged subjects became more aggressive with increasing group-density, whereas the normal subjects only became significantly more aggressive in the large group. The autists showed negligible aggression.

For social encounters, there was a significant main effect between groups ($F = 22·3$, d.f. 2/36, $P < 0·01$), and a significant interaction between groups and densities ($F = 2·8$, d.f. 4/36, $P < 0·05$). The normals showed progressively and significantly less social interaction with increasing group-size. The brain-damaged subjects showed significantly more interaction in the medium group, and the autists significantly less.

For time spent on the boundary, both the main effects between groups and between densities were significant ($F = 55·8$, d.f. 2/36, $P < 0·001$, and $F = 5·4$, d.f. 2/36, $P < 0·01$, respectively), as well as the groups × density interaction ($F = 7·9$, d.f. 4/36, $P < 0·001$). As density increased, the autists spent significantly more time on the boundary.

The finding that aggressive/destructive behaviour increased in both normal and brain-damaged subjects under high densities is in agreement with other animal work: Clarke[5] found that voles in a high-density population had more numerous scars from fighting and were in poorer health than animals living in a less dense population; Chitty[6] has suggested that intraspecific strife at high population densities severely limits the number of voles, that "populations change their nature as a result of crowding, and that a change in the condition of the individuals may be reflected in the vitality of their descendants"[7]; Calhoun[8] has described the emergence of a 'behavioural sink' as well as the sexual and social deviations manifested by Norway rats reared under high-density conditions. In fact the behavioural deviations of these rats ranged from 'phrenetic' activity to 'pathological withdrawal', and it is of interest that the brain-damaged and autistic subjects tended to show predominantly these extreme reactions respectively. In a large group two brain-damaged subjects were seen to bite other children, a behaviour not manifested by any other children.

The autists, indeed, showed behaviour characteristic of physiologically highly-activated organisms: they manifested little aggression, and with increasing density retreated more and more to the periphery of the room and often would be found sitting facing the wall. Their social interactions in the large group consisted almost entirely of approaches to the adults—to escape from the general 'mêlée', it seemed.

It is remarkable that despite the greater opportunity for social interaction with the larger groups, the normal subjects in fact showed less. These subjects were apparently endeavouring to minimize their social encounters, and this is an important point in view of Calhoun's finding that the frequency and 'velocity' of social encounters in rats were markedly increased at high densities, and of Leyhausen's[9] implied suggestion that humans living in high-density conditions might show 'crowd-addiction'.

Many writers and authorities, extrapolating from the infra-primate studies, are at present warning of the social dangers of human crowding[9–12]. There is, however, a regrettable lack of empirical evidence from studies of humans in different group densities. The findings of this preliminary study would suggest that the effects of crowding are complex, and that the nature or personality of the individuals involved in the interaction is a critical variable; furthermore, it is likely that in the human species, too, certain density-dependent regulatory mechanisms are capable of operating.

A fuller analysis of the behavioural changes with different group densities is in preparation. We are grateful to the Smith, Kline and French Foundation and the British Epilepsy Association for provision of film and equipment, and to the Nuffield Foundation for financial support.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 28

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (joint meeting with the British Society of Rheology, at the School of Pharmacy, Brunswick Square, London, W.C.1), from 9.30 a.m. to 5.15 p.m. —Symposium on "Chemistry and Rheology of Water Soluble Gums and Colloids".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 10 a.m.—Colloquium on "Making Plant Models".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. E. S. Clarke: "The Early History of the Nerve Cell".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with I.E.R.E. Television Group and the Television Society, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m. —Mr. R. Bridgen, Mr. K. E. Martin and Mr. P. L. Mothersole: "Semiconductors in Television Receivers".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Caravan Towing".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. E. Millard: "The Opposition to Reinforced Plastics in the Aircraft Industry".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. E. J. H. Corner, F.R.S.: "The Solomon Islands".

Tuesday, March 29

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at Battersea College of Advanced Technology, Battersea Park Road, London, S.W.11), at 10 a.m.—Symposium on "High-Temperature Technology".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. COMPUTER GROUPS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 2.30 p.m. and 5 p.m.—Symposium on "Computer Control in Industry—Equipment Design and Application Engineering".

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at the Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 6 p.m. —Annual General Meeting followed by Prof. R. H. S. Thompson: "The Nervous System and the Clinical Biochemist".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Meeting on "Rubber in Coaching Stock Construction".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Annual General Meeting, followed by "Basic Design Problems in Injection Moulding Equipment" (speaker from Demag AG)

Wednesday, March 30

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 3 p.m.—Meeting on "The Presentation of Navigational Information".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. Alexander Comfort. "Science and Longevity" (Trueman Wood Lecture).

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at "The Crown", St. Albans Road, Garston, Herts), at 7 p.m.—Mr. A. E. Bray: "The Role of Rubber and Plastics in the Motor-Car of the Future".

Thursday, March 31

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 2.30 p.m. and 5.30 p.m.—Symposium on "Monitoring of Ground and Airborne I.L.S. Equipment for Automatic Landing".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. S. Gillespie: "Tissue Binding of Noradrenaline" (review lecture).

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Snowfall Measurement and the Water Balance of Ice Caps", introduced by Dr. H. Lister.

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. C. H. Brissenden: "Refrigeration in the Dairy Industry".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1, Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Conversion from Coal to Oil Firing".

CROYDON NATURAL HISTORY AND SCIENTIFIC SOCIETY (at Friends' Meeting House, Park Lane, Croydon, Surrey), at 7.30 p.m.—Dr. D. T. Streeter: "The Management of Nature Reserves—Some Ecological Principles".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Mr. D. J. Iexander: "Some Aspects of Laboratory Planning".

Thursday, March 31—Friday, April 1

BRITISH INSTITUTE OF RADIOLOGY (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 10.30 a.m. on Thursday and 10 a.m. on Friday—Twenty-Seventh Annual Congress and Exhibition.

Friday, April 1

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Sequence Control of Plant Operations", opened by Mr. M. F. Binney, Mr. K. H. Higgens, Mr. T. K. M. Korytko and Mr. D. A. G. McInnes.

Monday, April 4

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. K. W. Pepper. "The Challenge of Synthetics to Leather".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS or ASSISTANT LECTURERS (2) (with a good honours degree in engineering, science or mathematics, and preferably postgraduate, industrial or research experience in one of the following fields: advanced propulsion systems, aero/space science, low density and/or high temperature gas dynamics, aircraft structures) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS—The Deputy Registrar, The University, Southampton (March 31).

LECTURER and an ASSISTANT LECTURER IN PHYSIOLOGY—The Secretary and Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 1).

ASSISTANT DIRECTOR (with a good honours degree in botany and experience in taxonomic work) OF THE UNIVERSITY BOTANIC GARDENS—The Registrar, The University, Liverpool, quoting Ref No. RV/482 (April 2).

LECTURER or ASSISTANT LECTURER (non-medical) IN THE DEPARTMENT OF PATHOLOGY—The Registrar, The University, Liverpool 3, quoting Ref. No. RV/476 (April 2).

SENIOR LECTURER (with high academic qualifications and the ability and desire to prosecute research) IN METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (April 4).

ASSISTANT LECTURERS IN LOW TEMPERATURE PHYSICS—The Registrar, University College of North Wales, Bangor, North Wales (April 5).

HEAD OF THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Principal, Lanchester College of Technology, Priory Street, Coventry (April 5).

CHAIR OF NUMERICAL ANALYSIS—The Assistant Registrar, The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (April 6).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Leicester (April 6).

CHAIR OF CHEMISTRY—The Secretary, University of Stirling, Stirling, Scotland (April 7).

LECTURER (with a higher degree such as Doctor of Philosophy and some postgraduate experience) IN ORGANIC CHEMISTRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (April 8).

SENIOR LECTURER IN STATISTICS—The Registrar, University College of Wales, Aberystwyth (April 8).

LECTURER or ASSISTANT LECTURER (preferably with special interests in analysis or functional analysis but applications from others with interests in pure mathematics are invited) IN THE DEPARTMENT OF MATHEMATICS—The Secretary, The University, Aberdeen (April 9).

LECTURERS (2) and an ASSISTANT LECTURER (with a good honours degree in mathematics, physics or engineering and a special interest in mathematics for engineers) IN ENGINEERING MATHEMATICS—The Secretary, The University, Aberdeen (April 9).

OFFICIAL FELLOW IN PHILOSOPHY—The Principal, Brasenose College, Oxford (April 12).

UNIVERSITY DEMONSTRATOR IN ORGANIC CHEMISTRY—Dr P. Maitland, University Chemical Laboratory, Lensfield Road, Cambridge (April 12).

LECTURER IN THE DEPARTMENT OF CHEMISTRY (Inorganic and Analytical) —The Secretary, The Queen's University, Belfast, Northern Ireland (April 13).

LECTURERS or ASSISTANT LECTURERS IN THE DEPARTMENT OF CHEMISTRY —The Registrar, The University, Manchester, 13, quoting Ref. 38/66 (April 13).

LECTURER IN PURE MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (April 15).

LECTURER IN THEORETICAL PHYSICS—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (April 15).

CHAIR OF EXTRACTION METALLURGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (April 16).

LECTURER (with a special interest in thermodynamics or mechanics of fluids) IN MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (April 16).

SECOND CHAIR (candidates should have special interests in control engineering) IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Registrar, The University, Sheffield (April 16).

SENIOR LECTURER IN MATHEMATICS or STATISTICS, a LECTURER or ASSISTANT LECTURER IN MATHEMATICS, a LECTURER or ASSISTANT LECTURER IN STATISTICS, a LECTURER or ASSISTANT LECTURER IN OPERATIONAL RESEARCH, and a LECTURER or ASSISTANT LECTURER IN NUMERICAL ANALYSIS—The Registrar, Bradford Institute of Technology, Bradford 7, Yorkshire (April 18).

PROFESSOR OF EDUCATION AND DIRECTOR OF THE INSTITUTE OF EDUCATION, Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 20).

LECTURER IN ANATOMY at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (April 22).

ASSISTANT LECTURER or LECTURER IN CHEMISTRY (the appointment may be made in any branch of organic chemistry)—The Registrar, The University, Sheffield (April 30).

JUNIOR DEMONSTRATOR (preferably with experience in some branch of endocrinology, preferably steroid biochemistry) IN THE DEPARTMENT OF PHYSIOLOGY—Prof. A. A. Harper, The Department of Physiology, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, 1 (April 30).

LECTURER (with interests in the theoretical and experimental aspects of modern control techniques) IN CONTROL ENGINEERING in the School of Engineering Science—The Registrar, University College of North Wales, Bangor, North Wales (April 30).

OFFICIAL FELLOW and LECTURER IN ENGINEERING—The President, Magdalen College, Oxford (April 30).

PROFESSOR OF PSYCHOLOGY—The Registrar, University Registry, Oxford (April 30).

THIRD CHAIR OF MATHEMATICS (Pure Mathematics) AT THE UNIVERSITY OF MELBOURNE, AUSTRALIA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 1).

ASSISTANT RESEARCH FELLOW or a RESEARCH FELLOW IN SOCIOLOGY OR ANTHROPOLOGY in the East African Institute of Social Research, Makerere University College (University of East Africa), Uganda—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 2).

SENIOR FELLOWS, a SENIOR RESEARCH FELLOW, FELLOWS, and RESEARCH FELLOWS IN THE DEPARTMENT OF NUCLEAR PHYSICS, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (May 13).

CHAIR OF VIBRATION STUDIES IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH—The Deputy Secretary, The University, Southampton (May 14).

POSTDOCTORAL RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICAL CHEMISTRY for the investigation of ion molecule reactions using a differentially pumped mass spectrometer—Dr. M. J. Henchman, Department of Physical Chemistry, The University, Leeds, 2 (May 31).

LECTURER or ASSISTANT LECTURER (graduate with an interest in yarn manufacture, including the spinning of natural and man-made fibres, and the bulking of continuous-filament yarns) IN THE DEPARTMENT OF TEXTILE TECHNOLOGY—The Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester 1 (June 4).

ASSISTANT (with a good honours degree and preferably postgraduate experience) IN CELL BIOLOGY—The Secretary of the University Court, The University, Glasgow.

BIOCHEMIST (preferably experience with chromatographic and electrophoretic techniques) to join a team studying aspects of copper and magnesium metabolism in animals—The Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh, 9.

LECTURER or ASSISTANT LECTURER (with a good honours degree in psychology) IN PSYCHOLOGY (Experimental Psychology/Social Psychology)—The Registrar, Goldsmiths' College (University of London), London, S.E.14.

LECTURERS or ASSISTANT LECTURERS (3) IN PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth.

PHYSICAL CHEMIST (capable of pursuing and/or supervising basic researches mainly on electrodeposition and electroforming)—The Secretary, The British Non-Ferrous Metals Research Association, Euston Street, London, N.W.1.

PROFESSOR OF BIOLOGY—The Registrar, The Chinese University of Hong Kong, Hang Seng Bank Building, 677 Nathan Road, Kowloon, Hong Kong.

RESEARCH ASSISTANT IN HAEMATOLOGY to work on problems related to iron deficiency anaemia and tissue fibrinolytic systems—Dr. A. Jacobs, Department of Pathology, Welsh National School of Medicine (University of Wales), Royal Infirmary, Cardiff.

RESEARCH ASSISTANT (post-graduate or post-doctoral, with a good degree in botany or horticulture, but students expecting to graduate this year may also apply) for investigation of the developmental physiology of the carnation—Prof. O. V. S. Heath, F.R.S., University of Reading Horticultural Research Laboratories, Shinfield Grange, Shinfield, Reading, Berkshire.

RESEARCH ASSISTANT (with a degree in either geography or geology and a special interest in geomorphology) IN GEOLOGY—The Registrar, University College of Wales, Aberystwyth.

RESEARCH ASSISTANTS (2) IN THE DEPARTMENT OF PHYSICS to undertake (*a*) spectroscopic measurements in discharge plasmas, and (*b*) scattering measurements using lasers—The Registrar, University of York, Heslington, York.

SCIENTIFIC OFFICERS (with an honours degree in chemistry or biology and under 29 years of age) IN THE SCIENTIFIC LABORATORY for work covering a wide range of inquiry and research in the field of forensic science—The Secretary, Room 164 (SO), New Scotland Yard, London, S.W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

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